

Supplementary Material

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

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Table S1. Pedigree and other information about the genotypes of sorghum used in this study.

S. No.	Genotypes	Pedigree	Remarks
1	ICSV 25275	(IS 19587 x B 24)-2-1-1-1	Post rainy salt tolerant sweet sorghum variety, developed at ICRISAT, Patancheru (India).
2	ICSV 25280	(ICSV 93046 x SSV 84)-7-2-1-3	Post rainy sweet sorghum variety, developed at ICRISAT, Patancheru (India).
3	RSSV 9	(RSSV 2 X SPV 462)-2-1-2-1	Both rainy and post rainy sweet sorghum variety, released by Mahatma Phule Krishi Vidyapeeth, Akola, (India) in 2010.
4	ICSSH 58	ICSA731 x ICSV93046	Both rainy and post rainy, salt tolerant, sweet sorghum hybrid variety, developed at ICRISAT, Patancheru (India).
5	M 35-1	Selection from Maharashtra, India	Most popular post rainy drought tolerant grain sorghum variety, released by Marathwada Agricultural University, Parbhani (India).
6	SPV 1411	Selection from GD 31-4-2-3	Popular post rainy grain sorghum variety, know as Parbhani Moti, released by Marathwada Agricultural University, Parbhani (India) in 2002.
7	PVK 801	[(IS 23528 × SPV 475) × (PS 29154)]-4-2-2-4	Popular rainy and mold tolerant grain sorghum variety released by Marathwada Agricultural University, Parbhani (India) in 2000.

Table S2. Phenological stage of plants, relative water content and sap volume at time of 1st harvest and number of days to 50% flowering and to physiological maturity in the sorghum genotypes. (♦) indicates lack of observations due to insufficient material.

	Stage at 1 st harvest		RWC(%) of flag leaf at 1 st harvest. Sap volume in 5 th internode in ml g ⁻¹ dry wt. at 1 st harvest (in parenthesis) Mean of n=5		Days to 50% flowering		2 nd harvest at physiological maturity stage (days)	
	Control (irrigated)	Drought (20 [•] days)	Control (irrigated)	Drought (20 [•] days)	Control (irrigated)	Drought (20 [•] days)	Control (irrigated)	Drought (recovered)
ICSV25275	Heading	Heading	100 (4.2)	67.3 (3.6)	83	71	118	106
ICSV25280	Heading	Heading	100 (4.2)	69.7 (3.1)	85	72	120	107
PVK801	Heading	Booting	100 (5.2)	75.6 (4.8)	81	71	116	106
RSSV9	Booting	Anthesis	99 (3.5)	69.5 (2.9)	76	69	111	104
ICSSH58	Heading	Heading	98 (4.4)	76.8 (5.3)	81	72	116	107
M35-1	Heading	Heading	100 (4.8)	68.2 (3.9)	80	68	115	103
SPV1411	Booting	Booting	100 (4.4)	74.1 (4.5)	81	71	♦	♦

[•]= Control plants received irrigation every ten days. One irrigation application was omitted for imposing drought stress. Irrigation was resumed at ten day intervals in both control and drought stressed plants up to physiological maturity.

Heading stage = plants with visible flag leaf.

Booting stage = 50 % of plants with visible heads.

Anthesis stage = 50 % of plants in bloom.

Table S3. Genes used for studies on transcriptional expression, primer sequences, expected product sizes and the annealing temperatures used during PCR. The gene sequences were obtained from Gramene database (<http://www.gramene.org>).

Gene number	Gene id	Primer sequence	Product size	Annealing Temperature °C
<i>Sucrose phosphate genes</i>				
SPS 1	Sb03g043900	FP 5' TGGCGATACCGATCTAGAGG 3' RP 5' CAGTCGTGAGCCACCAGTAG 3'	415	55
SPS 2	Sb04g005720	FP 5' ACCCTTTGCAGGATGTTGTC 3' RP 5' ACCGGTTTCTTGTTTGGTTG 3'	359	55
SPS 3	Sb05g007310	FP 5' TGTCGATACAGTGGGGCATC 3' RP 5' ATAACCCCAGCAGAGTGTGG 3'	360	50
SPS 4	Sb09g028570	FP 5' TGCTTCAATTGAGGGTATTGG 3' RP 5' TTGCAACAACGGAGAAACTG 3'	308	48
SPS 5	Sb10g025240	FP 5' GATGCTGTGGTCATTGTTGG 3' RP 5' TGCAGGGACCACACACATAG 3'	389	53
<i>Invertase genes</i>				
INV 1 (cell wall)	Sb01g008910	FP 5' AACCTCTACAAGCCGACCTTC 3' RP 5' GCCGTTAGACTCATATGCTACC 3'	303	50
INV 2 (cell wall)	Sb03g047060	FP 5' ACTTTGATCGATCGCTCTGC 3' RP 5' GGGTGTGCGACCATACTTTC 3'	367	50
INV 3 (vacuolar)	Sb04g000620	FP 5' ACCACGACTACATGGTCACG 3' RP 5' GTGGTGGCACACCATCATAC 3'	348	50
INV 4 (cell wall)	Sb06g031930	FP 5' CATACGGAGGATTCGTGGAC 3' RP 5' TCTTTCTCCCAACTCCCAAG 3'	369	50
<i>Sucrose Synthase genes</i>				
SUS 1	Sb01g035890	FP 5' GATGGCTGACTTCTTCGAG 3' RP 5' GATGTTGAGCCAGTATAGCC 3'	461	54
SUS 2	Sb04g038410	FP 5' CATCGAGGCAATGAACTGTG 3' RP 5' CCAGCTTCCTGAAATGAAGG 3'	411	55
SUS 3	Sb10g031040	FP 5' ACTAGCCAAACAGCGCTACC 3' RP 5' ATCTGGTCCGGTATCAGTGG 3'	403	49
<i>Internal control gene</i>				
EF1 α	Sb02g036420	FP 5' AGGAGCTTGAGAAGGAGCCCA3' RP 5' TCCACGCTCTTGATGACTCCA 3'	188	52

