

Study of Gene Effects for Stalk Sugar Yield and Its Component Traits in Sweet Sorghum [*Sorghum bicolor* (L.) Moench] Using Generation Mean Analysis

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Abstract. Generation mean analysis was carried out to estimate the nature and magnitude of gene effects for sugar yield and its component traits in sweet sorghum [*Sorghum bicolor* (L.) Moench. Six basic generations, namely P1, P2, F1, F2, BC1P1, BC1P2 of four crosses involving seven diverse parents were evaluated in rainy 2009. The mean performance of the F1 in all the crosses indicated dominant gene effect for all the characters. Simple additive-dominance model indicated presence of epistatic interaction. High positive additive \times additive interaction effects were found in all the crosses. Higher magnitude of dominance and dominance \times dominance gene interactions which were found minimizes the expression of heterosis leading to non-exploitation of crosses with duplicate epistasis. Reciprocal recurrent selection and/or biparental mating in early segregating generations has been suggested for development of high sugar yielding genotypes in view of the genotypes studied.

Key words: Sweet Sorghum, Generation Mean Analysis, Epistasis, Sugar yield.

Introduction

Sweet sorghum is similar to cultivated grain sorghum except for sugar rich stalks and is recognized widely as a potential source of biofuel. Besides having rapid growth, high sugar accumulation, and high biomass production potential, sweet sorghum has wider adaptability and offers comparable grain yields Reddy *et al.* (2008). It can be grown with limited water under minimal inputs and can be harvested within a span of four months. The economic superiority is contributed by characters such as stalk yield, stalk sugar content (Brix %), stalk juice extractability, content of non-reducing and reducing sugars and grain yield Bala Ravi *et al.* (1996). The sugar content in the juice extracted from sweet sorghum stalks varies from 16-23%. Sweet sorghum is best suited for ethanol production because of its higher fermentable sugar content in the stalk compared to sugarcane Reddy *et al.* (2008). The feasibility of converting stalk sugars to ethanol, syrup, jaggery on or near farms, and the adaptability of sorghum to a wide range of environments prompted researchers to evaluate the potential of sweet sorghum as an alternative crop for ethanol production Daniel *et al.* (1991). The bagasse after extraction of juice from sweet sorghum can be used for animal feed, vermi-composting and co-generation of power (Reddy *et al.*, 2005; Srinivasa Rao *et al.*, (2009). Further, the bagasse has a higher biological value than the bagasse from sugarcane when used as forage for animals, as it is rich in micronutrients and minerals Seetharama *et al.* (2002). Intake and growth trials with cattle using sweet sorghum bagasse and stripped leaves-based feed block (BRSLB) by International Livestock Research Institute (ILRI) and ICRISAT showed no significant differences between BRSLB and commercially produced sorghum stover-based feed block (CFB). In other words, sweet sorghum bagasse and stripped leaves provide a valuable, tradable feed resource that will potentially add considerable value

to a sweet sorghum biofuel value chain Blummel *et al.* (2009). The bagasse has similar levels of cellulose and sugarcane bagasse and therefore has a good prospect as a raw material for pulp product. The sweet sorghums have not been a major focus of commercial breeding programmes; hybrids have been developed between grain and sweet sorghums, usually for fodder or dual purpose use (grain and fodder). Thus, increasing stalk sugar yields is becoming an important objective in sweet sorghum breeding Murray *et al.* (2009). Genetic enhancement of the crop for increased sugar yield is very critical to make sweet sorghum more profitable to the farmers and the industry, while sustaining grain yield, juice volume, plant height, plant girth and other important components. The choice of an efficient breeding programme depends to a large extent on knowledge of the type of gene action involved in the expression of the character. The knowledge on nature of gene action for sugar yield and its component traits like Brix% and juice content in the breeding material can provide useful information for selecting proper breeding procedure for future genetic enhancement. Inheritance of stalk biomass, Brix% and stalk weight in sugar stalk was subject to both additive gene effect and non additive gene effect, but mainly controlled by non additive genes Zhou *et al.* (2005). However, the literature regarding inheritance of these traits and their genetic interactions in sweet sorghum is scanty. Keeping this in view, an attempt has been made to understand the gene action controlling sugar yield and its component traits through generation mean analysis using different lines of sweet sorghum with varied Brix% and juice content.

