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Research Paper

Biomass for direct combustion and lignocellulosic ethanol on different sorghum morphotypes in reponse to two plant densities

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ABSTRACT:*Biofuels and biomass for direct combustion emerge as technological alternatives to significantly reduce fossil fuel consumption. The objective was to study two plant densities and four morphological sorghum types effects on the calorific value and biomass attributes, which are determinant in the lignocellulosic ethanol production. Biomass dry yield (BDY), cellulosic ethanol yield (CEY), calculated from hemicellulose (Hcel) and cellulose (Cel) values, and higher heating value (HHV) were evaluated, among other determinations. Year effect was significant in most of the variables. During 21/22 growing season, significant effects in the hybrid×density interaction for total soluble solids (TSS) were detected. Changes in plant density, within the studied range, did not affect BDY, HHV, or CEY, but it caused morphological changes. A significant and positive correlation between BDY×CEY, HHV×Cel, and HHV×LDA was observed. Based on the results obtained, the plant density used in this study defined as low (120,000 plants/ha) could be recommended for the evaluated objectives and morphotypes. In both seasons, highest significant values of cellulosic ethanol yield (CEY), dry biomass yield (DBY), and higher heating value (HHV) corresponded to Sugargraze and Argensor 195Fb (non-photosensitive morphotypes).*

KEYWORDS: *Sorghum,Biomass,Bioenergetic aptitude,Plant density.*

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I. INTRODUCTION

The need to diversify the world's energy matrix and expand the spectrum of renewable sources has driven the search for sustainable technologies from both economic and environmental standpoints. Moreover, there is strong international pressure to reconcile a growing future energy demand with a reduction in CO2 emissions. In this scenario, biofuels and biomass for direct combustion emerge as technological alternatives, readily available, to significantly reduce fossil fuel consumption (Scott et al., 2015).

Ethanol from agricultural crops or bioethanol is poised as a potentially sustainable energy resource, offering long-term environmental and economic advantages compared to conventional fuels (Scott et al., 2015). Consequently, the production of ethanol from lignocellulosic biomass, grown in marginal areas, offers a sustainable alternative without occupying land intended for human or animal food production (Roozeboom et al., 2019).

On the other hand, biomass for direct combustion is a renewable energy source that does not contribute to global warming. In fact, it leads to a reduction in atmospheric carbon dioxide levels since it acts as a sink, potentially increasing soil carbon (Jiang et al., 2019). In this sense, sorghum is a multipurpose crop that provides grain and stems which can be used for sugar, alcohol, paper, syrup, various forms of animal feed (green forage, silage, hays, deferred feed, etc.), and eventually as a biofuel (Stamenkovic et al., 2020). The strategic advantage of this crop is its high productivity under unfavorable conditions, such as tolerance to water stress (Propheter et al., 2010), thermal stress (Druille et al., 2020), saline soils, poorly structured soils, and temporary flooding (Houx et al., 2013; Promkhambut et al., 2011). Even in these environments, it has the capacity to produce lignocellulose, sugar, and starch (Qiu et al., 2017). Moreover, it offers great morphological diversity among different biotypes (forage types, grain types, photosensitive types, etc.) with various morphological traits

(BMR's, stay-green, dry pith, etc.), all of them having high biomass production potential and potentially exploitable for bioenergy conversion. Sweet sorghum with juicy pith has been widely recognized as a source for sugar and ethanol production from its juice. However, its potential as a producer of biomass for direct combustion and ethanol from the residue still requires further experimentation (Tang et al., 2018; Fonseca de Almeida et al., 2019).

Traditionally, vegetative tissue was considered the energy capture source necessary for optimal grain yield. In this regard, lignocellulosic biomass production for biofuels adds value to vegetative tissue and leads to a paradigm shift to optimize plant architecture in bioenergy crops.

