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Relationship Between Shoot and Sugar-related Characters of Sweet Sorghum (Sorghum bicolor L.) Under Contrasting Moisture Regimes

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Dema Alhajturki, Majed Al Jamali, Adnan kanbar: Relationship Between Shoot and Sugar-related Characters of Sweet Sorghum (Sorghum Bicolor L.) Under Contrasting Moisture Regimes

ABSTRACT

Understanding the relationship between shoot and sugar morphological traits is an important objective for sweet sorghum breeding programs. Canonical correlation analysis has been adopted to find the shoot morphological characters that have the largest influence on sugar-related characters (Brix, juice yield and ethanol productivity) of sweet sorghum crop under contrasting moisture stress conditions. This study revealed that cane yield, green Leaf Area had the largest effect on sugar-related traits under well-watered condition, while cane yield and height showed the maximum contribution to sugar-related traits under low moisture stress. The results of redundancy showed that about 67% of the variability in the first linear function of the sugar-related characters is accounted for by the shoot morphological traits under control condition. And this value was reduced up to 52% under drought condition. The interrelationships clearly identified the importance of genotypes. demonstrating effective drought-tolerance traits, such as stem reserve carbohydrates and stem-sink capacity, in delaying the reproductive development, and thus enabling sweet sorghum to achieve economic yield under low moisture conditions .

Key words: Canonical correlation, sweet sorghum, drought, sugar-shoot traits.

Introduction

Drought is a multidimensional stress often coupled with heat stress affecting plant at various levels of their metabolic mechanisms (Blum, 1996), and is generally accepted as the most widespread abiotic stress experienced by crop plants (Quarrie *et al*., 1999). If plants are to survive this abiotic stresses they must have a range of morphological, biochemical and physiological mechanisms that enable them to grow and reproduce despite water limitations (Turner, 1997). Drought tolerance is defined as the relative ability to sustain plant function under dehydrated state and achieving an economic yield potential (Blum, 2005).

Many studies were conducted to investigate sweet sorghum as a drought-tolerant crop. Sweet sorghum [Sorghum bicolor (L.) Moench] is an annual warm season similar to Grain sorghum in grain production and almost like sugarcane for sugar-rich stalk and high sugar accumulation (Ratnavathi *et al*., 2004). As a C4 crop, sweet sorghum features rapid growth, low water requirement, high biomass production and wide adaptation. As a multi-purpose crop it has a great potential for food, fodder, feed, sugar, jaggery, syrup and most importantly fuel alcohol production.

Other putative roles of osmotic adjustment in sweet sorghum have been recently assembled under the term of "osmoprotection" which reserves high relative water content in leaves (Rontein *et al*., 2002). Numerous reports provides evidence on the association between high rate of osmotic adjustment and sustained yield or biomass under drought-stress by sustaining cellular turgor maintains and yield-forming processes during drought-stress conditions (Ali *et al*., 1999). Many studies showed that stem and leaf sheath of cereal crops are the organs where photosynthesis takes place while accumulating the photosynthetic assimilates in the pre-flowering and post-flowering stages (Slafer and Savin, 1994; Yang *et al*., 2007).

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Stem reserve mobilization have a significant role in supporting high yield under non-drought stress conditions, while it is noticeable that stem reserve is induced under drought stress during grain filling (Blum *et al*., 1994; Palta *et al*., 1994; Yang *et al*., 2001; Plaut *et al*., 2004). Stress that reduces plant water status and photosynthesis also induces stem storage conversion into soluble sugars like sucrose and glucose. Apparently, this is dehydration-tolerance process in sorghum and is linked to the stem capacity to store photosynthetic sugars. Non-senescence ('delayed senescence'; 'stay-green') is considered an important component for sustaining yield potential by sustaining leaves function and carbohydrates accumulation in stem under stress during dough stage (Tuinstra *et al*., 1998; Borrell and Hammer 2000; Sanchez *et al*., 2002). A resent study showed that non-senescent genotypes retain more of their photosynthate in their leaves and stems (Borrell and Hammer 2000).

