

Ethanol from maize hybrids in Brazil

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Abstract: *The aim was to investigate the differences between maize hybrids for their agronomic yield and their agro-energy potential. The study was conducted in Palotina and Assis Chateaubriand, Paraná, Brazil. The treatments were composed of 10 maize hybrids (DKB290 [non-GMO], DKB315, DKB340, DKB290, Formula, Defender, Status, 2B810, 30F53, BG7330), which were evaluated for grain moisture and yield, starch content, total soluble solids content, ethanol content in the juice and ethanol yield. The important factors for the result are related to grain yield and the quality of the harvested grains, and agronomic performance of these hybrids may differ according to each crop year. Hybrid 30F53 stood out positively for all variables. Hybrid DKB315 showed the highest ethanol yield and high grain yield, and insignificant grain quality was not enough to result in a decline in ethanol yield.*

Keywords: *Zea mays L., genotype, biofuel, grain yield, ethanol yield*

INTRODUCTION

Biofuels emerged as an alternative to minimize environmental impacts, and to meet new energy demands, reducing the import and production of fossil fuels, in addition to stimulating agricultural growth and providing better economic conditions for the agro-energy business (Sharma et al. 2020). Adequate understanding of the energy matrix requires knowledge of the different energy resources available that can be used in the current technological scenario. For each natural resource, major aspects and characteristics related to the energy industry are emphasized, adapting the resource for better use in the energy sector (Eckert et al. 2018, Correa et al. 2019).

When analyzing marketing aspects, biofuel production has increased, especially in the United States, Brazil, and the European Union (Condon et al. 2015). The improvement and study of raw materials for ethanol production is essential for the consolidation of this biofuel in the world energy matrix, in such a way that the main inputs for production are of saccharine and starchy source. Obtaining ethanol from starchy crops is not widespread in Brazil. However, the United States stands out for fermenting maize, with more than 90% ethanol production coming from this crop (Eckert et al. 2018), which has been used in this country since the mid-1900s (Bothast and Schlicher 2005).

Maize has favorable production characteristics, with high yield per planted area; in addition, its harvest is fully mechanized, and production and post-



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harvest technology is already consolidated in Brazil. Only industrial aspects related to ethanol production make the final product more expensive, so that, if there is the introduction of starchy ethanol, other starchy sources can be used, not limiting the processing (Ferragi and Naas 2015, Eckert et al. 2018). Ethanol production from maize requires the fragmentation of the starchy material. Dry milling is the most applied method in industry, in which maize is ground without water, generating maize flour, which is later mixed with water to form a solution. After solubilization, enzymes are introduced to convert the material into sugars, and then they are fermented by yeasts, forming ethanol (Wood et al. 2014, Szambelan et al. 2020).

Murthy et al. (2009) obtained ethanol concentration between 12.7 and 13.7%, in an analysis of 11 maize hybrids for ethanol production, with dry milling as a way of fractionating the material. The maize hybrid, the enzymes and yeasts used, in addition to the fermentation conditions, affect the final ethanol yield, and the starch content in the grains is not necessarily correlated with ethanol yield. The Brazilian scientific production related to maize ethanol is basically limited to market comparisons and net energy consumption between maize and various raw materials, not considering the capacity to produce ethanol. In addition, the use of maize in the Brazilian ethanol production chain will meet the demand for raw materials in the sugarcane off-season. Therefore, it is necessary to elucidate the potential for ethanol production from maize, as well as identifying possible differences between genetic materials and production environments. The aim of the present study was to investigate the differences between maize hybrids for their agronomic yield and their agro-energy potential.

MATERIAL AND METHODS

Location Description and Experimental Design

The study was conducted in two municipalities: Palotina, state of Paraná (PR), Brazil (lat 24° 20' 42.77" S, long 53° 51' 35.95" W, alt 305 m asl) and Assis Chateaubriand, PR, Brazil (lat 24° 16' 9.69" S, long 53° 39' 38.37" W, alt 406 m asl), in the second growing season of the 2014-2015 agricultural year, referring to the period from February to June 2015. According to the Köppen-Geiger classification, the climate of the region is Cfa, a warm temperate climate, with no defined dry season. and with hot summer. Weather conditions during the study period are illustrated in Figure 1. Soil of the experimental areas was classified as very clayey, with 7.8% sand, 10.6% silt and 81.6% clay in Palotina, 10% sand, 25% silt and 65% clay in Assis Chateaubriand.

