

Agroindustrial performance and heterosis in sweet sorghum using male-sterile lines with high stem sugar content

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Abstract: *The objective of this study was to evaluate the performance of hybrids and the heterosis in crosses of sweet sorghum using juicy sweet male-sterile lines and fertility-restoring lines with and without sensitivity to photoperiod. Thirty hybrids and six controls were evaluated in experiments laid out in a 6 × 6 triple lattice design. The genotypes differed for all traits. The general combining ability (GCA) of the R lines affected all traits, while the GCA of the A lines only affected juice extraction, total soluble solids, and tons of Brix per hectare (TBH). The photosensitive-restoring line CMSXS5022 had the highest positive GCA estimates for the fresh mass production and TBH traits. Heterosis was significant only for days to flowering and plant height. Overall, the photoperiod-sensitive hybrids stood out. The development of male-sterile lines requires better complementarity from restoring lines to reap the benefits of heterosis.*

Keywords: Sorghum bicolor, ethanol production, sorghum hybrids, multi-trait selection

INTRODUCTION


Growing global demand for food, fiber, and energy poses challenges in the economic, social, and environmental spheres (Shaffer 2019). Concerns about the environment have led the main global economies to seek alternatives to help reduce the use of fossil fuels, which emit large quantities of greenhouse gases (Skovgaard and van Asselt 2019, UNFCC 2021). Therefore, intensive research and efforts have been focused on the evolution of energy matrices, with greater use of renewable sources. Biofuels, such as biodiesel and bioethanol, are among the alternatives to replace fossil fuels (Joshi et al. 2017).

Sorghum (*Sorghum bicolor* (L.) Moench) is an agricultural crop suitable for various purposes, though most sorghum grown is used for grain production (Venkateswaran et al. 2019). Sorghum is also considered a bioenergy crop because it has good biomass production and high stem sugar concentration, and the genotypes with the highest sugar concentration are known as sweet sorghum varieties (Appiah-Nkansah et al. 2019). In Brazil, these sweet sorghum cultivars have been used as an alternative in the sugarcane off-season to produce ethanol and for cogeneration of electric energy from bagasse burning (Barcelos et al. 2016). There are commercial sweet sorghum lines and hybrids available on the market, but genetic improvement is necessary to increase crop yield.



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Hybridization has been the method most used in sweet sorghum breeding. Crosses have been made via cytoplasmic-genetic male sterility in the species (Smith and Frederiksen 2000). The male-sterile lines belong to group 'A', and their seeds are produced by crossing with isogenic but male-fertile 'B' lines. The hybrids are obtained by crossing 'A' lines with lines of the group of fertility-restoring lines called 'R' lines.

The hybrid cultivar is favored when it is superior to the average of the parents of the cross (heterosis) or to the average of the best parent (heterobeltiosis). For breeding by hybridization, generating lines that have good performance and that exhibit complementarity, i.e., genetic divergence, is essential. In sweet sorghum hybridizations, it is common for male-sterile lines to have aptitude for forage use, which results in worse averages for traits that behave in an additive manner, such as stem sugar content (Durães et al. 2020). Several studies have estimated the significance and magnitude of heterosis in sweet sorghum (Kumar et al. 2016, Lombardi et al. 2018, Aru et al. 2020, Chapara et al. 2020, Durães et al. 2020).

The search for increased sugar and biomass production has focused on other traits. Due to the sensitivity to photoperiod present in this species, sorghum is a short-day plant that flowers at day lengths of less than 12 h 20 min. Photoperiod sensitivity allows genotypes to have a longer period of vegetative growth, which confers greater biomass production to sensitive genotypes (Parrella et al. 2010). The presence or absence of juice in the stems should also be taken into account, as dry-stem genotypes show less juice extraction, reducing the yield of ethanol per hectare.

The objective of this study was to evaluate the performance of hybrids and the heterosis in crosses of sweet sorghum involving juicy sweet male-sterile lines and fertility-restoring lines with and without photoperiod sensitivity.