In spite of the surge in literature on drought tolerance in sweet sorghum during the past decades, clear picture on association between shoot morphological characters and sugar-related traits is yet to be determined. This is mainly because. most of the studies relied on simple correlation coefficients to analyze relationships. Simple correlations are inadequate to address this complex issue as shoot and sugar yield component traits are neither independent from each other nor among themselves. Therefore one has to consider the correlation between these two sets of variables, simultaneously. Canonical correlation, a well-known multivariate technique, has been established for similar situations, where one would like to measure the relationship between two sets of interrelated variables (Kanbar *et al*., 2009, 2011). In this experiment, canonical correlation analysis has been adopted to study the strength of association between the shoot and sugar morphological traits in ten sorghum varieties under normal and low-moisture stress conditions. Further, we intended to find the shoot morphological characters that have the largest influence on sugar traits under the two conditions.

Materials and methods

Plant Material:

Nine sweet sorghum and one grain local sorghum varieties were selected to evaluate some quantitative traits under contrasting moisture stress conditions. The local grain sorghum variety "Razinieh" was included as check line. Seeds of sweet sorghum were obtained from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India.

Phenotypic Evaluation:

Sweet sorghum cultivars and grain local check included in the study were grown in the farm of Abo Jarash, Faculty of Agriculture, Damascus, during summer 2010 for evaluation of anthesis date (days to 50% flowering), green leaf area (LA), cane height (cm), cane diameter (cm), cane yield (t/ha), Juice yield (kl/ha), Brix degree (%) and ethanol productivity (l/ha) at harvest date. The entries were planted on May 4 in five-row plots of 8 m long with a spacing of 0.25 m between the plants and 0.5 m between the rows. The experiment was laid out in a randomized complete block design (RCBD) separately for well-watered (WW) and low-moisture stress (LMS) conditions in three replications for each. In WW condition, irrigation was done every 15 days to maintain 70% field capacity, while LMS was imposed by withholding irrigation between 30 days after sowing and up to harvesting.

The plants were harvested for each cultivar in the dough stage (Undersander *et al*., 1990) when the stem represents a strong sink for available carbohydrates before getting remobilized to the seeds. The millable stalks were weighed to determine the yield (t/ha) then crushed to extract the juice using common sugar cane crusher after removing the leaves and panicles. Juice yield soluble solids concentration (Brix %) was recorded with a digital hand-held refractometer.

Theoretical ethanol yield was computed at 40 liters per ton of millable stalks yield according to method of Rao *et al*., (2004). ICRISAT follows similar procedure to calculate this parameter and advancing the materials.

Statistical Analyses:

Data were subjected to combined ANOVA over two moisture regimes for different characters in order to assess the variability among the genotypes and standard error of the treatment means using PROC ANOVA in SAS program (SAS Institute, Inc., 1996). Least significant difference (LSD) and co-efficient of variation were worked out using appropriate formulae. The environments were considered as fixed effects as they had been created artificially for this experiment.

Canonical correlation analysis was employed using PROC CANCORR procedures in the SAS program. shoot morphological characters and sugar-related traits were considered as independent (X) and dependent (Y) sets of variables, respectively. An overall test for statistical significance of all the four possible canonical correlations from zero was performed using Wilk's Lambda (Gittins, 1985). The Wilk's Lambda was computed using the formula:

$$\Lambda = \prod_{i=1}^{s}\left(1-C_i^2\right)$$

Where, Ci is the ith canonical correlation and s=min(p,q), p and q are the number of shoot-related and sugar morphological traits studied. Approximate F-test (as per SAS default) was used for assessing the statistical significance of the Wilk's Lambda. The structure correlations (Johnson and Wichern, 1998) were calculated using the following formula:

$$S_{i(j)k} = \frac{e_{ki(j)}\sqrt{\lambda_{i(j)}}}{\sqrt{\sigma_{kk}}}$$

Where, i(j)k =1,2,…p(q) depends on the number of characters studied in the shoot and sugar-related sets of variables, respectively; σ_{KK} is the variance of the kth variable and [$\lambda_{I(j)}$, $e_{ki(j)}$] are the eigenvalue-eigenvector pairs of the corresponding covariance matrices.