Material and Methods

Eight diverse genotypes of sweet sorghum (Table 1). Were selected based on their Brix% (5-16%) to provide the basic material in the study. Six generations viz., P₁, P₂, F₁, F₂, BC₁P₁ and BC₁P₂ of four inter-varietal crosses namely, ICSB 1×ICSB 38 (C1), ICSB 37×ICSR 48 (C2), ICSB 37×4487-3 (C3), ICSR 77×SSV 84 (C4) were developed and raised in randomized complete block design (RCBD) with three replications at International Crops Research Institute (ICRISAT) centre, Patancheru during the rainy 2009. The parents and F₁'s were planted in two rows of 2 m length; BC₁P₁ and BC₁P₂ were planted in six rows of 2 m length; F₂'s were planted in twelve rows of 2 m length each accommodating 20 plants in a row with spacing of 45 ×15 cm. All the recommended agronomic practices were adopted to raise a healthy crop. Ten plants from each of the parents and F₁'s, 35 plants from BC₁P₁ and BC₁P₂ generations and 120 plants from F₂ population were randomly selected avoiding border rows for recording data on days to 50% flowering, plant height (cm), stem girth (mm), stalk weight (g), cane weight (g), juice weight (g), juice volume (ml), Brix (%), bagasse (g) and sucrose (%). Sucrose was estimated using the Rudolphh Saccharimeter (Model: A21958 Autopol 880) according to manufacturers instructions. Sugar yield was calculated according to formula given by Daniel *et al.* (1991).

Statistical analysis: The adequacy of simple additive-dominance model to explain the gene action of characters was tested by applying the joint scaling test Cavalli (1952). Since the joint scaling test was positive, indicating the presence of interactions attempts were made to test the digenic epistatic model of Hayman (1958) as outlined by Mather and Jinks (1977). Successive mean effects [m] followed by one or more of the additive [d], dominance [h], additive × additive [i], additive × dominance [j] and dominance × dominance

[l] effects were fitted by the weighted least squares procedure and tested for goodness of fit. The chi-square value was compared with table χ^2 at (6-3) degrees of freedom. The significance of estimates of genetic parameters was tested by t-tests. The model showing the least mean residual sum of squares from the observed generation means was chosen for genetic interpretation of the data.

Results

The mean performance of P₁, P₂, F₁, F₂, BC₁P₁ and BC₁P₂ families of four crosses are shown in Table 2. The mean performance of the two parental lines for each cross were different from each other for all the eleven characters viz., days to 50% flowering, plant height, stem girth, stalk weight, cane weight, juice volume, Brix %, bagasse, sucrose and sugar yield. Also the mean performances of F₁ and F₂ for the above said characters were different from those of both the parents for each cross and they tended towards their respective female parents (P₂) which are of high Brix% and sucrose types except in the case of C4 where F₂ means were closer to the lower parent. The F₁ means were greater than the respective mid-parent mean values for all the given characters. The BC₁P₁ and BC₁P₂ family means were tended towards their respective parents and overlapped with each other which indicate gene interactions.

Joint scaling test revealed that (Table 3) both additive and dominance gene effects were highly significant for all the traits in all the crosses except in C4 for stem girth, in C1 for stalk weight, cane weight, juice weight, bagasse and sugar yield where only additive gene effects were non-significant. Similarly, dominant gene effects were non-significant for days to 50% flowering in C1, C2 and C3, for stem girth and Brix% in C1, for plant height, stalk weight, cane weight, juice weight and juice volume in C4. In general the estimates of dominance gene effects were positive and higher compared to additive gene effects which