On February 13, 2015, maize hybrids were directly sown in rows spaced 0.45 m apart. Plots were composed of six rows with 5 m in length. The usable area for evaluations was composed of the two central rows with 2 m in length. This was a randomized block design with four replications; the treatments were composed of 10 maize hybrids (Table 1). All management and phytosanitary treatments were carried out for the complete development of maize plants, with the harvest on July 14, 2015. The usable area of each plot was collected, and grains were packed in paper bags for further analysis.

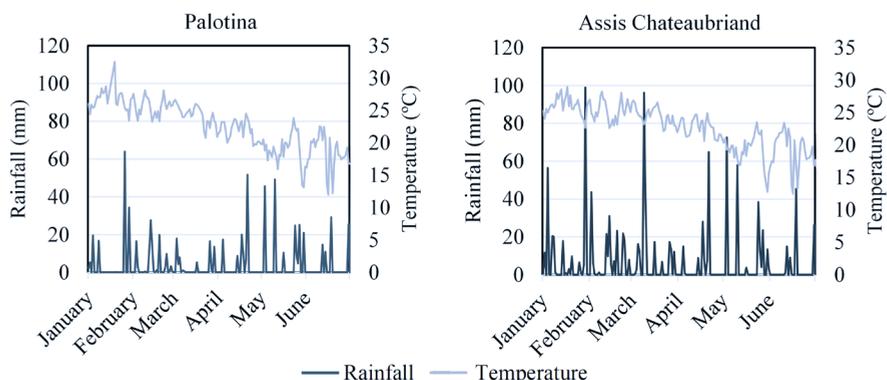


Figure 1. Daily rainfall and average temperature, between January 1st and June 30th, 2015, Palotina and Assis Chateaubriand, PR, Brazil.

Evaluations

Grain moisture and yield

Grain moisture was determined by the method of oven at 105 ± 3 °C (Brasil 2009), and grain samples with 50 g were analyzed in triplicate for each field plot. The containers used were oven heated at 105 °C for 30 min to eliminate any trace of moisture, then cooled in a desiccator. The first measurement was performed on an analytical balance with the container taken from the desiccator, and the value was measured (t) for further calculations. Whole grains were added to the container and weighed again, recording the resulting value (P).

After weighing, the containers were placed in an oven at 105 °C, and the drying time was counted only after the temperature was reached. Samples were kept in an oven for 24 h. After drying, the containers with the samples were taken from the oven, cooled in a desiccator, and later weighed, and their values were recorded (p). Moisture percentage calculations used Eq. 1:

$$\text{Moisture (\%)} = \frac{100(P - p)}{p - t} \quad \text{Equation 1}$$

Where:

P = initial weight, container weight plus wet grain weight;

p = final weight, container weight plus dry grain weight;

t = container weight.

Yield was estimated from the weight of grains in the usable area of each plot. Grain moisture content was determined as mentioned above, corrected to 13%, with results expressed in kg ha^{-1} .

Starch content

The analysis was performed according to an adapted methodology of the Instituto Adolfo Lutz (2008), in which 5 g ground sample was weighed in a porcelain crucible and subsequently treated with three 20 mL portions of ether. After stirring and decanting, the material was transferred to a 500 mL beaker, with 100 mL 70% alcohol, stirred and heated in a water bath at 83-87 °C for 1 h. After cooling, 50 mL of 95% alcohol were added and filtered through a dry filter. The residue was washed with 500 mL of 70% alcohol, and the washing solutions were combined and filtered.

The residue and the filter paper were transferred to a 500 mL flask, with 150 mL of water, and added with 5 drops of 10% sodium hydroxide solution. The mixture was heated in an autoclave at 1 atmosphere for 1 h, and after cooling, 5 mL of hydrochloric acid were added. It was heated again in an autoclave for 30 min and then neutralized with 10% sodium hydroxide solution. The material was transferred to a 500 mL volumetric flask and completed with distilled water. The material was stirred and dry filtered, and according to Eq. 2, the starch content in the material was determined.

$$\frac{100 \times A \times 0.9}{P \times V} = \%(m/m) \quad \text{Equation 2}$$

Where:

A : volume in mL of sample P g solution;

P : number of grams in the sample;

V : volume in mL used for titration.