In sorghum cultivation, plant spacing within rows is the management factor with the greatest impact on modifying plant architecture and consequently, the percentage variation of its components (panicle and stem+leaves) (Ottman and Ray, 2018). According to some reports, increasing the distance between plants within rows increases stem diameter and thereby the pith/bark ratio (Worley et al., 1991). This variation implies a favoring of the anatomical fraction of the stem containing a higher proportion of sugars (pith), providing an advantage for ethanol production from lignocellulosic residue, but without mentioning its effect on suitability for direct combustion. On the other hand, stem diameter has a positive association with sugar content and also with dry biomass yield (Adams et al., 2015, Tang, et al., 2018).

Thus, new breeding objectives and ideotypes of plants, along with appropriate management tools, should be established for production systems aimed at biofuels. There are still few or no experimental results in our productive region explicitly referring to the interaction of different sorghum morphotypes with different plant distribution patterns and their influence on biomass productivity and quality intended for direct combustion or cellulose ethanol.

This experiment was conducted to study the effect of two plant densities and four morphological types of sorghum on calorific value and different attributes of biomass, which are determinants in lignocellulosic ethanol production.

II. MATERIALS AND METHODS

Theexperimentswereconducted at Est. La Esperanza (35°3'29.88"S/58°50'6.13"W), located in the Cañuelas district, duringthe 2020/21 and 2021/22 growingseasons.

ThetrialsweresownbetweenNovember 15th and November 30th. In bothseasonslandpreparationwas done underconventionaltillage. At thebeginningoftheexperimentsoiltestsindicatedthefollowing: 2020/21: 4.4% organicmatter, soil pH 6.3, 83.3 ppm extractable P, 16.2 ppm N-NO3-, and soybean as a previouscrop; 2021/22: 3.7% organicmatter, soil pH 7.1, 65.4 ppm P, 20.1 ppm N-NO3-, and maizesilage a apreviouscrop. Plantingwas done using experimental machinery. A preventiveweed control wascarriedoutbyapplying 2.2 kg/ha ofatrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] + 1 L/ha of Dual Gold® (s-metolachlor) as pre-emergencetreatment. AllplantedseedsweretreatedwithConcep III®. At planting, fertilizationwas done with 100 kg/ha ofNitrocomplex Zar (21-17-3 + 1% MgO + 5% S + 0.1% Zn). In the 2020/21 season, a preventive control againstsugarcaneaphid (Melanaphissorghi) wasconductedusingSulfoxaflor+Lambdacyhalothrin (11%) formulation.

Thehybridswereselectedbasedonplantarchitecturedifferences and morphophysiologicalcharacteristics. Thematerialsusedwere: ACA785BMR® (BMR photosensitivemorphotype), Green Feed® (conventionalphotosensitivemorphotype), Sugargraze® (sweetsorghummorphotypewithlowpanicleproportion), and Argenfor195Fb (experimental hybrid, lowpanicleproportionmorphotype, drypith).

Twoplantdensitieswereevaluated: 6 plants/linear meter and 12 plants/linear meter, equivalentto 120,000 and 240,000 plants ha-1, respectively.

Experimental design in eachseasonwerearranged in randomized complete blocks withthreereplications. Eachplotconsistedonninerows, 5.2 m long and 0.5 m apart; withinninerowsdensitieswereassignedtotwo central rows and treatmentswereseparatedbytworowstominimizebordereffects.

Thecriteriafordetermininghharvest time wasadjustedtoachieveanequivalentplantdrymattercontentamongmorphotypes. Forphotosensitivemorphotypes (ACA785BMR and Green Feed), harvestwas done whentheplantsreached 0.6 m of basal chlorosis, and fortheremainingmaterials, itwas done whentheyreachedtheharddoughgrainstage (E8-2) (Torrecillas et al., 2011, Tamang et al., 2011).

Theharvestmethodologyinvolvedcuttingtwo linear meters (2m) in eachtreatment, and after separating and discardingthepaniclefraction in thosehybridsthathadit, itsweightwasrecorded. Thisweightconstitutedtheyieldofgreenmatterfromthevegetativefraction per plot. A representativesub-samplewasdried at 60ºC up toconstantweight in a forced-air oven toestimatedryweight and thentocalculatedrymattercontent (DM).