The practical importance of the canonical correlations was obtained by calculating the redundancy measure (RM) according to Sharma (1996) using the formula:

$$RM_{Vi\backslash Wi} = AV\left(Y/V_i\right)\times C_i^2$$

Where, AV(Y/V_i), variance extracted, is the average variance in Y variables that is accounted for by the canonical variate Vi (is the linear combination of the sugar-related characters). Total redundancy for the Y variables ($RM_{y/x}$) was computed as:

$$RM_{Y/X} = \sum_{i=1}^{q} RM_{Vi/Wi}$$

Where, RM Vi/Wi is the redundancy measure as explained above.

Results and discussion

Statistical analysis, ANOVA, revealed significant differences among the varieties for all the characters studied under WW and LMS conditions (table 1). Highly significant genotype X environment interaction (G × E) were observed for all the traits except Brix degree (%) and green leaf area (LA), indicating that total soluble sugar increased by decreasing soil moisture which helped maintaining green leaf area under LMS condition. Further, significant variance due to genotype X moisture levels was indicative of their different response to two-moisture regimes.

Table 1: Analysis of variance and statistical parameter for sweet sorghum varieties under WW and LMS conditions.

S.O.V.	d.f.	Leaf Area (cm^2)	Cane height (cm)	Cane diameter (cm)	Cane yield (t/ha)	Juice yield (kl/ha)	Brix (%)	Days to 50 % flowering	Ethanol productivity (l/ha)
Replication/Env.	2	2414975.3	8.71	0	1.32	0.47	2.11	0.05	1230.79
Condition (C)	1	1428079.5	1664.26**	1.66**	243.77**	57.90**	35.26**	3465.60**	384339.27**
Varieties (V)	9	3644517.82**	8873.34**	2.10**	2124.93**	487.12**	12.62**	1182.06**	3415371.47**
(C * V)	9	1182185.5	60.85**	0.29**	11.81**	2.56**	3.00	289.60**	23659.68**
Error	38	1309161.8	7.08	0.03	1.41	0.78	1.94	0.05	2984.11
Mean	WW	962.3b	125a	2.46a	25.18a	8.59a	15.86b	83.10b	1004.85a
	LMS	832.6a	114.50b	2.13b	21.15b	6.63b	17.40a	98.30a	844.78b
SE±	WW	315.2	7.06	0.12	3.32	1.65	0.39	2.78	133.42
	LMS	105.09	6.55	0.1	3.32	1.53	0.3	2.73	133.81
C.V%	WW	131.42	2.02	7.4	5.69	12.85	9.08	0.38	5.8
	LMS	4.8	2.45	8.55	4.09	8.09	7.98	0.00	5.52
L.S.D	WW	2781.7	4.35	0.31	2.45	1.89	2.47	0.54	100.10
	LMS	68.691	4.81	0.31	1.48	0.92	2.38	0.00	80.015

Canonical correlation analysis was carried out to identify how shoot-related traits influence the sugar-related characters (juice, Brix and ethanol productivity) under contrasting moisture regimes in nine varieties of sugar sorghum and one local grain sorghum cultivar. The result of canonical correlation analysis showed that the first canonical significant (about 0.99) under both WW and LMS conditions, while the second canonical was 0.83 and 0.71 under WW and LMS conditions, respectively. No significant differences were observed for LMS condition at third canonical variation. Squared canonical correlation showed that 98.9% of variability in Brix, ethanol and juice yields were explained by the first linear combination of the two sets of characters under LMS conditions (table 2).

Table 2: The first three canonical correlations between shoot morphological and sugar-related traits, squared canonical correlation, cumulative proportion, approximate F and significance level under WW and LMS conditions in sweet sorghum.

