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Research Article

# Path analysis in the evaluation of ethanol and culm yield components in sweet sorghum[1]

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## ABSTRACT

Path analysis is a methodology able of splitting the correlation coefficients into direct and indirect effects, helping the breeder in the study of quantitative traits. This study aimed to use path analysis to quantify such effects on the yield of theoretical ethanol, exerted by the variables total and culm dry matter yield, soluble solids content at harvest and efficiency of broth extraction, and on culm yield, by the agronomic variables average plant height at harvest, number of days until flowering, green matter yield and percentage of culms in the green matter yield. The soluble solids content variable presented the greatest total (0.8844) and direct (0.5969) effects on the yield of theoretical ethanol, indicating a great dependence of the main variable by the explanatory variable. The largest indirect effect was via culm yield (0.2820). The four variables considered as dependent (average plant height, number of days until flowering, percentage of culms in the yield of green matter and green matter yield) showed positive values for the total effect on culm yield. The largest direct effect (0.8010) was exerted by the green matter yield.

KEYWORDS: *Sorghum bicolor* L., biofuels, genotypic correlation.

## RESUMO

Análise de trilha na avaliação de componentes de produção de etanol e de colmos em sorgo sacarino

Análise de trilha é uma metodologia capaz de desdobrar os coeficientes de correlação em efeitos diretos e indiretos, auxiliando o melhorista no estudo de caracteres quantitativos. Objetivou-se utilizar a metodologia para quantificar esses efeitos na produção de etanol teórico, exercidos pelas variáveis produção de matéria seca total e de colmo, sólidos solúveis na colheita e eficiência de extração de caldo, e na produção de colmo, pelas variáveis agronômicas altura média das plantas na colheita, número de dias até o florescimento, produção de biomassa verde e porcentagem de colmos na produção de biomassa verde. A variável sólidos solúveis apresentou os maiores efeitos total (0,8844) e direto (0,5969) sobre a produção teórica de etanol, indicando grande dependência da variável principal pela explicativa. O maior efeito indireto foi via produção de colmos (0,2820). As quatro variáveis consideradas como dependentes (altura média das plantas, número de dias até o florescimento, porcentagem de colmos na produção de biomassa verde e produção de biomassa verde) apresentaram valores positivos de efeito total sobre a produção de colmo. O maior efeito direto (0,8010) foi exercido pela produção de biomassa verde.

PALVRAS-CHAVE: *Sorghum bicolor* L., biocombustíveis, correlação genotípica.

## INTRODUCTION

Brazil currently stands out in the ethanol production. In 2018, 35.3 million $m^3$ of biofuel were produced by the Brazilian agribusiness (Brasil 2020). Almost all was produced from sugarcane; however, recent studies have highlighted sweet sorghum as a potential species to be cultivated in the off-season of sugarcane for biofuel production (Masson et al. 2015, Kavya et al. 2020).

Sorghum (*Sorghum bicolor* L.) is cultivated worldwide and nationally, mostly for grain yield (Prasad & Sridhar 2020). The genotypes that fit into the saccharine group combine high biomass yield and high content of fermentable sugars in their culms. Other important features are the short cycle, fully mechanizable production and good performance when cultivated under conditions of high temperatures and low water availability. It is also responsive to crop treatments (Kavya et al. 2020).

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The similarity between sweet sorghum and sugarcane (*Saccharum* spp.), regarding the way of storing simple sugars in the culms, makes their broth extraction processes analogous (Biondo et al. 2019). So, sweet sorghum is a great alternative for ethanol production in the off-season of sugarcane.

In areas planted with the objective of producing ethanol, the final yield is calculated from the volume of produced biofuel. For sweet sorghum, the ethanol comes from the broth present in the culms. Both the ethanol yield per hectare and culms yield are quantitative variables, influenced by a large group of agronomic and technological variables (Martins et al. 2017).

Path analysis is a methodology proposed by Wright (1921), that is capable of jointly analyzing a group of variables in which one is considered to be the main one and the others to be explanatory, being the result of the analysis the quantification of the direct and indirect effects of the latter on the principal. The application of such methodology becomes relevant in the genetic breeding initial stages of a crop to be explored for new purposes. For a better understanding of the complex relationship between the sorghum different variables and the ethanol final production, the path analysis is applicable and offers results with relevant information (Kavya et al. 2020).