Basedonthe DM, thedrybiomassyield per unitarea (DBY) wascalculated.

Beforeeachharvest, in the central sectionoftheplant and onfiverepresentativeplants in a situationof complete competition, stalkdiameter (Diat, mm), and total soluble solids (TSS) in thejuiceweremeasuredusing a digital handheldrefractometer (ATAGO PAL-1, Atago USA). The TSS variable wasexpressed in °Brix.

Thefollowingqualitydeterminationsweremadeonthesamples:

AcidDetergentLignincontent (%) (ADL).
Hemicellulosecontent (%) (Hcel).
Cellulosecontent (%) (Cel).
Calculatedbasedontheexpressions:
Hcel = NDF-AFD
Cel = AFD-ADL
In vitro digestibilityof DM (%) (Dig).

Thedeterminationsof Neutral DetergentFiber (NDF), AcidDetergentFiber (ADF), and AcidDetergentLignin (ADL) werecarriedoutusingthefilter bag technique in an ANKOM220 incubator (ANKOM technology Corp., Fairport, NY) (Vogel et al., 1999). TheDigdeterminationswereperformedusingNIR's (NIRS Foss 6500).

High HeatingValue (HHV) (Kcal/kg) wascarriedoutonallsamples at Faq-Univ. Nac. del Litoral (Santa Fé, Argentina), following ASTM D-2382 standard.Cellulosicethanolyield (CEY) wascalculatedbasedonhemicellulose and cellulosevalues, accordingto Zhao et al. (2012).

Bothseasonswereanalyzedseparately, conducting a double ANOVA betweenhybrids and densities, and the mean comparisonswerecarriedoutusing a LeastSignificantDifference (LSD) test with a significancelevelof $p<0.05$. Pearson'sphenotypiccorrelationsamongtheevaluated variables werestudied.Forallstatisticalanalyses, INFOSTAT software wasused (Di Rienzo et al., 2020).

III. RESULTS AND DISCUSSION

3.1. Climate scenario

Table 1 shows rainfall distribution for the two seasons, compared to the 35-year historical record. The 2020/21 growing season had the highest accumulation during the November to February period (50% more than 2021/22). Additionally, the value obtained in January allowed entering the crop's critical period with an intermediate level of soil recharge, followed by a month of regular distribution (although below the historical average). The subsequent season (2021/22) showed nearly 237 mm less than the historical average, which was evidenced by difficulties in implantation and during the yield determination period (January and February).

Mean temperature, based on the monthly average of each season, fell within the normal range of historical records.Averaging between growing seasons and considering the hybrids that headed, the earliest material (Sugargraze) reached the mid-bloom stage (E6) 79 days after emergence, while Argenfor195Fb did so in 87 days. Plant height ranged from 3.84 (Green Feed) to 2.62 m (ACA785BMR). In BMRhybrid, described with a height above 2.60 m, a low lodging incidence was observed, which did not compromise evaluation of the experimental plots in both experiments.

Since both seasons were highly contrasting, significance of interactions with the environment was observed in most evaluated traits. In relation to the above and to properly discriminate the incidence of the main effects (Hybrid and density), two seasons were analyzed separately.

Table 1. Rainfall distribution (mm) and mean temperature (°C) on critical crop phase and historical 35-yr on 2020/21 and 2021/22 growing seasons

Mes	Growingseasons				Historical	
	2020/21		2021/22		(1960-1996)	
	mm	°C	mm	°C	mm	°C
Nov	134	21,3	21	20,8	123,3	19,6
Dic	25	22,6	22	22,5	101,6	22,3
Ene	121	23,9	102	24,9	88,2	23,9
Feb	63	22,9	54	22,8	122	22,7
Total	343		199		435,1	