	Canonical correlation		squared canonical correlation		cumulative proportion		approximate F		P-value	
	WW	LMS	WW	LMS	WW	LMS	WW	LMS	WW	LMS
1	0.998	0.993	0.996	0.986	0.989	0.984	30.28	13.15	0.0001	0.0001
2	0.832	0.715	0.692	0.511	0.995	0.998	4.92	1.81	0.0140	0.0460
3	0.762	0.334	0.582	0.112	1.000	1.000	5.57	0.63	0.0120	0.6060

The intra-set structural correlation which gives the magnitude and direction of the contribution of variables (cane yield, cane height, cane diameter, days to 50% flowering and green leaf area) to the varieties (Brix, ethanol and juice yield) within domain are presented in (table 3 and 4) for the above characters. Among the shoot morphological traits, cane yield showed the maximum contribution (99%) to the first canonical variant in both conditions followed by LA (42%) under control. Days to 50% flowering was the most influential character in forming the second canonical variant (about 94%) followed by LA (about 86%) under WW condition. Under LMS condition, cane yield (99.8%) and cane height (61.1%) had the highest contribution to the first canonical variant. Results suggested that cane yield was the most important shoot-related traits under both WW and LMS conditions.

Table 3: Structure correlation between shoot-related traits and their (first three) canonical variates under WW and LMS conditions in sweet sorghum.

Traits	WW			LMS		
	V1	V2	V3	V1	V2	V3
Cane yield (t/ha)	0.996	0.043	0.05	0.998	0.003	0.031
Cane height (cm)	0.309	-0.604	-0.38	0.611	-0.34	0.557
Cane diameter (cm)	-0.313	0.488	0.044	0.061	0.525	-0.177
Days to 50 % flowering	0.197	0.939	-0.05	0.463	0.697	-0.306
Leaf Area (cm^2)	0.426	0.858	-0.087	0.341	0.76	-0.287

Many studies showed that stem sugar accumulation is an effective yield-supporting mechanism under drought stress (Hossain *et al*., 1990; Pheloung and Siddique 1991; Gavuzzi *et al*., 1997; Yang *et al*., 2002; Asseng and van Herwaarden 2003; Plaut *et al*., 2004). Studies which traced photosynthetic products movement with [14CO2] feeding methods have shown that the stem is a major sink to carbohydrates accumulation during development period at soft dough stage (Hume and Campbell, 1972; Alove and Schrader, 1975). On the other hand, genotypes differ in their patterns of stem sugar accumulation (Daynard *et al*., 1969). This showed that cultivars superior in cane component (cane diameter and cane height) gives the highest value of cane yield, similar result found by (El-Razed, 2009). In this regard, numerous reports showed that individual stalk performance and stalk yield differed greatly among sweet sorghum varieties and most of these variations appeared to be genetical (Bapat *et al*., 1987, El-Karim *et al*., 1999, Saleh, 2004). Parsada *et al*., (2008) reported that panicle initiation can be delayed 2-25 days and flowering by up to 59 days under drought stress conditions. Delayed senescence, referred to as stay-green in sorghum, leads to post-flowering drought tolerance (Blum, 1986) and increases photosynthesis per unit green leaf area under water stress. Hence, minimizing growth inhibition by reducing leaf area, leaf size and leaf dry matter accumulation (Borrell *et al*., 2001). Similar results were found by McBee and Duncan., (1984) who reported that sorghum genotypes with stay-green traits gave higher levels of cane yield and stem carbohydrates under stress conditions. However studies by Borrell *et al*., (2009) suggested that sorghum genotypes possessing the stay-green trait had a significant yield advantage under water-limited conditions compared with genotypes not possessing this trait. Among the sugar-related traits, ethanol yield had the highest contribution (99%) to the first variant under both conditions followed by juice yield (72%) under WW and Brix % (56%) under LMS condition. In second variate, Brix (%) had the highest contributing character 59.3% and 51.6% under WW and LMS, respectively.

Table 4: Structure correlation between sugar-related traits and their (first three) canonical variates under WW and LMS conditions in sweet sorghum.