Sometimes, as a result of performing a path analysis, it is observed that the characteristics evaluated are strongly correlated. This phenomenon is called multicollinearity, and causes a bias in the analysis results. From the detection and estimation of the degree of multicollinearity, the researcher must assess the need to apply an adequate methodology to mitigate such adverse effect (Olivoto et al. 2017, Zuffo et al. 2020).

Thus, this study aimed to estimate the magnitude of direct and indirect effects of the main technological and agronomic variables on ethanol and culm yield components in sweet sorghum using path analysis.

## MATERIAL AND METHODS

For this study, sweet sorghum genotypes were evaluated for ethanol production, in 2014. The trial was conducted at the experimental field of the Instituto Agronômico de Pernambuco, in Vitória de Santo Antão, Pernambuco state, Brazil (08º07’05”S, 35º17’29”W and altitude of 156 m). The soil chemical characterization, classified as Haplic Podzol (FAO 2014), and the the meteorological data during the period of the experiment are shown, respectively, in Tables 1 and 2.

A total of 65 progenies F6, from the crossing of IPA 467-4-2 (male parent) and IPA 2502 (female parental) varieties, were evaluated, totaling 80 genotypes.

The experiment was carried out in randomized blocks, with three replications (80 x 3), totaling 240 experimental plots. Each plot had 6 rows and a total area of 4.80 m$^2$ (6.0 x 0.8 m). The two lateral rows were considered as borders, totaling 3.20 m$^2$ (4 x 0.80 m) of useful area per plot.

Table 1. Soil analysis performed in the area 1 (block 1) and 2 (blocks 2 and 3).

| Area | P | pH | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $Al^{+3}$ | $H^{+}$ | S | CEC |
|---|---|---|---|---|---|---|---|---|---|---|
| | mg dm$^{-3}$ | $H_2O$ | $cmol_c$ dm$^{-3}$ | | | | | | | |
| 1 | 80 | 7.20 | 2.55 | 1.45 | 1.00 | 0.42 | 0.00 | 0.74 | 5.4 | 6.2 |
| 2 | 26 | 6.50 | 2.55 | 1.05 | 0.14 | 0.27 | 0.00 | 1.56 | 3.7 | 5.3 |

Table 2. Meteorological data from the Instituto Agronômico de Pernambuco experimental station, in Vitória do Santo Antão, Pernambuco state, Brazil, from February to June 2014.

| Data | Months | | | | | Total |
|---|---|---|---|---|---|---|
| | February | March | April | May | June | |
| Rainfall (mm) | 88.4 | 84.2 | 78.6 | 110.2 | 120.6 | 482.20 |
| Average maximum air temperature (ºC) | 37.3 | 33.9 | 35.4 | 32.1 | 31.1 | - |
| Average minimum air temperature (ºC) | 21.7 | 22.3 | 22.6 | 22.2 | 21.1 | - |
| Relative humidity (%) | 72.0 | 72.0 | 70.0 | 75.0 | 71.0 | - |

The sowing was carried out with 20-30 seeds $m^{-1}$, and thinning was performed at 15 days after planting, keeping 12 plants $m^{-1}$.

For irrigation, a microsprinkler system was used, and, to prevent pest attacks, the following pesticides were applied: Decis (10 mL diluted in 20 L of water) at 40 days after planting, and ant killer MIREX-S at 15 days before and 15 days after planting.

The evaluated variables were: average plant height at harvest (cm); yield of culms (t $ha^{-1}$); total dry matter yield, obtained by weighing the sample of five plants per dry plot in a forced air circulation oven at 65 ºC, for 72 hours, with value expressed as t $ha^{-1}$; broth extraction efficiency, obtained by weighing the broth extracted from the culm of five plants in each plot, using an electric mill, with value expressed as %; number of days until flowering; total green matter biomass yield, obtained by weighing the fresh sample of five plants per plot, with value expressed as t $ha^{-1}$; percentage of culms in the total green matter yield, obtained by PC = (SCW/FSW) x 100, where: SCW is the weight (g) of the sample with culms and FSW the weight (g) of the fresh sample; percentage of total dry matter, obtained by PDM = (DSW/FSW) x 100, where: DSW is the weight (g) of the dry sample and FSW the weight (g) of the fresh sample; soluble solids content at harvest, estimated by a portable refractometer, with result expressed as ºBrix; yield of the theoretical ethanol, adapted from Vasilakoglou et al. (2011): YTE = ART x SWE x 6.5 x 0.85 x (1.0/0.79), where: ART is the total sugar content (%), 6.5 the conversion factor of ethanol from sugar, 0.85 the efficiency in the fermentation process and (1.0/0.79) the specific gravity of the alcohol.