3.2. Experiment 2020/21

Significance was not found in the Hib×Den interaction in any of the studied variables (Table 2). Significant effects of hybrids were recorded for SST, where Sugargraze and Green Feed outperformed the rest (10 and 9.9°, respectively). In this regard, we found agreement with Kanbar et al. (2021), and to some extent, the reading of degrees brix is widely accepted as a reflection of sucrose content since a positive and significant correlation between brix and sucrose has been confirmed through the evaluation of numerous genotypes (Morey et al., 2018). Considering Diat and averaging between densities, the conventional photosensitive hybrid had the lowest performance (16.05 mm), significantly surpassed by the rest of the materials. Density substantially influenced this variable, with ACA785BMR and Argenfor195Fb obtaining the highest value (23.6 mm) in the low population density setup, compared to Green Feed hybrid in high density (12.6 mm) (Table 3).

The VCS analysis indicated significant differences among hybrids but not between densities, placing Argenfor195Fb at the top of the ranking (4042 kcal/kg), differing from the other silage morphotype (3825 Kcal/kg) (Table 2). This behavior could be partially explained by the fact that this hybrid had the highest value of Cel content, which is related to calorific value (Castro et al., 2015).

Likewise, it was observed that the BMR morphotype had a similar behavior to its non-BMR counterpart, ranging between the conventional photosensitive and the silage morphotype. This aligns with findings by Souza et al. (2024), who obtained a range of values between 16 and 17.5 MJ/kg.

Table 2. Means of Total soluble solids (TSS), Hemicellulose content (Hcel), DM Digestibility (Dig), High heating value (HHV), Dry biomass yield (DBY) and Cellulosic ethanol yield (CEY), obtained on 2020/21 growing season.

Hybrid	TSS (°Brix)	Hcel (%)	Dig (%)	HHV (Kcal/kg)	DBY (tDM/ha)	CEY (L/ha)
Green Feed	9,95$^{\alpha}$ a	27,11 b	45,08 b	3941 ab	29,57 b	9928 b
ACA785BMR	4,92 b	30,43 a	57,01 a	3885 bc	27,35 b	8937 b
Argensor195Fb	6,18 b	26,49 b	39,94 c	4042 a	32,91 a	11033 a
Sugargraze	10,0 a	27,55 b	43,41 b	3825 c	26,28 b	8715 b
			ANOVA			
Hyb	*	*	*	*	*	*
Den	ns	ns	ns	ns	ns	ns
Hyb×Den	ns	ns	ns	ns	ns	ns

Means averaged across densities, means within a column followed by the same letters are not significantly different at 5% probability level according to LSD test; ANOVA: * and ns: significant and non significant at 5% probability level, respectively.

Table 3. Stalk diameter (Diat), values of two densities and four sorghum morphotypes, during 2020/21 and 2021/22 growing seasons.

Density	**Green Feed**	**ACA785BMR**	**Argenfor195Fb**	**Sugargraze**
		mm		
		2020/2021		
Alta	12,6 d	15,3 cd	15,1 cd	15,7 c
Baja	19,5 b	23,6 a	23,6 a	22,1 ab
		2021/2022		
Alta	15,9 b	16,2 b	14,4 b	14,6 b
Baja	21,9 a	23,8 a	23,2 a	22,0 a

Means within a row followed by the same letters are not significantly different at 5% probability level according to LSD test.

Significance was detected in the performance of hybrids for RBS, with Argenfor195Fb showing better performance (32.91 t MS/ha) and nearly 7 t MS/ha difference from the Sugargraze silage. This result is related to the hybrid's cycle and is in line with findings by Olson et al. (2013), who observed that greater biomass accumulation is linked to a longer vegetative period, higher leaf area index, increased interception, and greater efficiency in the use of solar radiation.