Ethanol

Analyses were performed in triplicate of each sample, subjected to dry hammer milling and sieving through a 1.0

Table 1. Description of maize hybrids used

Hybrid	GMO technology	Grain type
DKB290	Non-GMO	Semi-dented
DKB315	YieldGard™ VT Pro™ (PRO)	Hard
DKB340	YieldGard™ VT Pro™2 (PRO2)	Semi-hard
DKB290	YieldGard™ VT Pro™3 (PRO3)	Semi-dented
Formula	Agrisure™ CB/LL (TL)	Semi-hard
Defender	Agrisure™ Viptera (VIP)	Hard
Status	Agrisure™ Viptera™ 3110 (VIP3)	Hard
2B810	Power Core™ (PW)	Semi-hard
30F53	Optimum™ Intrasect (YHR)	Semi-hard
BG7330	Herculex™ I (HX)	Semi-hard

GMO: genetically modified organisms

mm mesh (Murthy et al. 2009). After milling, 50 g of the material was suspended in water to a 25% (w/w) concentration solution of starchy material.

Hydrolysis

The method for enzymatic hydrolysis of maize starch was based on and adapted from Murthy et al. (2009). Maize solutions with water were heated in a metabolic water bath in a Dubnoff orbital shaker at 80 °C for gelatinization, then the sample pH was adjusted to 5.5. After pH adjustment, 0.28 mL of Termamyl 2X alpha-amylase enzyme (Novozymes®) was added at 90 °C for 90 min under stirring.

Saccharification

To complete the starch cleavage, with saccharification, starch was cooled to 60 °C with pH adjusted to 4.2 (using 1.0 N sulfuric acid), added with 0.56 mL glucoamylase enzyme AMG 300L (Novozymes®) and urea (1 g L⁻¹ juice) under stirring for 90 min.

Total soluble solids content

The determination of the amount of available solute in the medium is possible once the refractive index of the aqueous solution is known. This property is used to determine the concentration of soluble solids in aqueous solutions, and these solids are mostly free sugars. The refractive index for a pure substance is constant, under constant conditions of temperature and pressure, and can be used to identify it. The presence of water-soluble solids results in a change in the refractive index. Total soluble solids content was measured before and after fermentation. First, it was verified how much glucose was released in the aqueous solution with the enzymatic activity, being measured as initial soluble solids; later, with fermentation, the yeasts would have, hypothetically, converted most of the glucose, and the soluble solids content would tend to be lower than the initial amount, being designated as final soluble solids.

The analysis was performed with a digital refractometer, which after calibration with water has the values in °Brix for the analyzed samples. In case of temperature changes (above or below 20 °C), corrections were made in the final values according to the tabulations of Instituto Adolfo Lutz (2008).

Fermentation

After hydrolysis and saccharification, the material was fermented with the yeast *Saccharomyces cerevisiae*, which is resistant to high concentrations of alcohol at a concentration of 3% (m: v). The process was carried out at 30 °C for 72 h in a fermentation reactor, keeping the solution under stirring (Murthy et al. 2009).

Ethanol content in juice

The alcohol content was evaluated using the ebulliometric method, with an ebulliometer, which checks the increase in the boiling temperature of the solvent when a non-volatile solute is dissolved in it (Carvalho et al. 2008). First, the ebulliometer test was performed with water only. Water was added to the condenser attached to the ebulliometer and to the reservoir under it; the instrument was heated to boiling. The temperature was recorded as a calibration for the subsequent analysis of the alcohol content.

For final analysis, the fermented juice was added in the condenser and in the reservoir; the temperature at which the sample boiled was observed and, together with the calibrated ruler, the alcoholic percentage of the mixture was determined.

Statistical analysis

Data were tested by analysis of variance (ANOVA) using the F-test ($p \leq 0.05$). The treatment means were compared by Tukey's test, at 5% level. Analyses were run in Sisvar 5.6 software (Ferreira 2011).