Traits	WW			LMS		
	W1	W2	W3	W1	W2	W3
Ethanol productivity (l/ha)	0.999	0.002	0.036	0.996	0.039	-0.077
Juice yield (kl/ha)	0.728	-0.297	0.617	-0.536	-0.277	0.797
Brix (%)	0.701	0.593	0.395	0.561	0.516	-0.647

Since sweet sorghum ethanol is extracted from the stem, the higher the cane yield the higher would be the ethanol yield. This explained why stalk yield trait was the most important sugar-related trait showing the maximum contribution to the variation between the varieties under both WW and LMS conditions. Our results are compatible with these found by kumar *et al*., (2008). As mentioned earlier, theoretical ethanol productivity was calculated using cane yield. Thus, the higher the variate in stalk dimensions (height and diameter) the more variation between cultivars for ethanol yield.

The contribution of the linear function of all shoot-morphological characters for expression of ethanol yield was 95.5% from the first and 99.6% percent from the first three linear functions under WW condition (table 5). Under LMS conditions, the contribution of the linear function of all the shoot-morphological characters for expression of ethanol yield was approximately 98% in first two and first three linear functions. Influence of shoot-related traits on juice yield was maximized from third linear combination as it was reached from 52.9% in case of first linear function to 81.2% for the third linear function under WW conditions. Under LMS condition, there was less contribution of the linear functions of the shoot-morphological traits for expression of juice yield. Similarly, the contribution of the individual shoot morphological traits to the first three linear function of the Brix (%) trait showed about 82.5% and 49.3% under WW and LMS conditions, respectively.

Table 5: Cumulative proportion of sugar-related traits contributing to the first three canonical variates of shoot morphological traits under WW and LMS conditions in sweet sorghum.

Traits	WW			LMS		
	1	2	3	1	2	3
Ethanol productivity (l/ha)	0.955	0.995	0.996	0.979	0.979	0.980
Juice yield (kl/ha)	0.529	0.59	0.812	0.283	0.322	0.939
Brix (%)	0.490	0.734	0.825	0.310	0.446	0.493
canonical R-squared	0.997	0.692	0.582	0.986	0.511	0.112
cumulative redundancy	0.671	0.773	0.877	0.524	0.583	0.622

This result was expected as the ethanol yield was calculated based on stalk yield, so any change in stem-related traits will add variation in ethanol yield. Geng *et al*., (1989) found that the contribution of shoot-related traits in sugar yield was more under WW condition compared with LMS condition. But this result was in contrast to massacre *et al*., (1996), who showed no difference is juice yield and Brix (%) at the time of harvest between irrigated and drought stressed sweet sorghum. The influence of shoot-related traits was decreased from WW to LMS conditions and that could be explained by the restriction of shoot growth while root growth continue under LMS to maintain open stomata at lower water potential (Burke, 2007). Table 5 shows that, for the first canonical correlation, although the independent canonical variable explains 99.7% and 98.6% of the variance in the sugar-related traits under WW and LMS conditions, respectively, the independent canonical variable (shoot-related traits) was only able to predict 87.7% of the variance in the individual original dependent variable (sugar-related traits) for the third canonical correlation under WW condition and this value was reduced to 62% under LMS condition.

In conclusion, our results showed sweet sorghum as a drought-tolerance crop. Canonical correlation analysis showed the importance of shoot-morphological characters in WW and LMS conditions by looking at the cumulative redundancy. Approximately, 62% of the variation in the sugar-related traits under drought stress was due to reduction in shoot-morphological traits. In selection nurseries, an effective and successful selection for yield under drought stress most likely involve a genetic shift towards dehydration-avoidance genotype pattern by reducing the water loss from plant under drought stress featuring early flowering, small plant size and small leaf area all of which negatively affect high yield potential (Blum, 1988). drought-tolerance can be combined with high yield potential if selection is designed to recombine high yield and drought-tolerance mechanism and relative dehydration-avoidance factors that are not associated with lower yield potential like osmotic adjustment.

This study revealed the association between traits which combine to help genotypes to delay reproductive development so they can achieve an economic yield potential under dehydration conditions especially in the semi-arid areas where unexpected patterns of drought and heat stress are common during agricultural seasons.

Thus, selection for drought-tolerant genotypes should consider and combine the morphological traits associated with an economic yield potential and afford plant ability to sustain dehydration status in the given environment.

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