MATERIAL AND METHODS

Experiment locations

The experiments were conducted in two locations in the state of Minas Gerais, Brazil: 1) Lavras, at the experiment site of the Center for Scientific and Technological Development at the Muquém farm (lat 21° 14' S, long 45° 00' W, alt 918 m asl), belonging to the Federal University of Lavras, Lavras, MG. The mean annual temperature is approximately 19.4 °C, and the cumulative annual rainfall is 1,500 mm. The climate is Cwa in the Köppen classification system. The soil is classified as *Latossolo Vermelho-Amarelo*, with gentle slopes. 2) Sete Lagoas, MG, at the experiment site of Embrapa Maize and Sorghum (lat 19° 27' S, long 44° 14' W, alt 767 m asl). The mean annual temperature is approximately 23 °C, and the mean cumulative annual rainfall is 1,400 mm. The climate is Cwa. The soil is classified as *Latossolo Vermelho*. The cumulative rainfall in Lavras in the period from planting to harvest was 623 mm, while in Sete Lagoas it was 795 mm. The mean relative humidity was 72% in Lavras and 73.5% in Sete Lagoas.

Genotypes evaluated

A total of 36 genotypes were studied, composed of 30 experimental hybrids and six controls. The control group consisted of six genotypes: two commercial hybrids – N31L5010 (Nexteppe Sementes do Brasil) and CV198 (Monsanto); one hybrid – CMSXS5501A × CMSXS5021 from the Embrapa breeding program; and the varieties (lines) BRS 511, CMSXS643, and CMSXS646 from the Embrapa breeding program.

To obtain the experimental hybrids, A lines were crossed with R lines of sweet sorghum in a partial diallel cross design (Table 1). The photoperiod-insensitive hybrids were obtained by crossing an insensitive female (*ma1 ma1*) with an insensitive male (*ma1 ma1*), and the photoperiod-sensitive hybrids were obtained by crossing an insensitive female (*ma1 ma1*) with a sensitive male (*Ma1 Ma1*). The sensitive genotypes were dry-stemmed, obtained by crossing juicy-stemmed females (*dd*) with dry-stemmed males (*DD*), but both parents had sugar in the stems. The lines used as parents in the crosses came from the sweet sorghum breeding program of Embrapa Maize and Sorghum.

Experiment plan and implementation

The experiments at each location were set up in a 6 × 6 triple lattice design. A plot consisted of two 5-m rows spaced 0.7 m apart. Planting was performed on November 30, 2017, in Lavras, and on October 26, 2017, in Sete Lagoas. The genotypes were harvested when the grain had reached the milk stage.

Table 1. Codes and descriptions of genotypes evaluated regarding pedigree (A line ♀ × R line ♂) and photoperiod sensitivity (PS)

Code	Pedigree	PS*	Code	Pedigree	PS
1	CMSXS5502 A × BRS 508	I	19	CMSXS5502 A × 201717B062	I
2	CMSXS5503 A × BRS 508	I	20	CMSXS5503 A × 201717B062	I
3	CMSXS5504 A × BRS 508	I	21	CMSXS5504 A × 201717B062	I
4	CMSXS5505 A × BRS 508	I	22	CMSXS5505 A × 201717B062	I
5	CMSXS5506 A × BRS 508	I	23	CMSXS5506 A × 201717B062	I
6	CMSXS5507A × BRS 508	I	24	CMSXS5507 A × 201717B062	I
7	CMSXS5502 A × CMSXS643	I	25	CMSXS5502 A × CMSXS5022	S
8	CMSXS5503 A × CMSXS643	I	26	CMSXS5503 A × CMSXS5022	S
9	CMSXS5504 A × CMSXS643	I	27	CMSXS5504 A × CMSXS5022	S
10	CMSXS5505 A × CMSXS643	I	28	CMSXS5505 A × CMSXS5022	S
11	CMSXS5506 A × CMSXS643	I	29	CMSXS5506 A × CMSXS5022	S
12	CMSXS5507 A × CMSXS643	I	30	CMSXS5507 A × CMSXS5022	S
13	CMSXS5502 A × CMSXS646	I	31	CMSXS5501 A × CMSXS5021	S
14	CMSXS5503 A × CMSXS646	I		CMSXS643	I
15	CMSXS5504 A × CMSXS646	I		CMSXS646	I
16	CMSXS5505 A × CMSXS646	I		BRS 511	I
17	CMSXS5506 A × CMSXS646	I		N31L5010	I
18	CMSXS5507 A × CMSXS646	I		CV198	I