Table S4. Nested ANOVA for total sugar content (response) in peduncles and 5th internodes of seven sorghum genotypes at early reproductive stage. Sources of variation (factors) were (a) Genotypes and (b) Treatments (genotypes). The data was log transformed to normalize the distribution.

Source of variation	Degrees of freedom	Mean square values	F ratio	p value
<i>Early reproductive stage (Peduncles)</i>				
Genotype	6	0.009	12.28	0.000
Treatment (Genotype)	7	0.048	7.33	0.000
Error	56	0.007		
<i>Early reproductive stage (5th internodes)</i>				
Genotype	6	0.424	12.12	0.000
Treatment (Genotype)	7	0.253	7.33	0.000
Error	56	0.035		

Table S5. Multivariate Analysis of Variance (MANOVA) of responses (reducing sugars, total sugars, osmolarity and sap volume) in the varieties belonging to Group I (ICSSH 58 and SPV 1411) and Group II (ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35-1). Ratios of the values of these parameters measured under drought and irrigated conditions were considered as responses. The approximate F values, numerator and denominator degrees of freedom and p-values corresponding to Wilks' Lambda other test statistics (Lawley-Hotelling and Pillai's) are indicated.

Criterion	Test Statistic	Approx F	Degrees of Freedom		p value
			Num	Denom	
<i>Group I</i>					
Wilks'	0.25441	3.663	4	5	0.094
Lawley-Hotelling	2.93061	3.663	4	5	0.094
Pillai's	0.74559	3.663	4	5	0.094
<i>Group II</i>					
Wilks'	0.54815	0.715	16	52	0.766
Lawley-Hotelling	0.68637	0.665	16	62	0.816
Pillai's	0.53076	0.765	16	80	0.719

Table S6. Multivariate Analysis of Variance (MANOVA) of responses (sucrose synthase in synthesis and cleavage directions, sucrose phosphate synthase and neutral and acid invertases) in the varieties belonging to Group II (ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35-1). Since Group I (ICSSH 58 and SPV 1411) consisted of only two genotypes (with three replicates) and a larger number of variables (responses), MANOVA was restricted to only the two invertases, since they showed significant changes across treatments. Ratios of the values of these parameters measured under drought and irrigated conditions were considered as responses. The approximate F values, numerator and denominator degrees of freedom and p-values corresponding to Wilks' Lambda other test statistics (Lawley-Hotelling and Pillai's) are indicated.

Criterion	Test Statistic	Approx F	Degrees of Freedom		
			Num	Denom	p value
<i>Group I</i>					
Wilks'	0.74769	0.506	2	3	0.647
Lawley-Hotelling	0.33746	0.506	2	3	0.647
Pillai's	0.25231	0.506	2	3	0.647
<i>Group II</i>					
Wilks'	0.02691	2.058	20	20	0.057
Lawley-Hotelling	8.27171	1.861	20	18	0.095
Pillai's	2.00048	1.801	20	36	0.061

Table S7. Nested ANOVA for stalk yield, grain yield, juice yield and Brix content of six sorghum genotypes at physiological maturity stage. Sources of variation (factors) were (a) Genotypes and (b) Treatments (genotypes). The data was log transformed to normalize the distribution.

Source of variation	Degree of freedom	Mean square values	F ratio	p value
<i>Stalk yield/plant</i>				
Genotype	5	0.006654	27.70	0.000
Treatment (Genotype)	6	0.016461	68.53	0.000
Error	24	0.000240		
<i>Grain yield/plant</i>				
Genotype	5	0.021392	10.93	0.000
Treatment (Genotype)	6	0.018590	9.50	0.000
Error	24	0.001957		
<i>Juice yield/plant</i>				
Genotype	5	0.057390	65.72	0.000
Treatment (Genotype)	6	0.033421	38.27	0.000
Error	24	0.000873		
<i>Brix/plant</i>				
Genotype	5	0.053057	52.30	0.000
Treatment (Genotype)	6	0.006297	6.21	0.000
Error	24	0.001014		