are negative. Significance of chi-square values were reported for all the traits in four crosses. Six parameter model (Table 4) of Jinks and Jones (1958) revealed significance of main genetic effects, [d] and [h] in general. However, additive [d] gene effects were not significant for stalk weight, cane weight, juice weight, Brix %, bagasse and sucrose in C1 and for stem girth in C3. In the same manner dominance [h] gene effect was not significant for Brix% in C3. The magnitude of dominance gene effects [h] was substantially higher than that of additive gene effects [d] in all the crosses for eleven characters. The magnitudes of dominance×dominance [l] gene effects were higher than additive×additive [i] and additive×dominance [j] gene effects irrespective of direction of their effects for all the characters in four crosses. Further, the net sign of dominance×dominance [l] gene effects were positive in general for characters studied and in particular for Brix% and sugar yield in all the crosses.

Discussions

The mean values of F1 and F2 families tended towards that of sweet sorghum parent (P2) except in case of C4 where F2 means were closer to P1 which may be ascribed to large error variance (Table 2). The F1 means were greater than the respective mid-parent mean values for all the given characters, indicating dominance for important traits like plant height, stalk yield, juice yield, Brix% and sucrose content conforming the report of Semenova (1988). The BC₁P₁ and BC₁P₂ family means were tended towards their respective parents and overlapped with each other which indicate gene interactions. Chi-square significance of joint scaling test indicated the inadequacy of additive-dominance model which in turn indicated the presence of non-allelic interactions in the present study involving four crosses suggesting possible involvement of digenic interactions for the eleven traits under examination (Table 3).

Significance of additive and dominance gene effects was observed in all the crosses revealing the importance of both of these in such a way that the negative sign associated with additive effects [d] in all the four crosses for each trait indicates the combination of genes from both the parents did not add up to the improvement of the characters suggesting dominance effect (Table 4). Importance of both additive and non-additive gene effects for sugar traits of sorghum was revealed in previous reports by Ramalingam and Rangasamy (1987); Saxena *et al.* (1999). Further, dominance component [h] of generation mean observed was positive and greater in magnitude than additive gene effect [d] for all the characters in the four crosses which strengthen the fact that dominance component played a major role in the inheritance of all these characters. The sign for dominance effect is a function of the F1 mean value in relation to the mid-parental value and indicates which parent is contributing to the dominance effect (Cukadar-Olmedo and Miller, 1997). The predominant role of non-additive gene action for plant height, stem girth, total soluble solids (Brix %), stalk yield and juice yield in sweet sorghum was reported by Sankarapandian *et al.* (1994). Similarly Gupta and Baliwal (1976) reported non-additive gene action for total soluble solids (Brix %). The negative sign found associated with the dominance effect for days to 50% flowering in C1 and C3 indicate the dominance effect for decreasing alleles as it reduced the number of days to 50% flowering in hybrid combinations in which it was close to their lower parent. In contrary, Dangi *et al.* (1978) reported predominant role of additive gene action for days to 50% flowering as well as for plant height and stem thickness. Among the digenic interactions, additive×additive (i) interaction was positive for most of the characters in all the four crosses, except for days to 50% flowering in C1 and C2, plant

height in C3 and for Brix% in C1 and C3 respectively (Table 4). It was found that magnitude of *i* (additive×additive) was significant and higher than both *j* (additive×dominance) and *l* (dominance×dominance) components revealing the presence of associated pair of genes for all the traits in four crosses. Opposite signs of dominance [*h*] and dominance× dominance (*l*) gene effects revealed duplicate epistasis for all the traits except for days to 50% flowering in C4 revealing consistency of gene action over crosses. The negative sign associated with additive (*d*) and positive sign associated with additive×additive (*i*) component in the four crosses for majority of traits indicate positive additive gene action consisted of positive additive digenic interaction whereas, the balance of the additive gene effects of the genes controlling these traits was negative. Kearsay and Jinks (1968) suggested that the two parental lines have equal opportunity to contribute to the expression of additive by additive effects when averaged cross all possible F2 genotypes. Accordingly the combination of genes from both the parents would have contributed to expression of sugar yield and its component traits in the crosses under study.