* I = Insensitive; S = Sensitive

Traits evaluated

The following traits were evaluated: Days to flowering (DTF, days) – number of days from sowing to the flowering of at least 50% of the plants in the plot. Plant height (HGT, m) – mean height (m) of five plants randomly selected from the plot, measured from the soil surface to the tip of the panicle using a tape measure. Fresh mass production (FMP, t ha⁻¹) – the plants of the plot were cut at 5.0 cm from the soil surface, then weighed (without panicles) on a hanging scale. Juice extraction (EXT, %) – six plants were randomly sampled per plot, without panicles. In Lavras, juice extraction was performed using a double-tandem sugarcane mill with 10" × 14" rollers. The extraction percentage was calculated as the ratio between the weight of the juice and the weight of the six stems. In Sete Lagoas, the plants were shredded and homogenized, and then a subsample of 500 ± 0.5 g was collected for juice extraction in a hydraulic press, with a constant pressure of at least 250 kgf cm⁻² applied to the sample for 1 min. The weight (g) of the juice extracted from the subsample was recorded. The extraction percentage was calculated using the formula: $EXT = \text{weight of juice} / 500 \times 100$. Total soluble solids content (TSS, %juice) was determined using a digital refractometer with automatic reading. Tons of Brix per hectare (TBH, t ha⁻¹) was determined from the expression: $TBH = FMP \times TSS$.

Statistical-genetic analysis

Statistical analysis

Multilocation analysis was performed according to the following model:

$$y_{ijkl} = \mu + a_l + r_{i(l)} + b_{j(i)} + g_k + ga_{kl} + e_{ijkl}$$

where y_{ijkl} is the observation of the portion of block j within replicate i at location that received genotype k ; μ is a constant associated with the observations; a_l is the effect of location l ; $r_{i(l)}$ is the effect of replication i within location l ; $b_{j(i)}$ is the effect of block j within replicate i at location l , $b_{j(i)} \sim N(0, \sigma_b^2)$, where σ_b^2 is the variance of blocks within the replicates; g_k is the effect of genotype k ; ga_{kl} is the effect of the interaction of genotype k with location l ; and e_{ijkl} is the experimental error associated with observation y_{ijkl} , $e_{ijkl} \sim N(0, \sigma_e^2)$, where σ_e^2 is the error variance.

The homogeneity of the residual variances of the locations was tested by Levene's test implemented in the car R package (Fox and Weisberg 2019). Statistical analyses were performed using the lme4 R package (Bates et al. 2015) in the R software (R Core Team 2019). From the fitted models, the adjusted phenotypic means of the genotypes at each location were estimated using the emmeans R package (Lenth 2020). The precision and quality of the experiments were

measured by selective accuracy using the estimator $r_{gg} = \sqrt{1 - 1/F_g}$, where F_g is Snedecor's F value for the genotype effect (Resende and Duarte 2007).

Joint diallel analysis

The general and specific combining abilities were estimated using the procedure described by Geraldi and Miranda Filho (1988) according to the following model:

$$y_{mm'l} = \mu + a_l + l_m + l_{m'} + s_{mm'} + la_{ml} + la_{m'l} + sa_{mm'l} + e_{mm'l},$$

where $y_{mm'l}$ is the mean of the hybrid between the m^{th} A line and m'^{th} R line; μ is the overall mean; a_l is the effect of the l^{th} location; l_m is the effect of the general combining ability (GCA) of the m^{th} A line; $l_{m'}$ is the effect of the GCA of the m'^{th} R line; $s_{mm'}$ is the effect of the specific combining ability between the m^{th} A line and m'^{th} R line; la_{ml} is the interaction effect between the GCA of the m^{th} A line and the l^{th} location; $la_{m'l}$ is the interaction effect between the GCA of the m'^{th} R line and the l^{th} location; $sa_{mm'l}$ is the interaction effect between the specific combining ability between the m^{th} A line and m'^{th} R line and the l^{th} location; and $e_{mm'l}$ is the experimental error associated with observation $y_{mm'l}$, $e_{mm'l} \sim N(0, \sigma_e^2)$, with σ_e^2 being the mean error variance.