In order to carry out the present study, the results of the aforementioned experiment were used, in which the presented correlation coefficients between the technological variables and evaluated agronomic variables were obtained, and later to perform the path analysis proposed by Wright (1921), able to partition the coefficient values into direct and indirect effects and exerted on a base variable.

In order to unfold the correlation coefficients existing between the technological variables (total dry matter yield, culm yield, soluble solids content and efficiency of broth extraction) and the theoretical ethanol, a path analysis was performed, considering this as an independent variable.

Montgomery et al. (2012) proposed the following classification for the effect of multicollinearity in relation to the condition number (CN): presence of weak multicollinearity (CN < 100), presence of moderate to strong multicollinearity (100 < CN > 1,000) and presence of severe multicollinearity (CN > 1,000).

Path analyzes performed under a high degree of multicollinearity, without the application of any method capable of reducing its effects, may result in estimates of effects that do not match the biological explanations regarding the analyzed variables (Olivoto et al. 2017).

If all six technological variables were considered as explanatory for the yield of the theoretical ethanol in a path analysis, it would be necessary to apply a high value of the constant k on the diagonal of the correlation matrix X'X to stabilize the data, what could lead to the unreliability of the results of the analysis (Carvalho 1995).

Salla et al. (2015) mention the identification and subsequent removal of variables causing a high degree of bias of the analysis as an alternative to minimize or avoid the effects generated by multicollinearity. To mitigate these estimation problems resulting from collinearity, simulations were performed excluding one variable at a time from the analysis, to identify the most plausible choice to make.

To unfold the existing correlations between the evaluated agronomic variables (average plant height, number of days until flowering, total green matter yield and percentage of culms in the yield of green matter), a path analysis was performed, considering culm yield as the main variable.

The exclusion of any explanatory variable from the analysis was not considered to contour the effects of multicollinearity, given its little bias effect. The matrix eigenvalue analysis test (X'X), which estimates the approximate degree to which the explanatory variables are linearly related, was performed to detect the presence of collinearity between them (Carvalho 1995). To mitigate such effects, a methodology called crest analysis was applied, in which a constant k within the range 0 < k > 1 is added on the diagonal of the correlation matrix X'X, selecting the lowest possible value of k with the ability to stabilize most of the analysis coefficients (Carvalho 1995). In this analysis, the addition of the constant k = 0.09 to the correlation matrix X'X = 2.192 was performed.

In the crest analysis, it is important to ensure that the applied constant k maintain the variance inflation factor (VIF) values as small as possible, since this factor is also an indicator of the degree of collinearity of the data. When it presents values lower than 10, it is considered that there is a reliability of results. In the performed analysis, the highest VIF value was 4.35 (Olivoto et al. 2017).

The path analysis, as well as the tests for the presence of multicollinearity, were performed with the Genes software (Cruz 2016).

## RESULTS AND DISCUSSION

The percentage of total dry matter variable was excluded from the analysis, because it was detected by the matrix eigenvalue analysis test (X'X) that it was responsible for a high degree of multicollinearity among the analyzed data (Salla et al. 2015).

The coefficients of genotypic correlations among the characters total dry matter yield, soluble solids content, efficiency of broth extraction, culm yield and yield of theoretical ethanol are expressed in Figure 1.

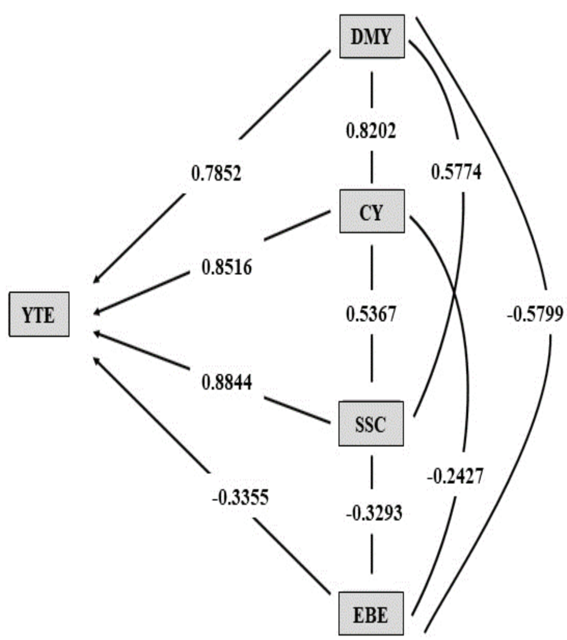


Figure 1. Diagram of the genotypic correlation coefficients among the characters total dry matter yield (DMY), soluble solids content (SSC), efficiency of broth extraction (EBE), culm yield (CY) and yield of theoretical ethanol (YTE).