The magnitude of heterosis is influenced by non-allelic interactions. Non-allelic interactions are known to either reduce or enhance the extent of heterosis depending upon their direct ion and magnitude of

action. Confounding epistatic effects in the study suggested that inheritance of these traits is complex and polygenic Warnock *et al.* (1998). Higher magnitude of dominance gene effects and dominance gene interactions could not be exploited for heterosis breeding due to presence of duplicate epistasis in the present crosses as it minimizes the manifestation of heterosis Kearsay and Pooni (1996). Hence, selection for high sugar yielding genotypes would be effective if dominance and epistatic effects were first reduced by few generations of selfing. Then biparental mating followed by intermating of selected progeny and selection in subsequent segregating generation or population improvement methods may possibly serve the purpose of developing high sugar yielding genotypes of sweet sorghum.

Conclusions

Generation mean analysis of four crosses in the present study explicated the presence of epistasis for the characters involved. The presence of epistasis has important implications for any plant breeding program. Although the results of this experiment may be applicable to the germplasm used herein, the identification of dominance and epistatic effects suggest that additional research is necessary to further advance the breeding of sweet sorghum.

Table 1. Characters of Parental Lines of Sweet Sorghum Used in the Study

Cross	Parent	Important character	Brix%
C1	ICSB 1	Medium grain yielding	Low
	ICSB 38	High grain yielding	High
C2	ICSB 37	Medium grain yielding	Low
	ICSR 48	High grain yielding	High
C3	ICSB 37	Medium grain yielding	Low
	ICSV 25274	High grain yielding	High
C4	ICSR 77	High grain yielding and medium maturity	Low
	SSV 84	High grain yielding and late maturity	High

Table 2. Generation Means of the Families for Sugar Yield and its Component Traits in Sweet Sorghum During Rainy 2009
Mean ± Standard error