The significance of the effects of the general and specific combining abilities was assessed using Student's *t*-test at 5% significance. This diallel analysis was performed on the Genes software (Cruz 2013). The genotypes were graphically represented by biplots of the standardized phenotypic means, where each column in the two-way matrix corresponds to the combination between trait and location, according to the procedure described by Yan and Tinker (2006). The graphs were prepared using the GGEBiplots R package (Dumble 2017).

Decomposing the genotype × environment interaction

The mean square of the genotype × location interaction (QM_{ga}) was decomposed into non-crossed or simple (QM_{ga_s}) and crossed or complex (QM_{ga_c}) parts, as proposed by Cruz and Castoldi (1991), using the following equation:

$$QM_{ga} = QM_{ga_s} + QM_{ga_c}$$

where $QM_{ga_s} = \frac{(\sqrt{Q_l} - \sqrt{Q_{l'}})^2}{2} + c\sqrt{Q_l Q_{l'}}$ and $QM_{ga_c} = \sqrt{(1-r)^3 Q_l Q_{l'}}$, where Q_l and $Q_{l'}$ are the mean squares of the genotypes at locations l and l' , respectively; r is the correlation coefficient between the means of the genotypes at locations l and l' ; and $c = 1 - r = \sqrt{(1-r)^3}$.

RESULTS AND DISCUSSION

Statistical analysis

In the selective accuracy estimates, the experiments showed high accuracy according to the classification of Resende and Duarte (2007), ranging from approximately 91% for TBH to 100% for DTF (Table 2). Thus, the selection had high reliability based on the experimental data. The evaluated genotypes differed for all traits, with marked variations among the experimental hybrids. Depending on the trait, the experimental hybrids had average performance equal to or inferior to the controls. However, this contrast should be interpreted with caution, since the group of experimental hybrids was composed of 30 treatments, while there were only six controls. The experimental hybrids with inferior performance decreased the mean of the group, but there were hybrids with performance superior to all the controls, such as CMSXS5501A × CMSXS5021 (Table 4).

There was significant genotype × location interaction for all traits, except for HGT (Table 2). Regarding the type of the genotype × location interaction, simple interaction predominated only for DTF (94%) and TBH (59%), whereas TSS (82%), FMP (53%), and EXT (97.7%) showed a higher proportion of complex interaction. According to Cruz et al. (2014), the predominance of simple interaction is not a detriment to ranking, since the order of classification of the genotypes does not change, but when there is a predominance of complex interaction, the analysis of the mean between locations can lead to the selection of poorly adapted genotypes. Given the significance and nature of the genotype × environment interaction, in each location we decomposed the performance of the hybrids into the variables EXT, DTF, FMP, TBH, and TSS, as well as the mean between locations for HGT (Table 4).

Table 2. Summary of the joint analysis of the traits days to flowering (DTF), plant height (HGT), fresh matter production (FMP), juice extraction (EXT), total soluble solid content (TSS), and tons of Brix per hectare (TBH) and the selective accuracy

Source of variation	df	F _c – Snedecor					
		DTF	HGT	FMP	EXT	TSS	TBH
Location (L)	1	1939.05*	0.08	85.54*	934.74*	27.50*	39.76*
Genotype (G)	35	628.63*	25.75*	8.10*	5.43*	8.29	5.99*
Experimental hybrids (H)	29	647.32*	21.10*	5.37*	5.38*	7.72*	3.68*
GCA A	5	1.39	1.2	1.97	2.74*	4.17*	2.97*
GCA R	4	4679.97*	140.14*	32.55*	31.77*	43.03*	15.93*
SCA A × R	20	2.27*	2.27*	0.78	0.76	1.54	1.41
Controls (C)	5	607.20*	55.38*	23.18*	6.97*	11.43*	17.07*
H vs. C	1	165.54*	12.34*	11.91*	0.20	11.05*	17.91*
G × L	35	43.17*	1	1.99*	1.56*	2.88*	2.90*
H × L	29	47.26*	1.05	1.56*	1.76*	2.29*	2.08*
GCA A × L	5	0.24	0.98	1.47	4.65*	1.75	0.99
GCA R × L	4	337.20*	0.78	5.71*	1.31	10.18*	8.84*
SCA A × R × L	20	1.03	1.13	0.75	1.13	0.84	1
C × L	5	27.86*	0.45	4.37*	0.68	5.06*	7.00*
(H vs C) × L	1	0.87	2.21	2.74	0.04	9.11*	6.26*
Selective accuracy (%)		100	98	94	90	94	91

* Significant at 5% probability by the F test.