RESULTS AND DISCUSSION

The hybrid with the highest moisture content in the grains was BG7330H in Palotina, with 33.96% moisture, without differing from hybrids Status, 2B810 and 30F53. Regarding the lower moisture content, this was observed for the Formula

hybrid, with 21.92%. For Assis Chateaubriand, no differences were detected between hybrids (Figure 2A). The moisture present in the grains was a result of the high rainfall, which delayed the harvest, also affecting the amount of material harvested, as loss from lodging was observed.

In Assis Chateaubriand, the 30F53 hybrid had higher yield compared to hybrids DKB290, DKB315 and Formula, but without differing from the others. In Palotina, hybrids DKB315 and 2B810 were superior to all the others (Figure 2B). Differences between locations are due to soil properties (Freddi et al. 2006, Nunes et al. 2018), nutritional requirements of plants (Lázaro et al. 2013), and water availability, mainly from tasseling until the beginning of grain filling, with an average of 10 days without rainfall in the period (Bergamaschi et al. 2006, Andrea et al. 2018)

For Assis Chateaubriand, hybrid Status had 69.4% starch in its composition, while hybrid DKB290 had the lowest percentage of starch, 62.4%. In Palotina, hybrid 30F53 had the highest starch content, 67.85%, and the hybrid with the lowest starch content was DKB290, with 60.77% (Figure 3A). Water stress at the R₂ stage of grains affects photosynthesis and the formation of starch granules, causing losses in the final production of starch (Luz et al. 2014, Liu et al. 2020). The R₂ stage occurred between the second half of March and the first half of April, when there was a drop in rainfall, with an average rainfall of 70 mm for the experimental fields, but which was not accentuated to the point of generating large starch losses in all hybrids. Presence of rot grains in some samples was also observed, as moisture above 20%