Cross	Family	Days to 50% Flowering	Plant Height (cm)	Stem Girth (mm)	Stalk weight (g-plant)	Cane weight (g-plant)	Juice weight (g-plant)	Juice volume (ml-plant)	Brix %	Bagasse (g-plant)	Sucrose (%)	Sugar yield (g-plant)
C1	P1	66±0.39	115.67±1.83	16.70±0.58	160.80±8.11	117.40±6.37	31.77±3.90	29.40±2.63	6.63±0.16	85.00±3.20	6.22±0.33	2.12±0.25
	P2	64±0.16	133.83±1.47	24.56±1.13	165.97±10.41	113.13±5.51	24.00±1.40	23.30±1.23	12.63±0.18	87.03±3.41	8.29±0.18	3.02±0.11
	F1	59±0.61	147.50±2.92	20.84±0.87	364.73±23.84	244.2±15.00	170.97±5.01	93.20±5.56	10.13±0.30	172.67±9.59	7.08±0.23	17.92±0.73
	F2	64±0.26	144.21±1.35	15.88±0.20	195.55±4.96	136.33±3.32	33.49±1.21	30.86±1.11	12.25±0.13	105.21±2.12	10.05±0.13	4.27±0.18
C2	BC1P1	61±0.4	144.10±2.08	23.80±0.54	395.91±11.56	272.52±8.16	100.12±3.80	91.69±3.31	12.58±0.22	167.00±4.55	10.22±0.20	12.65±0.56
	BC1P2	62±0.3	145.26±1.96	21.75±0.35	322.30±10.14	222.99±7.49	67.50±3.38	64.17±2.91	11.51±0.22	148.45±4.11	10.13±0.19	7.89±0.42
	P1	65±0.34	141.33±1.08	16.82±0.34	174.60±5.07	95.43±4.79	18.83±0.79	19.37±2.21	12.27±0.36	76.90±1.28	11.58±0.24	2.26±0.12
	P2	80±0.34	157.67±2.13	18.86±0.74	330.83±23.59	246.20±18.63	46.30±3.90	42.9±3.73	17.67±0.30	185.00±10.09	14.47±0.31	8.40±0.80
C3	F1	73±0.36	223.33±1.55	22.53±0.43	680.10±28.72	537.70±23.45	157.57±9.32	45.3±8.69	19.47±0.11	357.60±14.66	17.39±0.19	30.52±1.70
	F2	72±0.38	171.53±1.71	16.78±0.20	292.40±7.65	216.64±6.04	48.45±1.73	45.06±1.67	16.32±0.12	163.99±3.85	14.16±0.12	8.39±0.33
	BC1P1	69±1.11	167.33±3.12	21.72±0.36	463.12±22.20	323.41±16.36	78.65±5.43	73.18±5.00	15.8±0.26	226.18±10.41	13.87±0.27	13.43±1.10
	BC1P2	78±0.35	201.92±3.62	23.10±0.28	604.49±15.80	459.22±12.98	121.12±4.27	114.00±4.00	18.74±0.18	317.55±7.93	16.65±0.19	22.95±0.88
C4	P1	65±0.33	130.50±1.29	16.01±0.59	143.27±10.10	93.37±6.23	20.53±1.69	18.6±1.55	8.47±0.47	75.13±4.15	6.00±0.25	1.92±0.24
	P2	81±0.12	304.17±2.71	17.45±0.63	569.03±31.18	481.70±29.56	135.33±10.55	130.73±9.94	18.83±0.29	322.97±15.46	17.14±0.28	26.08±2.27
	F1	81±0.22	323.00±1.29	20.17±0.36	786.30±22.95	691.67±19.35	168.73±5.54	156.27±5.04	19.90±0.20	469.77±16.16	16.96±0.19	33.81±1.21
	F2	78±0.37	276.62±2.51	18.74±0.14	618.67±14.97	532.78±13.29	130.30±4.56	124.56±4.58	18.83±0.15	398.78±	9.90±0.15	25.19±0.92
C4	BC1P1	68±0.97	243.85±5.83	20.70±0.28	607.90±30.14	489.42±26.18	118.04±6.04	112.77±6.00	17.31±0.25	344.89±19.28	14.54±0.30	21.26±1.32
	BC1P2	75±0.58	309.14±2.15	23.31±0.32	882.84±24.83	796.87±21.63	200.80±9.23	210.84±8.76	18.57±0.20	559.05±14.57	16.00±0.23	36.66±1.54
	P1	60±0.29	167.5±1.90	18.23±0.39	249.2±12.15	200.37±10.86	73.90±7.89	58.57±3.40	12.43±0.38	136.93±6.68	11.53±0.38	9.06±0.85
	P2	85±0.22	285.67±2.36	16.91±0.30	521.00±27.31	421.57±18.61	121.67±5.02	111.97±7.30	19.33±0.20	281.67±11.69	17.09±0.40	23.50±1.03
C4	F1	73±0.59	298.5±5.64	20.41±0.48	751.07±41.62	632.40±38.83	171.20±10.19	161.03±9.49	18.93±0.31	418.57±15.47	16.70±0.36	32.94±2.12
	F2	59±0.19	169.59±0.85	18.63±0.22	304.12±6.99	233.95±5.32	68.20±2.12	65.99±2.10	12.50±0.12	161.60±3.09	10.95±0.14	8.73±0.30
	BC1P1	59±0.2	173.57±1.53	19.76±0.33	345.44±12.47	247.37±9.41	81.53±4.21	78.49±4.16	12.13±0.17	162.52±4.84	10.45±0.16	10.19±0.56
	BC1P2	70±0.61	287.14±4.82	21.37±0.31	782.11±30.37	689.21±27.35	174.03±7.67	168.93±7.34	18.58±0.27	478.94±18.09	16.84±0.27	33.86±1.53

Table 3. Joint Scaling Test for Assessing the Adequacy of Additive-Dominance Model for Sugar Yield and its Related Traits in Sweet Sorghum.