In the collinearity diagnostic test performed among the five variables considered to explain the theoretical ethanol (total dry matter yield, culm yield, soluble solids content, efficiency of broth extraction and percentage of total dry matter), the condition number (CN) found was 130.31 (CN > 100), indicating the degree of collinearity, which was considered significant among these characteristics. The best scenario found to contour the multicollinearity effect was due to the exclusion of the variable percentage of dry matter. The degree of multicollinearity detected among the four remaining variables (total dry matter yield, culm yield, soluble solids content and efficiency of broth extraction) was of weak magnitude, in which a value of 26.12 (CN < 100) was found. According to Montgomery et al. (2012), collinearity effects at this level do not constitute serious problems for the analysis.

A path analysis was performed to estimate the direct and indirect effects of the variables total dry matter yield, soluble solids content at harvest, efficiency of broth extraction, culm yield and yield of theoretical ethanol (Table 3).

Considering the total effect of the explanatory characteristics on the yield of theoretical ethanol, the variables total dry matter yield, culm yield and soluble solids content showed positive correlation coefficients, and a negative value was found only in the total effect of the broth extraction efficiency.

The soluble solids content variable presented the largest total effect (0.8844) and the greatest direct effect (0.5969) on the theoretical ethanol, indicating a great dependence of the main variable by the explanatory variable. The largest indirect effect was via culm yield (0.2820). For the same crop, Figueiredo Júnior et al. (2018) obtained a genotypic correlation coefficient of r = 0.5798 between the soluble solids content and ethanol yield per hectare, while Souza et al. (2012) obtained such coefficient at the value of r = 0.78 and Lombardi et al. (2015) estimated r = 0.86, referring to the phenotypic relationship between such characters.

Characteristics that have a high heritability and high correlation with a quantitative characteristic are likely to be used in the indirect selection. Oliveira (2015) and França et al. (2016) estimated $H^2$ values > 0.7 for soluble solids content in studies of genetic parameters in sorghum. Therefore, the measurement of soluble solids content may be considered of great importance for studies on biofuel yield, which can

Table 3. Direct and indirect effects of the primary variables total dry matter yield (DMY), soluble solids content at harvest (SSC), efficiency of broth extraction (EBE), culm yield (CY) on the base variable and yield of theoretical ethanol (YTE).

| Variables | Effects | Estimates | Total |
|---|---|---|---|
| DMY | Direct effect on YTE | 0.0045 | 0.7852 |
| | Indirect effect via CY | 0.4310 | |
| | Indirect effect via SSC | 0.3447 | |
| | Indirect effect via EBE | 0.0051 | |
| CY | Direct effect on YTE | 0.5254 | 0.8516 |
| | Indirect effect via DMY | 0.0037 | |
| | Indirect effect via SSC | 0.3204 | |
| | Indirect effect via EBE | 0.0051 | |
| SSC | Direct effect on YTE | 0.5969 | 0.8844 |
| | Indirect effect via DMY | 0.0026 | |
| | Indirect effect via CY | 0.2820 | |
| | Indirect effect via EBE | 0.0029 | |
| EBE | Direct effect on YTE | -0.0088 | -0.3355 |
| | Indirect effect via DMY | -0.0026 | |
| | Indirect effect via CY | -0.1275 | |
| | Indirect effect via SSC | -0.1966 | |
| Determination coefficient | | 0.9818 | |
| Residual variable effect | | 0.1347 | |

be used in indirect selection for ethanol yield, having the additional advantage of being easily measured.

The total and direct effects exerted by culm yield presented values similar to those by soluble solids content, but of lesser magnitude, presenting 0.8516 for total effect and 0.5254 for direct effect on yield of theoretical ethanol. Almost all of the indirect effect of this variable is due to soluble solids content (0.3204).