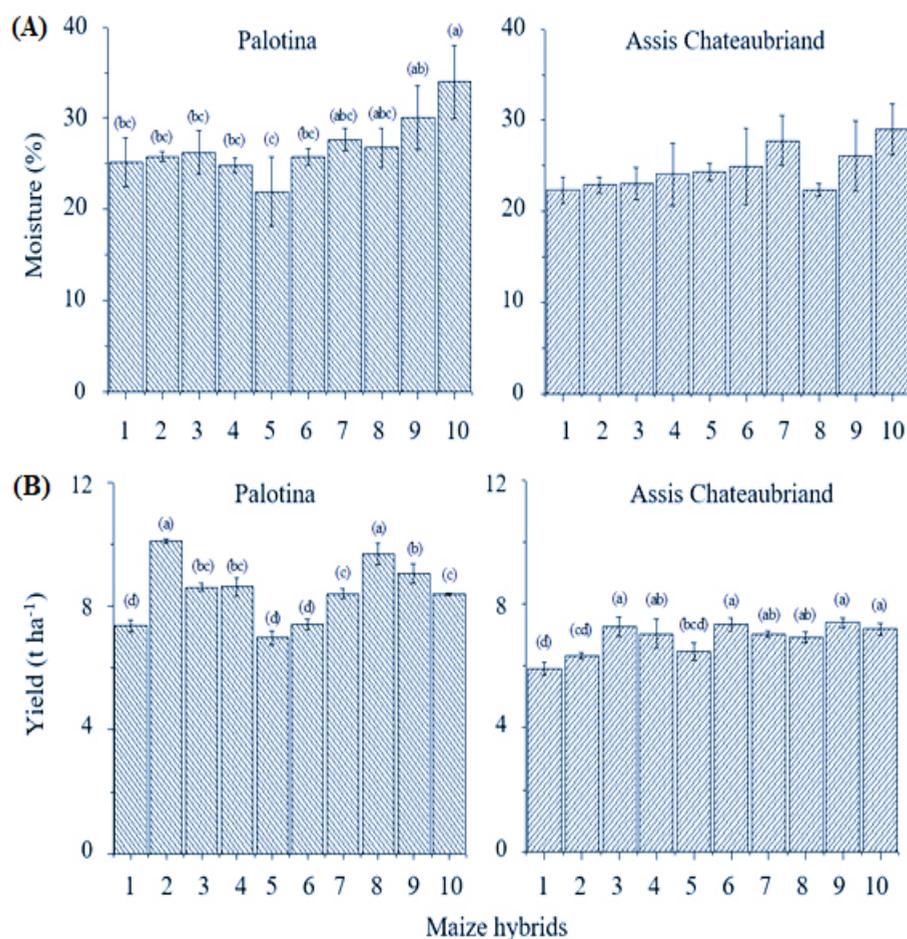


Figure 2. Grain moisture **(A)** and grain yield **(B)** of maize hybrids (1: DKB290 [non-GMO], 2: DKB315, 3: DKB340, 4: DKB290, 5: Formula, 6: Defender, 7: Status, 8: 2B810, 9: 30F53, 10: BG7330). The upper whiskers on the bars characterize the sample standard deviations. Bars with the same letter do not differ from each other by Tukey's (1949) test, at 5% level. Bars without letters do not differ from each other by F-test, at 5% level.

promoted ear rot and the development of fungi in the grains, and a consequent drop in the starch content. Excessive rainfall can result in lower starch content (Fejér et al. 2022).

ANOVA did not indicate a significant effect ($P > 0.05$) on the initial soluble solids content, in any of the two locations. An average of 8.8 °Brix was observed for the hybrids in Palotina, and 10 °Brix in Assis Chateaubriand (Figure 3B). After 72 h of fermentation, the final soluble solids content was measured, that is, the amount of simple sugars that were converted by yeasts in an anaerobic medium. Only in Assis Chateaubriand there was a difference between the hybrids, in which hybrid 30F53 YH showed lower °Brix after fermentation (2.03); the hybrid with the lowest sugar consumption was Defender, with 3.28 °Brix (Figure 4A). The higher the °Brix, the lower the consumption of sugars from the medium; consequently, the lower the ethanol production (Santos et al. 2018, Kavya et al. 2020). However, the values only consider the final value obtained after fermentation, and not the consumption between the initial and final °Brix in each hybrid, because when this difference is taken into account, other hybrids show higher and lower consumption of sugars. The hybrids with higher consumption of simple sugars were: DKB290, for Assis Chateaubriand, and 30F53, for Palotina; as for lower sugar consumption, hybrid 2B810, for Assis Chateaubriand, and DKB340, for Palotina.

The ethanol content in the juice was evaluated by ebulliometry; ANOVA evidenced no significant effect ($p > 0.05$) of the hybrids, in both locations. An average of 5.21% ethanol content was observed for the hybrids in Palotina, and 6.29% in Assis Chateaubriand (Figure 4B). The content of ethanol present in the fermented juice is usually between 7% and 10%