Sl. No	Character	Cross	m	[d]	[h]	Chi-square
1	Days to 50% Flowering	C1	65.20**	0.77**	-5.36	47.93**
		C2	72.77**	-7.91**	0.49	27.99**
		C3	73.31**	-8.14**	7.79**	146.14**
		C4	71.15**	-12.45**	-13.93	1482.36**
2	Plant height (cm)	C1	126.59**	-7.68**	30.96**	25.66**
		C2	147.61**	-8.12**	70.88**	89.65**
		C3	217.07**	-85.74**	106.44**	18.61**
		C4	215.13**	-52.67**	-63.03	1382.83**
3	Stem girth (mm)	C1	18.50**	-2.32**	-1.05	365.26**
		C2	17.52**	-1.42**	3.91**	376.32**
		C3	18.01**	-1.43**	3.03**	189.71**
		C4	17.63**	0.14	3.58**	64.61**
4	Stalk weight (g.plant)	C1	161.23**	4.75	163.79**	328.88**
		C2	253.03**	-84.33**	247.23**	333.71**
		C3	402.08**	-254.18**	455.42**	56.13**
		C4	344.41**	-115.40**	27.89	309.76**
5	Cane weight (g.plant)	C1	114.00**	6.17	106.41**	308.78**
		C2	167.28**	-77.97**	222.21**	292.11**
		C3	340.85**	-246.24**	408.04**	64.50**
		C4	286.46**	-105.53**	-31.13	337.29**
6	Juice weight (g.plant)	C1	16.74**	-2.83	68.08**	729.58**
		C2	33.26**	-14.97**	60.33**	278.20**
		C3	87.22**	-66.12**	92.66**	29.72**
		C4	78.88**	-35.33**	11.01	240.00**
7	Juice volume (ml.plant)	C1	25.12**	3.25*	34.45**	416.95**
		C2	30.02**	-15.00**	59.32**	277.21**
		C3	88.14**	-69.02**	80.45**	53.79**
		C4	76.33**	-24.99**	10.57	232.64**
8	Brix %	C1	12.35**	0.85**	-1.37	152.22**
		C2	14.42**	-2.96**	4.93**	34.96**
		C3	15.45**	-3.17**	5.02**	144.83**
		C4	13.69**	-4.92**	1.15**	555.04**
9	Bagasse (g.plant)	C1	85.18**	0.62	75.25**	203.78**
		C2	132.19**	-55.99**	141.68**	278.85**
		C3	228.66**	-152.40**	321.81**	83.62**
		C4	174.53**	-63.74**	27.82*	507.03**
10	Sucrose (%)	C1	9.56**	1.03**	1.03**	169.37**
		C2	12.72**	-1.83**	4.24**	69.38**
		C3	12.67**	-3.98**	4.48**	58.87**
		C4	12.15**	-4.05**	0.92*	388.33**
11	Sugar yield (g.plant)	C1	2.59*	0.39	6.43**	283.00**
		C2	5.33**	-3.15**	11.75**	278.34**
		C3	15.80**	-13.86**	19.82**	15.38**
		C4	13.86**	-6.87**	-5.44	390.47**

* Significance at P = 0.05 ** Significance at P = 0.01

Table 4. Estimates of Additive, Dominance and Digenic Epistatic Gene Effects of Sugar Yield and Related Traits in Sweet Sorghum.