The great magnitude of direct and indirect effects exerted on yield of theoretical ethanol, via soluble solids content and culm yield, indicates the possibility of performing a simultaneous selection for this pair of characteristics, when an increase in the ethanol yield in L $ha^{-1}$ is desired.

The total effect of total dry matter yield on the obtained yield of theoretical ethanol was satisfactory (0.7852), but this was basically due to the indirect effects via culm yield (0.431) and via soluble solids content (0.3447), with its direct effect being practically null (0.0045). Oliveira (2015) obtained correlation coefficients of a positive genotypic nature between total dry matter yield and soluble solids content, and between total dry matter yield and average number of culms per hectare, with values equal to 0.43 and 0.65, respectively. Such results show once again the high influence that the variables culm yield and soluble solids content exert on yield of theoretical ethanol.

A total negative correlation of efficiency of broth extraction with yield of theoretical ethanol was obtained, and its partition into direct and indirect effects also resulted in the estimation of only negative values. The efficiency of broth extraction exerted positive indirect values via the other technological characteristics, but not significant, all being less than 0.01.

Santos et al. (2014), studying sweet sorghum, estimated genotypic correlations among 10 variables, and the negative values for r were attributed to the correlation of the efficiency of broth extraction with almost all the studied characteristics, except for the estimate of reducing sugars, with which it was positively correlated. Lombardi et al. (2015) obtained a negative phenotypic correlation coefficient ($r = -0.17$) between efficiency of broth extraction and ethanol yield per hectare.

The efficiency of broth extraction is a quantitative characteristic influenced by several factors, which go beyond those related to the plant, also including those referring to the industrial process of broth extraction. The results of this study, as well as those aforementioned, indicate that this variable has a negative effect on ethanol yield, not being indicated to be used in indirect selection for purposes of genetic gains in such character.

Given the weak nature of multicollinearity, it was not necessary to exclude any variables from the analysis. To mitigate the adverse effects of the presence of multicollinearity, the crest analysis was applied (Carvalho 1995).

The culm yield variable had an expressive total effect on theoretical ethanol (0.8516), as well as a high degree of direct pathway effect (0.5254) on this variable (Table 3). Culm yield is considered a quantitative variable, having agronomic characters as yield components. For a better understanding of these relationships, a path analysis was performed from the unfolding of the estimated correlation coefficients between such variables (Figure 2), in which the total culm yield was considered as an independent variable, and average plant height, number of days until flowering, percentage of culms in the yield of green matter and total green matter yield were considered as dependent variables (Table 4).

The estimation of culm yield is extremely important, given its high correlation with yield of theoretical ethanol by direct and indirect means, and also because it presents the morphological part to be used for the extraction of the product that will be commercialized, when the cultivation is directed to the yield of alcohol. In areas of cultivation intended for silage and human feed, such importance is attributed to grain yield per hectare. The culm yield was studied in more detail by performing a path analysis, this being considered as the main variable, and the agronomic variables average plant height at harvest, flowering, total green matter yield and percentage of culms as dependents (Table 4).

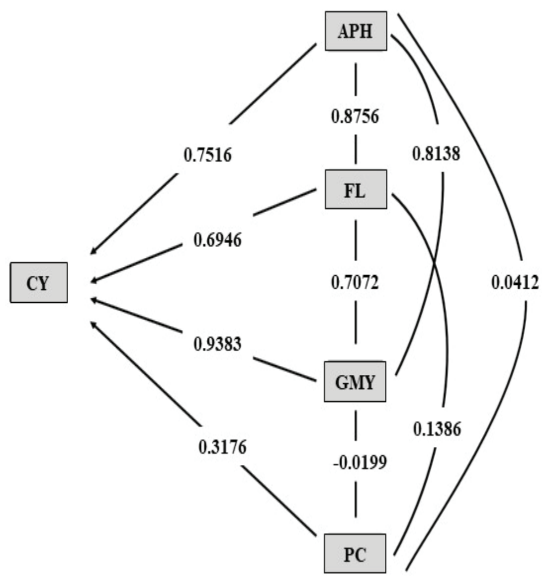


Figure 2. Diagram of the genotypic correlation coefficients among the characters average plant height (APH), flowering (FL), total green matter yield (GMY), percentage of culms in GMY (PC) and culm yield (CY).