On the other hand, the ACA785BMR morphotype clearly differentiated (by over 10 percentage points) from the remaining morphotypes for the Dig variable, highlighting the advantages of the trait without showing a decrease in yield or agronomic performance due to its presence. In this sense, it was observed that this hybrid had almost 40% less LDA value than the top-ranked one (Argenfor195Fb) (Table 2). The low LDA value observed in ACA785BMR suggests that the presence of the bmr allele gives the biomass significant differences in cell wall composition, besides promoting adequate fiber digestibility. It is noteworthy that when the goal is the production of second-generation ethanol, the fact that this material combines high RBS and Dig and low LDA will result in lower energy demand for the hydrolysis process of lignocellulosic residue (Cotton et al., 2013). Regarding LDA, density also generated significant differences, although of little practical relevance, as the difference oscillated in values close to 5% (Data not shown).

Since REcel is strongly linked to RBS, the same hybrid ranking as that found for RBS was observed, with values ranging from 11000 to 8700 l/ha.

Cellulose and hemicellulose are the main polysaccharides in biomass, and their content impacts enzymatic hydrolysis yield and consequently the amount of ethanol obtained. In this experiment, the BMR morphotype significantly exceeded the rest for Hcel (30.16%), which aligns with reports by Dien et al. (2009) and Fonseca de Almeida et al. (2019). At the same time, ACA785BMR recorded the lowest value for Cel, and in this sense, these results also agree with those obtained by Fonseca de Almeida et al. (2019)."

3.2. Experiment 2021/22

SignificantHib×Den interaction for the SST reveals that some of the hybrids examined in this study responded better than others to density variation, where for example, Sugargraze obtained the highest reading (12.7°) at low density (Table 4). Likewise, both ACA785BMR and Argenfor195Fb had the lowest °Brix values, with no significant differences between densities.

Similar to the 2020/21 season, the hybrids significantly differed for Cel, and again Argenfor195Fb achieved the highest value. Considering Diat, a similar trend was found to the previous season, whereby the low density in the photosensitive BMR morphotype obtained a value nearly 10 mm higher than Argenfor195FB at high density. In experiments conducted at an equivalent density (116,000 plants/ha), Suwarti et al. (2018) obtained analogous results regarding the pattern of stem diameter variation and magnitude, although their relation with plant height differs from that found in the present study (Data not shown).

The differences found between hybrids for Hcel again highlight the superiority of the BMR trait and the better performance of ACA785BMR (Table 5).

Averaging between densities, Argenfor195Fb and the conventional photosensitive morphotype significantly differed from the rest for VCS. In this sense, the values found in this experiment for a morphotype similar to Green Feed are lower than those reported by Castro et al. 2015.

Table 4. Total soluble solids (TSS), values of two densities and four sorghum morphotypes, during 2021/22 growing season.

Densidad	Green Feed	ACA785BMR	Argenfor195Fb	Sugargraze
		°Brix		
Alta	5,33 cd	4,70 d	4,53 d	7,57 b
Baja	6,73 bc	4,30 d	4,93 cd	12,70 a

Means within a row followed by the same letters are not significantly different at 5% probability level according to LSD test.

Similar to the 2020/21 season, only the hybrid effect was significant for RBS, where Sugargraze achieved the highest yield (25.12 t MS/ha), which, due to environmental constraints, represented nearly 7 t MS/ha less than that season.

In this season, only the hybrid effect was detected for LDA, highlighting Argenfor195Fb behavior and confirming that as the lignin/holocellulose ratio increases, so does the VCS (Hodgson et al., 2010, Maksimuk et al., 2021).When considering Dig, the same value pattern as the previous season was reiterated, with the BMR morphotype standing out with a difference of almost 17 percentage points compared to the Argenfor195Fb hybrid (Table 5). In this sense, it is well established the superiority of the BMR trait in terms of MS digestibility compared to its non-BMR counterpart (Oliver et al., 2005, Yerka et al., 2015).

Table 5. Means of cellulose content (Cel), hemicellulose (Hcel), acid detergent lignin (ADL), DM digestibility (Dig), high heating value (HHV), dry biomass yield (DBY) and Cellulose ethanol yield (CEY) during 2021/22 growing season.