Genetic analysis

There was divergence in the GCA of the A lines for the traits EXT, TSS, and TBH, while the R lines were divergent for all traits. Therefore, there was less genetic divergence between the male-sterile lines than fertility-restoring lines (Table 2). Other studies with diallels of sweet or biomass sorghum in Brazil and abroad found similar results (Vinaykumar et al. 2011, Bunphan et al. 2015, Lombardi et al. 2018, Oliveira et al. 2019). This reality alerts us to the need to develop A lines focusing not only on the *per se* performance, such as of the A lines used in this study, which had been bred for higher sugar content, but also on the genetic variability within this group. The photosensitive parent CMSXS5022 of the R group had strongly positive and significant GCA estimates for the traits of interest, FMP and TBH, although it had lower stem sugar content, as evidenced by the negative GCA estimate for TSS (Table 3). This line conferred photoperiod sensitivity to all crosses in which it was a parent, and these hybrids showed good rankings, especially for biomass (FMP), and thus also for sugar content (TBH) (Table 4).

Table 3. Estimates of general combining ability effects for group A lines and for group R lines for each trait and location

Line	DTF		HGT	FMP		EXT		TSS		TBH	
	Lavras	Sete Lagoas		Lavras	Sete Lagoas	Lavras	Sete Lagoas	Lavras	Sete Lagoas	Lavras	Sete Lagoas
Group A											
CMSXS5502	0.62	1.42*	-0.09*	-7.43	-1.48	-3.14	-0.05	-1.04*	-0.41	-1.503	-0.431
CMSXS5503	-0.18	-0.35	0.02	-1.48	-1.17	-3.32	1.12	0.11	-0.01	-0.136	-0.244
CMSXS5504	-0.11	-0.15	0.04	1.81	0.9	-0.26	0.3	-0.55	-0.12	-0.091	0.166
CMSXS5505	0.42	0.32	0.01	1.73	-0.77	-0.95	-1	0.43	0.29	0.38	0.015
CMSXS5506	-0.91	-0.48	0.01	6.5	-0.21	8.62*	-0.74	-0.21	0.18	0.756	0.026
CMSXS5507	0.16	-0.75	0.02	-1.12	2.73	-0.94	0.37	1.26*	0.07	0.594	0.466
Group R											
BRS 508	-7.98*	-15.35*	-0.09*	-5.75	-3.36	1.62	1.07	1.41*	1.17*	-0.122	0.04
CMSXS643	-8.31*	-12.96*	-0.17*	-7.23*	-2.75	1.4	4.42*	-0.41	0.18	-1.276*	-0.28
CMSXS646	-9.70*	-16.85*	-0.18*	4.78	-1.84	4.08*	2.19*	2.22*	1.16*	2.059*	0.29
201717B062	-10.26*	-17.41*	-0.31*	-13.01*	-3.26	1.12	3.09*	-2.07*	0.12	-2.643*	-0.37
CMSXS5022	36.24*	62.57*	0.75*	21.22*	11.22	-8.23*	-10.78*	-1.15*	-2.63*	1.983*	0.32

Regarding the specific combining ability (SCA) estimates, there was significance only for DTF and HGT (Table 2), while for the traits of economic importance, SST, FMP, and TBH, there was not. While finding similar magnitudes of the experimental precision metrics, other studies have observed significant SCA values (Rocha et al. 2018, Lombardi et al. 2018, Durães et al. 2020). It is important to note that most of the diallel analyses (such as those performed in the studies mentioned) that found significance for SCA evaluated both parents and F₁ plants. According to Yao et al. (2013), the inclusion of parents in diallel analyses produces biased estimates of heterosis by attributing the additive × additive epistatic effect to SCA. Therefore, the effects of the SCAs in those studies are most likely overestimated. For a better evaluation of the effects of SCA, the analysis should be performed using only the F₁ plants in the model, as done in the present study. Therefore, there was no significant effect of SCA on the main target traits of sweet sorghum, FMP, EXT, TSS, and TBH, when using new female genotypes (A lines) with aptitude for sweet sorghum varieties.