Table 4. Direct and indirect effects of the primary variables average plant height (APH), flowering (FL), total green matter yield (GMY) and percentage of culms in GMY (PC) on the base variable culm yield (CY).

| Variables | Effects | Estimates | Variance inflation factor | Total |
|---|---|---|---|---|
| APH | Direct effect on CY | 0.0453 | 4.35 | 0.7516 |
| | Indirect effect via FL | 0.0378 | 2.04 | |
| | Indirect effect via GMY | 0.6518 | 1.39 | |
| | Indirect effect via PC | 0.0123 | 0.00 | |
| FL | Direct effect on CY | 0.0431 | 3.18 | 0.6946 |
| | Indirect effect via APH | 0.0397 | 2.58 | |
| | Indirect effect via GMY | 0.5665 | 1.05 | |
| | Indirect effect via PC | 0.0412 | 0.02 | |
| GMY | Direct effect on CY | 0.8010 | 2.51 | 0.9383 |
| | Indirect effect via APH | 0.0369 | 2.23 | |
| | Indirect effect via FL | 0.0305 | 1.33 | |
| | Indirect effect via PC | -0.0060 | 0.00 | |
| PC | Direct effect on CY | 0.2975 | 1.13 | 0.3176 |
| | Indirect effect via APH | 0.0019 | 0.00 | |
| | Indirect effect via FL | 0.0060 | 0.05 | |
| | Indirect effect via GMY | -0.0159 | 0.00 | |
| Coefficient of determination | | 0.9101 | | |
| k value used | | 0.0945 | | |
| Residual effect of the variable | | 0.2999 | | |
| Determinant of the correlation matrix | | 0.2192 | | |

The presence of multicollinearity was observed, however of magnitude classified as weak (CN = 26.12), not resulting in serious estimation problems.

The four variables considered as dependent (average plant height at harvest, flowering, percentage of culm and total green matter yield) showed positive values for total correlation effect on culm yield. Three of these characteristics (total green matter yield, average plant height at harvest and flowering) showed total effect values greater than 0.69, being considered of great magnitude on the main variable, indicating that these can be classified as components of culm yield per hectare.

The total effects of the average plant height at harvest and flowering on culm yield were considered high, with the respective values of 0.7516 and 0.6946. However, both showed non-significant direct effects, below 0.05. This effect on the main variable was basically due to indirect effects via total green matter yield, this being responsible for 87.53 % of the average plant height at harvest effect and 81.54 % of the flowering effect.

In studies carried out with the crop, Cunha & Lima (2010) estimated heritabilities of 97.05, 58.21 and 74.76 %, respectively for the variables plant height, flowering and green matter weight. Such results reinforce an indication of the possibility of indirect selection via these characters, since they have a high heritability.

Among the agronomic variables, the average plant height at harvest deserves to be highlighted as a yield component for presenting heritability values close to 1. In a study carried out by Araújo et al. (2014), a value of $h^2$ 0.98 was estimated for this variable.

The highest total correlation coefficient obtained was that for total green matter yield (0.9383), being the direct effect exerted on culm yield by this variable (0.8010) greater than the total effects exerted by each of the remaining variables. Such a high magnitude correlation indicates the total green matter yield as the main characteristic responsible for the multicollinearity present in the sample, even being of a weak nature (CN < 100) (Montgomery et al. 2012, Zuffo et al. 2020).

The total green matter yield is easier to estimate than the culm yield and, since this pair of characters is so positively correlated, the selection for genetic gains in culm yield may be performed via total green matter yield.

The percentage of culms showed the smallest value for total effect on culm yield (0.3176), this being practically due to the direct effect of the variable, indicating a positive correlation with the independent variable, but of low magnitude, in relation to the other estimated variables.

Most of the studies on path analysis in sorghum already published have been performed to evaluate the interference of explanatory variables on grain yield, in view of the history of use of the crop for human and animal feed. Thus, due to the recent interest in sorghum cultivation for ethanol yield, path analysis studies considering this as the main variable may constitute an important tool in breeding programs with such interest.

## CONCLUSIONS

1. The variables total soluble solids content and culm yield are potential characters to be used in indirect selection to increase the volume of ethanol produced per hectare;
2. The efficiency of broth extraction had negative effects on the theoretical ethanol yield variable, both directly and indirectly, via the other analyzed variables;
3. The selection of plants for higher efficiency of broth extraction imply in a decrease in ethanol yield;
4. The variables average plant height, flowering and total green matter yield are potential characters to be used in the indirect selection of culm yield.

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