Hybrid	Cel (%)	Hcel (%)	ADL (%)	Dig (%)	HHV (Kcal/kg)	DBY (tMS/ha)	CEY (L/ha)
Green Feed	36,55 b	26,73 b	5,72 b	43,50 b	3936 ab	15,52 c	5079 c
ACA785BMR	32,63 c	30,16 a	3,98 c	57,48 a	3814 b	21,36 b	6947 b
Argensor195Fb	38,11 a	26,82 b	6,52 a	40,64 c	4035 a	19,89 b	6688 b
Sugargraze	36,31 b	27,01 b	5,33 b	44,83 b	3847 b	25,12 a	8232 a
ANOVA							
Hyb	*	*	*	*	*	*	*
Den	ns	ns	ns	ns	ns	ns	ns
Hyb×Den	ns	ns	ns	ns	ns	ns	ns

Means averaged across densities, means within a column followed by the same letters are not significantly different at 5% probability level according to LSD test; ANOVA: * and ns: significant and non significant at 5% probability level, respectively.

Phenotypic correlations between the variables are expressed in Table 6. Understanding correlation estimates allowed the evaluation of the degree of association between two traits. These values show positive or negative influence between the variables, facilitating the prediction of other behaviors.

The highest values of significant and positive correlation were observed between RBS×REcel (0.98), Dig×Hcel (0.78), and LDA×Cel (0.73).

In both seasons, no significant association was found between SST and Diat, which contradicts reports by Tang et al. 2018, although the latter used morphotypes with a greater aptitude for sugar accumulation in the stem. Conversely, a significant and positive association was observed between VCS and Cel and VCS and LDA, which is entirely consistent with observations by Maksimuk et al. 2021.

The variation in plant density within the range studied did not affect biomass yield, higher calorific value, or theoretical yield of cellulose ethanol. However, it was observed to promote morphological changes such as stem diameter, similar to that reported by Snider et al. 2012. Along the same lines, May et al. 2016, evaluating a conventional photosensitive hybrid in a range of 80,000 to 140,000 plants/ha, also did not verify the influence of population density on biomass yield.

Based on the results obtained, the plant density used in this study, defined as low (120,000 plants/ha), could be recommended for the objectives and morphotypes evaluated. In fact, the cost of sorghum seed has significantly increased in recent years, so establishing an optimal plant stand helps to optimize costs in this energy matrix. In both seasons, highest significant values for cellulose ethanol (REcel), dry biomass yield (RBS), and higher calorific value (VCS) corresponded to Sugargraze and Argensor 195Fb (non-photosensitive morphotypes). Although the presence of the BMR trait determined significantly higher digestibility values, it did not impact the energy productivity. It is worth noting that the four morphotypes studied here represent a wide range of hybrids present in the market, and the significant differences between morphotypes are of sufficient relevance for the selection of more suitable genotypes for energy conversion. Those morphotypes with high biomass yield are predominantly composed of structural carbohydrates (hemicellulose, cellulose, and lignin), therefore, such biomass can be used for combustion through pyrolysis but also for conversion to second-generation ethanol.

Table 6. Estimates of the Pearson phenotype correlation coefficients between the traits: SST,Cel, Hcel, ADL, Dig, Diat, DBY, HHVand CEY.

Trait	SST	Cel	Hcel	ADL	Dig	Diat	DBY	CEY
Cel	0,32*							
Hcel	-0,28	-0,85*						
ADL	0,08	0,73*	-0,63*					
Dig	-0,31*	-0,89*	0,78*	-0,88*				
Diat	0,04	-0,10	0,05	-0,08	0,13			
DBY	0,27	0,14	0,03	0,01	-0,10	-0,06		
CEY	0,29	0,18	0,02	0,03	-0,13	-0,07	0,98*	
HHV	-0,14	0,46 *	-0,33*	0,44*	-0,37*	0 01	0,05	0,07

* significant at 5% probability level.

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