Table 4. Adjusted phenotypic means for each genotype in the trait/location combinations. L: Lavras; S: Sete Lagoas; DTF: days to flowering; HGT: plant height; FMP: fresh mass production; EXT: juice extraction; TSS: total soluble solid content; TBH: tons of Brix per hectare

Genotype	DTF_L	DTF_S	HGT	FMP_L	FMP_S	EXT_L	EXT_S	TSS_L	TSS_S	TBH_L	TBH_S
CMSXS5502 × BRS_508	75.67 f	84.33 e	3.19 b	49.14 b	38.90 a	37.20 a	61.78 c	15.33 a	16.53 a	7.51 a	6.43 a
CMSXS5503 × BRS_508	74.67 f	82.67 e	3.11 b	46.97 b	42.90 a	36.89 a	65.53 c	14.2 a	16.4 a	6.58 a	6.99 a
CMSXS5504 × BRS_508	77 f	82.67 e	3.12 b	56.86 b	46.38 a	42.81 a	62.06 c	16.77 a	16.27 a	9.64 a	7.55 a
CMSXS5505 × BRS_508	75.67 f	84.33 e	3.09 b	55.25 b	41.14 a	37.81 a	62.66 c	14.77 a	16.9 a	8.19 a	6.95 a
CMSXS5506 × BRS_508	74 f	81.33 e	3.09 b	59.36 b	39.48 a	46.49 a	63.28 c	15.07 a	16.53 a	8.92 a	6.51 a
CMSXS5507 × BRS_508	79 f	86.67 e	3.13 b	42.50 b	44.62 a	38.31 a	66.27 a	15.73 a	15.6 b	6.60 a	6.97 a
CMSXS5502 × CMSX643	75.33 f	87.00 e	3.09 b	43.94 b	42.81 a	36.05 a	68.27 a	11.9 b	14.83 b	5.11 a	6.37 a
CMSXS5503 × CMSX643	77.33 f	86.00 e	2.87 b	40.44 b	42.19 a	38.74 a	71.67 a	13.57 b	15.43 b	5.53 a	6.51 a
CMSXS5504 × CMSX643	74.67 f	84.67 e	3.10 b	57.14 b	44.86 a	39.26 a	67.08 a	11.97 b	15.03 b	6.96 a	6.70 a
CMSXS5505 × CMSX643	77.33 f	87.00 e	3.04 b	50.00 b	47.14 a	40.03 a	65.69 c	15.17 a	15.77 a	7.47 a	7.41 a
CMSXS5506 × CMSX643	74 f	85.67 e	3.06 b	60.97 b	41.10 a	47.64 a	63.48 c	13.1 b	16.43 a	8.00 a	6.72 a
CMSXS5507 × CMSX643	75.33 f	86.00 e	3.06 b	48.67 b	39.00 a	36.47 a	65.51 c	15.27 a	14.77 b	7.45 a	5.76 a
CMSXS5502 × CMSX646	75.67 f	86.00 e	2.94 b	56.89 b	43.81 a	41.41 a	61.56 c	14.77 a	15.87 a	8.42 a	6.95 a
CMSXS5503 × CMSX646	74 f	84.67 e	3.08 b	65.64 b	36.24 a	38.46 a	65.73 c	17.13 a	16.23 a	11.26 a	5.88 a
CMSXS5504 × CMSX646	74 f	82.67 e	3.06 b	53.11 b	48.05 a	45.42 a	66.46 a	14.47 a	16.27 a	7.52 a	7.80 a
CMSXS5505 × CMSX646	74.67 f	81.33 e	3.08 b	60.47 b	41.62 a	42.23 a	65.29 c	16.37 a	16.37 a	9.94 a	6.81 a
CMSXS5506 × CMSX646	73 f	81.33 e	2.95 b	71.22 a	44.24 a	43.86 a	64.11 c	18.07 a	16.9 a	12.92 a	7.47 a
CMSXS5507 × CMSX646	74.33 f	77.00 e	3.09 b	65.89 b	48.62 a	42.85 a	65.17 c	15.97 a	16.5 a	10.47 a	8.00 a
CMSXS5502 × 201717B062	74.33 f	84.00 e	2.93 b	39.19 b	40.76 a	37.80 a	64.70 c	11.3 b	15.07 b	4.54 a	6.16 a