Sl. No	Character	Cross	m	[d]	[h]	[i]	[j]	[l]	Type of Epistasis			
1	Days to 50% Flowering	C1	74.24**	0.95**	-26.68**	-8.95**	-4.09**	11.44**	D			
		C2	65.92**	-7.56**	17.24*	6.70**	-4.02	-10.20*	D			
		C3	99.05**	-8.11**	-66.88**	-25.59**	1.16	49.37**	D			
		C4	49.17**	-12.71**	16.79**	23.27**	3.508	6.85*	C			
2	Plant Height (cm)	C1	122.86**	-9.08**	60.71**	1.88	15.84*	-36.08**	D			
		C2	97.08**	-8.16**	171.50**	52.41**	-52.84**	-45.26*	D			
		C3	217.83**	-86.83**	129.98**	-0.49	43.07**	-24.81	D			
		C4	-16.56	-59.08**	429.46**	243.14*	-108.9**	-	114.40**	D		
3	Stem Girth (mm)	C1	-6.94**	-3.93**	63.49**	27.57**	11.96**	-35.71**	D			
		C2	-4.70**	-1.02*	58.68**	22.54**	-0.71	-31.45**	D			
		C3	3.64**	-0.72	43.84**	13.08**	-3.77**	-27.32**	D			
		C4	9.86**	0.65*	24.55**	7.71**	-4.55**	-14.01**	D			
4	Stalk Weight (g.plant)	C1	-490.8**	-2.58	1756.6**	654.2**	152.40**	-	767.73**	D		
		C2	-712.8**	-78.11**	2628.1**	965.6**	-126.49*	-	1235.1**	D		
		C3	-150.64	-212.8**	2140.3**	506.7**	-124.10	-	1203.3**	D		
		C4	-653.8**	-135.9**	2426.5**	1038.9**	-601.5**	-	1021.6**	D		
5	Cane Weight (g.plant)	C1	-	330.42**	2.13	1292.4**	445.69**	94.80**	-717.7**	D		
		C2	-	527.89**	-75.38**	1912.5**	698.71**	-120.85*	-846.9**	D		
		C3	-153.93	-	194.16**	1901.2**	441.47**	-	226.56**	1055.6**	D	
		C4	-	626.94**	-	110.60**	2183.6**	937.91**	-	662.47**	-924.3**	D
6	Juice Weight (g.plant)	C1	-173.3**	3.88	483.1**	201.2**	57.47**	-138.8**	D			
		C2	-173.1**	-13.73**	555.7**	205.7**	-57.48**	-225.0**	D			
		C3	-38.5	-57.40**	468.0**	116.4**	-50.72*	-260.8**	D			
		C4	-140.7**	-23.88**	523.6**	238.5**	137.22**	-211.6**	D			
7	Juice Volume (ml.plant)	C1	-161.9**	3.05*	516.0**	188.2**	48.92**	-260.9**	D			
		C2	-162.9**	-11.76**	523.9**	194.1**	-58.10**	-215.6**	D			
		C3	-74.2**	-56.06**	564.8**	148.9**	-84.00**	-334.3**	D			
		C4	-145.9**	-26.70**	540.34**	231.1**	127.49**	-	233.40**	D		
8	Brix %	C1	10.90**	0.60	8.33**	-0.80	0.93	-11.31**	D			
		C2	11.15**	-2.70**	12.33**	3.80**	-0.48	-4.02**	D			
		C3	17.18**	-5.18**	3.84	-3.53**	7.85**	-1.13	D			
		C4	4.50**	-3.45**	17.60**	11.37**	-5.99**	-3.17*	D			
9	Bagasse (g.plant)	C1	-	124.03**	-1.01	620.28**	210.05**	39.13**	-	323.57**	D	
		C2	-	300.56**	-54.05**	1200.0**	431.51**	-74.64**	-	541.87**	D	
		C3	-13.69**	-	123.91**	1166.4**	212.74**	-	180.49**	-	682.97**	D
		C4	-	427.76**	-72.36**	1510.6**	637.06**	488.10**	-	664.26**	D	
10	Sucrose (%)	C1	4.02**	0.22	19.43**	4.48**	3.72**	-14.75**	D			
		C2	8.62**	-1.44**	13.36**	4.40**	-2.65**	-4.59**	D			
		C3	12.04**	-4.56**	7.47**	0.52	6.21**	-2.56**	D			
		C4	3.58**	-2.77**	16.40**	10.72**	-7.21**	-3.28**	D			
11	Sugar Yield (g.plant)	C1	-21.14**	0.57*	66.22**	23.98**	8.36**	-30.78**	D			
		C2	-33.85**	-3.07**	104.62**	39.18**	-12.90**	-40.24**	D			
		C3	-1.07	-12.08**	70.18**	15.08**	-6.63	-35.29**	D			
		C4	-36.91**	-7.21**	112.70**	53.19**	-32.91**	-42.85**	D			

* Significance at P = 0.05 ** Significance at P = 0.01, D = Duplicate, C = Complementary

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