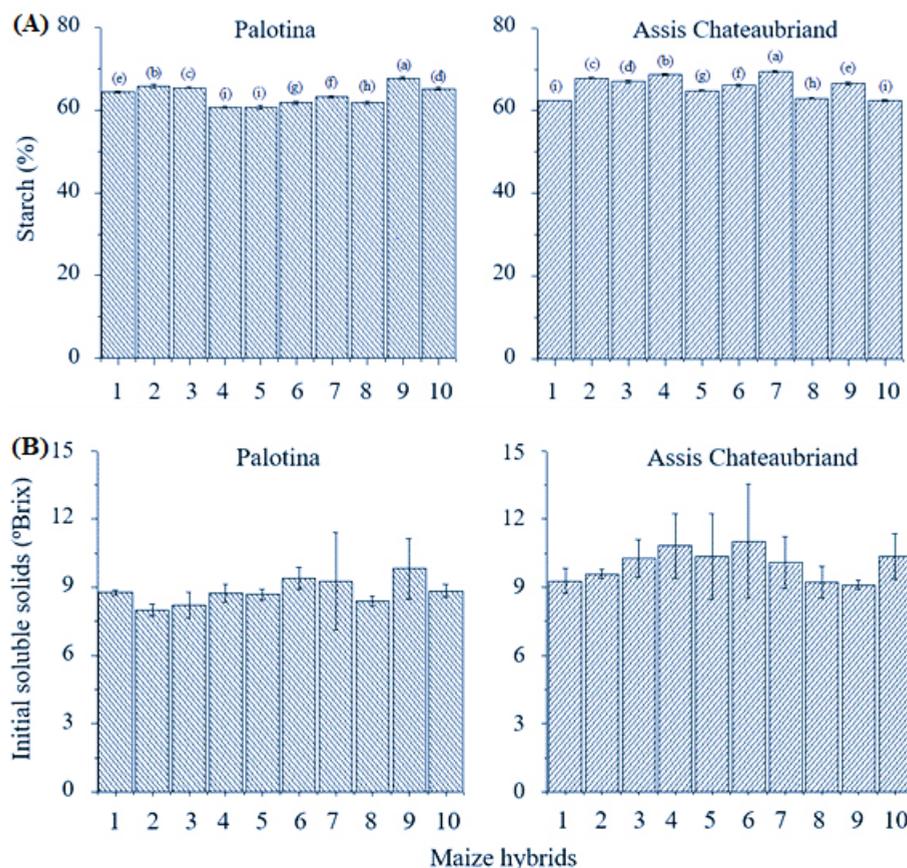


Figure 3. Starch (A) and initial soluble solids (B) of maize hybrids (1: DKB290 [non-GMO], KB290, 2: DKB315, 3: DKB340, 4: DKB290, 5: Formula, 6: Defender, 7: Status, 8: 2B810, 9: 30F53, 10: BG7330). The upper whiskers on the bars characterize the sample standard deviations. Bars with the same letter do not differ from each other by Tukey's (1949) test, at 5% level. Bars without letters do not differ from each other by F-test, at 5% level.

(EMBRAPA 2015). The observed content can be explained by the amount of inoculum, insufficient for the conversion of available sugars into ethanol, as the percentage adopted here was 3% (m: v) of *S. cerevisiae* (Baptista et al. 2013), and 10% inoculum cells is industrially used for fermentation (Gallardo et al. 2011). The presence of contaminants during