CMSXS5503 × 201717B062	73.33 f	78.00 e	2.90 b	45.56 b	40.43 a	32.60 a	65.52 c	12.13 b	15.63 b	5.55 a	6.31 a
CMSXS5504 × 201717B062	73.67 f	82.33 e	2.99 b	46.83 b	45.24 a	40.27 a	65.43 c	11.43 b	15.57 b	5.37 a	7.04 a
CMSXS5505 × 201717B062	74.67 f	82.67 e	2.89 b	47.39 b	40.10 a	41.67 a	65.75 c	12.33 b	15.47 b	5.91 a	6.20 a
CMSXS5506 × 201717B062	73.33 f	83.00 e	2.85 b	48.97 b	45.19 a	43.63 a	66.89 a	10.22 b	14.37 b	5.52 a	6.48 a
CMSXS5507 × 201717B062	73 f	79.67 e	2.87 b	38.56 b	42.33 a	40.54 a	65.42 c	13.6 b	15.83 a	5.33 a	6.72 a
CMSXS5502 × CMSXS5022	122 b	160.52 a	3.48 b	60.81 b	54.33 a	23.32 a	56.07 d	11.03 b	11.67 c	7.06 a	6.24 a
CMSXS5503 × CMSXS5022	119.67 b	162.00 a	4.18 a	81.11 a	60.38 a	28.16 a	49.77 d	13.03 b	12.27 c	10.55 a	7.38 a
CMSXS5504 × CMSXS5022	120 b	162.00 a	3.96 a	82.25 a	48.00 a	22.41 a	53.10 d	12.13 b	12.27 c	10.19 a	6.03 a
CMSXS5505 × CMSXS5022	119.67 b	161.33 a	4.00 a	82.67 a	54.19 a	24.95 a	48.23 d	13.04 b	12.93 c	10.52 a	7.01 a
CMSXS5506 × CMSXS5022	121 b	161.33 a	4.14 a	79.11 a	56.95 a	52.95 a	51.17 d	12.04 b	12.67 c	8.45 a	7.25 a
CMSXS5507 × CMSXS5022	119 b	162.00 a	3.99 a	85.94 a	67.10 a	28.60 a	52.10 d	15.23 a	13.63 c	13.26 a	9.18 a
CMSXS5501A × CMSXS5021	128 a	162.00 a	4.68 a	121.00 a	71.71 a	25.98 a	51.11 d	15.53 a	14.35 b	18.84 a	10.11 a
CMSXS643	88.33 c	97.33 b	3.03 b	49.83 b	43.38 a	45.05 a	66.59 a	15.1 a	15.03 b	7.50 a	6.49 a
CMSXS646	81.33 e	88.67 d	3.04 b	56.69 b	36.19 a	37.43 a	65.87 a	18.83 a	16.7 a	10.66 a	6.05 a
BRS 511	84.67 d	92.33 c	3.04 b	50.33 b	33.10 a	46.32 a	65.79 b	17.57 a	15.2 b	8.77 a	5.06 a
N31L5010	83 e	93.33 b	3.18 b	60.03 b	61.95 a	38.99 a	62.37 c	9.97 b	14.4 b	5.90 a	9.03 a
CV198	73 f	89.00 d	3.13 b	63.81 b	47.38 a	32.82 a	62.97 c	16 a	15.8 a	10.42 a	7.48 a

Genotypes followed by the same letter in the column belong to the same cluster (Scott–Knott at 5% significance).

Analysis of the interaction between genetic and location effects showed that there was an interaction of the GCA of the R lines with location for all traits except for HGT. The GCA and SCA of the A lines showed no interaction with location, except for EXT (Table 2). The GCA and SCA components were analyzed at the mean of the locations for the HGT trait. For the other traits, the genetic components (GCA and SCA) were analyzed for each location separately, due to the existence of interaction between the GCA of the R lines and location.

Performance analysis of hybrids

The performance of the hybrids was evaluated graphically using a genotype-by-trait × location biplot (Figure 1). The first and second principal components captured 80.87% of the variation present in the data. The vector of the average environment coordination, represented by the black line with an arrow, indicates the genotypes with the highest score, considering all the traits evaluated. Thus, the best-ranked genotypes overall were the CMSXS5501A × CMSXS5021 control (31 in the graph) and the CMSXS5507A × CMSXS5022 experimental hybrid (30 in the graph). The CMSXS5501A × CMSXS5021 control showed a performance far superior to the average of the others. It also showed a stable performance profile in the different traits, followed by experimental hybrid 30, whose performance was better for the FMP, HGT, and DTF traits.

The photoperiod-sensitive genotypes stood out; four of the five best-ranked genotypes were photoperiod-sensitive (Figure 1), and these genotypes had mean biomass production higher than 54 t ha⁻¹ (Table 4). Similarly, four of the five genotypes with the best performance were experimental hybrids (30, 17, 28, and 26 in the graph), and all of these genotypes had an estimated biomass production greater than 44 t ha⁻¹. These experimental hybrids were also superior to all commercial controls except for CMSXS5501A × CMSXS5021.

According to Schaffert et al. (2011), the ideal genotype should produce at least 60 t ha⁻¹ of biomass. Several experimental hybrids showed potentials above this threshold, such as hybrid 25, all the crosses containing the R line CMSXS5022, and hybrids 14 and 17. Most of these hybrids were sensitive to photoperiod, but some insensitive genotypes still approached the ideotype (Table 4). According to Lombardi et al. (2015), TBH is the trait that best correlates with ethanol production per hectare. We used this trait for indirect selection for ethanol production. Despite the correlation with TBH, no equivalence could be drawn between the 3600 L ha⁻¹ threshold of the ethanol production ideotype and TBH production. Such a link needs to be established, if possible, by other experiments. Therefore, this study could not identify which experimental hybrids reached the minimum liters of ethanol per hectare required for viability in the industry, and we can only identify the best genotypes among those evaluated in the experiment.

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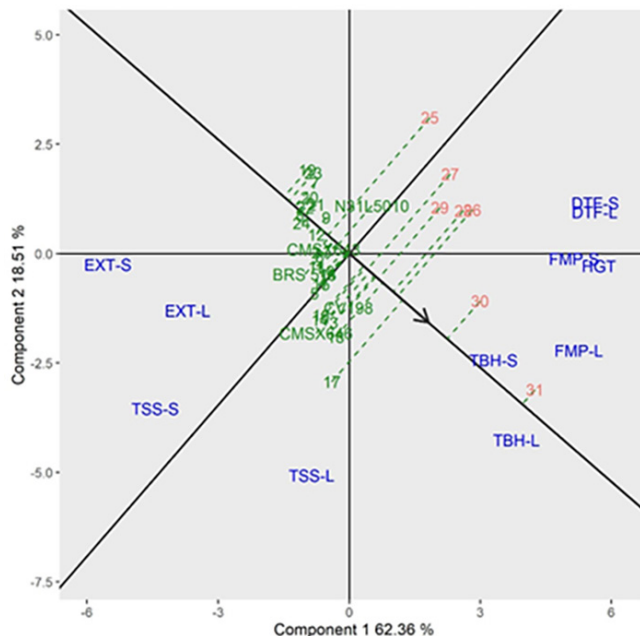


Figure 1. Representation of genotypes for the traits EXT, DTF, HGT, FMP, TSS, and TBH in the decompositions for Lavras (L) and Sete Lagoas (S) under the average vs. stability perspective. Genotypes represented in red are photoperiod-sensitive, those in green photoperiod-insensitive. The axis with the arrow indicates the genotype with average performance [average tester coordinate (ATC) abscissa], and the axis in bold perpendicular to this one passing through the origin is the ATC ordinate. The projection of the genotypes on the ATC abscissa represents the performance of each genotype in relation to the average performance.


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