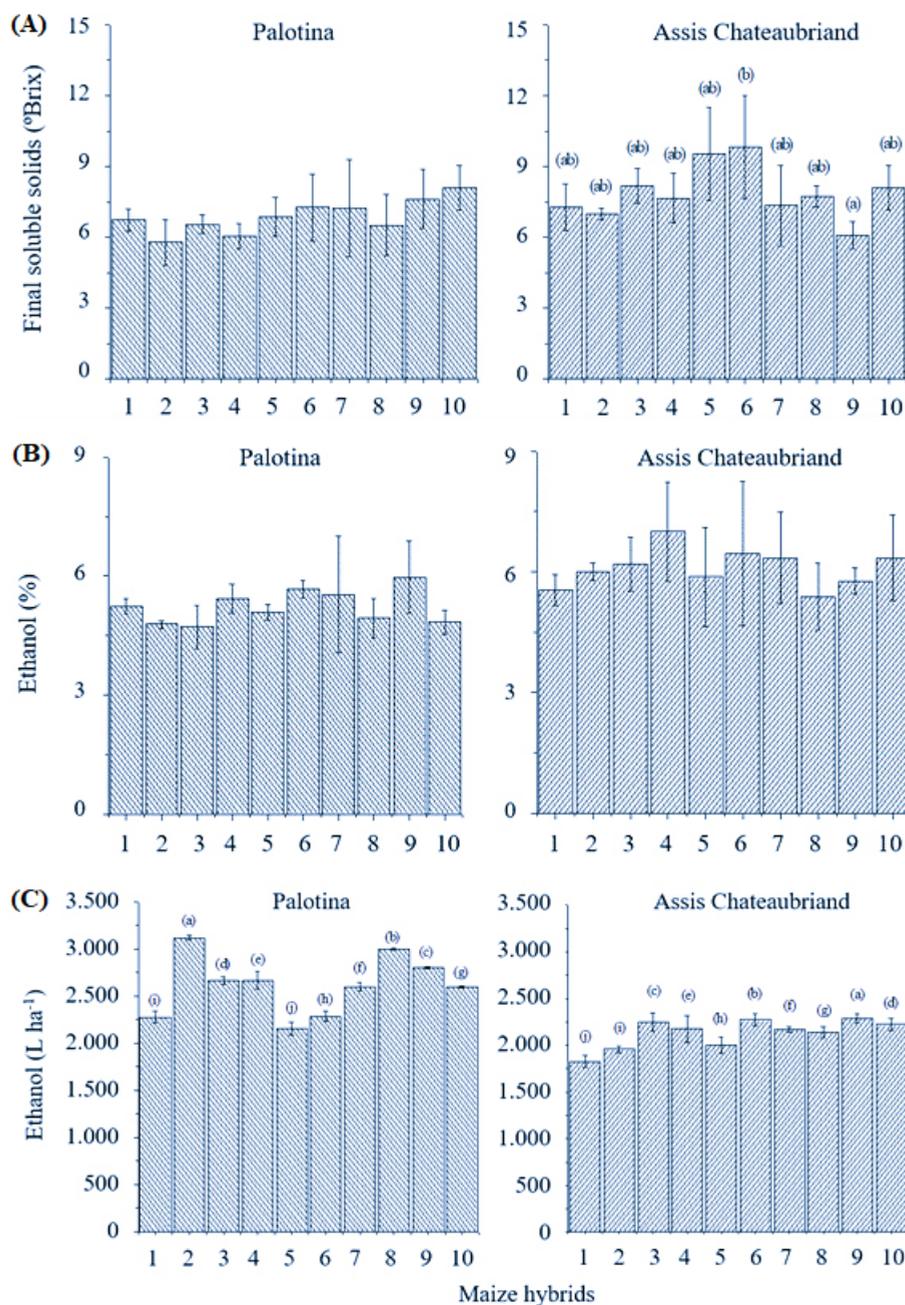


Figure 4. Final soluble solids (A), ethanol in juice (B), and ethanol yield (C) of maize hybrids (1: DKB290 [non-GMO], 2: DKB315, 3: DKB340, 4: DKB290, 5: Formula, 6: Defender, 7: Status, 8: 2B810, 9: 30F53, 10: BG7330). The upper whiskers on the bars characterize the sample standard deviations. Bars with the same letter do not differ from each other by Tukey's (1949) test, at 5% level. Bars without letters do not differ from each other by F-test, at 5% level.

fermentation can result in damage to the inoculum, which influences the yield. When contamination occurs, sucrose degradation and acid production are high, generating yeast intoxication (Brexó and Sant'Ana 2017). The ethanol content may have been altered by some contamination prior to the fermentation process, coming from the experimental fields, and may not have been accounted for at the end of the procedure.

In Assis Chateaubriand, the 30F53 hybrid had the highest ethanol yield (2,287 L ha⁻¹), and the hybrid with the lowest conversion to ethanol was DKB290 (non-GMO), with 1,827 L ha⁻¹. In Palotina, hybrid DKB315 showed 3,121 L ha⁻¹, and the hybrid that produced the least was Formula, with 2,158 L ethanol ha⁻¹ (Figure 4C). The yields in terms of L ethanol ha⁻¹ emphasize that the biomass yield ratio (kg ha⁻¹) was correlated in more than 99% for the two experimental fields. When comparing the yields in kg grains ha⁻¹ and L ethanol ha⁻¹, the same hybrids stood out, for both higher and lower production, indicating that the aspects of biomass quantity are important for biofuel yield. Increases in maize yield allow for greater ethanol production, while minimizing the use of new land for maize production (Mussato et al. 2010, Brown et al. 2014, Eckert et al. 2018, Souza-Abud and Silva 2019, Moreira et al. 2020).

Maize has great potential for ethanol production due to its biological characteristics that can substantially contribute to the demands of biofuels, especially in the sugarcane off-season. The importance of introducing this crop, already used for energy production in other countries, has been demonstrated, which can offer high resilience about fuel stocks in the off-season, minimizing product price variations, as well as generating stability in the ethanol market. The results obtained experimentally demonstrate that the study of more sites of implementation, as well as new hybrids, different sowing times, technologies, and managements, is indispensable for future works, to define specific hybrids, according to the soil and climate characteristics of each Brazilian region suitable to produce maize destined for the biofuel industry.

CONCLUSION

The factors important for the result are related to grain yield and the quality of the harvested grains, and agronomic performance of these hybrids may differ according to each crop year. Hybrid 30F53 stood out positively for all variables. Hybrid DKB315 showed the highest ethanol yield and high grain yield, and insignificant grain quality was not enough to result in a decline in ethanol yield.

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