Early selection of sugarcane using path analysis

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ABSTRACT. The aim of this study was to analyze sugarcane (*Saccharum officinarum*) biometric and technological data, obtained at different timepoints, using path analysis. The experiment was conducted in União, PI, Brazil, and evaluated 12 sugarcane genotypes (RB036066, RB9438, RB935744, RB021764, RB021754, RB021534, RB966229, RB977540, RB863129, and RB987935, and the varieties RB92579 and RB867515 as controls) in a randomized block design with four replications. Data were collected at six timepoints that were spaced 30 days apart (90, 120, 150, 180, 210, and 240 days). Direct and indirect effects of the following production components were compared: stalk length, stalk diameter, internode length, number of tillers, number of green leaves, and stalk dry matter. The technological variables evaluated were total recoverable sugar, degrees Brix, tons of polarization (pol, apparent sucrose content) per hectare, juice purity, fiber, juice pol, and tons of sugarcane per hectare. The coefficients of determination were high in all path analyses, suggesting that the components evaluated explained a large part of the variation in stalk production and in the technological variables. Stalk diameter was the trait that best correlated with stalk dry matter yield at all timepoints, with positive values that were higher than the residual effect. This demonstrates the possibility of obtaining significant gains via indirect selection for stalk dry matter yield via stalk diameter or via stalk diameter and number of tillers. The technological variables degrees brix and juice pol were the traits that best correlated with total recoverable sugar production, indicating that they could be used to indirectly select for total recoverable sugar.

Key words: *Saccharum officinarum* L.; Relationship between traits; Indirect selection

INTRODUCTION

The study of relationships between variables is necessary to measure the existence and/or intensity of interactions between traits. In general, these relationships are evaluated through correlations that, in plant breeding for instance, are important, because correlations between traits allow for selection based on a single trait to result in simultaneous changes in one or more characters of agronomic importance. Consequently, many breeding programs use correlations. Correlation analysis allows the breeder to evaluate the degree of association between two traits and the practical viability of indirect selection, which, in some cases, may lead to more rapid progress than direct selection (Cruz et al., 2006; Ferreira et al., 2007).

The correlations evaluated in plant breeding can be of a phenotypic, genotypic, or environmental nature. Phenotypic correlations have genetic and environmental causes, but only genetic causes are heritable and used in breeding programs (Cruz et al., 2004; Esposito et al., 2012). However, despite being an easily obtained statistical parameter, the magnitude of a correlation must be interpreted carefully, because this process is complicated by its significance, the importance of the traits, the effect of two or more traits, and the influence of the environment on their expression (Falconer and Mackay, 1996).

Path analysis can distinguish between the direct and indirect effects of variables that influence a basic or main variable of greater interest (Cruz et al., 2006). The technique, introduced by Wright (1921, 1923) and described in detail by Li (1956, 1975), identifies miscorrelations between two traits that may not necessarily be related by direct cause-and-effect, because of the influence of a third trait. Path analysis has been widely used by plant breeders in a variety of crops, e.g., soybean (Peter et al., 2014), corn (Faria et al., 2015), common bean (Cabral et al., 2011), green bean (Araujo et al., 2012), cowpea (Moura et al., 2012; Santos et al., 2014), rice (Marchezan et al., 2005), wheat (Kavalco et al., 2014), cotton (Hoogerheide et al., 2007; Farias et al., 2016), sweet sorghum (Lombardi et al., 2015), and sugarcane (*Saccharum officinarum*) (Kang et al., 1983; Reddy and Reddy, 1986; Sukhchain and Sain, 1997; Ferreira et al., 2007; Silva et al., 2009; Souza et al., 2011; Esposito et al., 2012). However, studies of this nature are still necessary, because different population structures, environments, and management strategies should be considered.

The current study evaluated different genotypes at different timepoints and the genetic consequences of relationship between traits. Our results will facilitate the manipulation of these genotypes and indicate their most suitable traits, which would be indispensable when performing indirect selection on sugarcane because it reduces the amount of work necessary when assessing a large number of genotypes in the initial stages of a breeding program.

In view of the above considerations, and given the need for honing the process of selection of promising genotypes in sugarcane breeding programs, the present study was conducted to quantify, by path analysis, the direct and indirect effects of production components on the yield of stalks per hectare, and of technological variables on the total recoverable sugar (TRS) at six data collection periods.

MATERIAL AND METHODS

The experiment was conducted on June 7, 2013, at Companhia Vale do Parnaíba alcohol and sugar mill that belongs to the Olho D'água group, which is in União, PI, Brazil (04°52'09"S, 42°52'45"W, 67 m above mean sea level). According to Thornthwaite and Mather (1955), the climate of the region is classified as dry sub-humid, with a moderate water surplus in the summer and a water shortage from July to December.

A randomized block design with four replications was adopted, and included 12 sugarcane genotypes (RB036066, RB9438, RB935744, RB021764, RB021754, RB021534, RB966229, RB977540, RB863129, and RB987935, and the varieties RB92579 and RB867515 as controls) obtained from the sugarcane breeding programs of the Inter-University Network for the Development of Sugar and Alcohol Sector (Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro, RIDESA).

The plot was composed of two 5-m furrows spaced 1.4 m apart. Each linear meter of furrow received six sugarcane cuttings with three buds each (totaling 18 buds per linear meter). Base fertilization was based on a chemical analysis of the soil (Table 1), and consisted of 500 kg/ha of 06-28-22 (N, P and K, respectively) formulation that was applied in the planting furrow.

Table 1. Chemical characteristics of the soil in the experimental area.

Depth (m)	pH ($CaCl_2$)	P (Mehlich)	K	Ca	Mg	Al	H+Al	AlS	BS
		mg/dm^3		Cmol$_c$/dm^3				%	
0.00-0.20	7.0	94.3	0.59	2.14	0.99	0.0	0.47	0.0	88.80
0.20-0.40	7.0	100.2	0.53	2.34	0.86	0.0	0.49	0.0	88.30
0.40-0.60	6.9	100.3	0.52	2.39	0.84	0.0	0.50	0.0	88.20

BS, base saturation; AlS, aluminum saturation.

The following traits were evaluated from September 2013 to February 2014: stalk length (SL) in meters, which was the distance from the base of the stalk to the first visible leaf, and obtained by randomly sampling stalks from each tussock; stalk diameter (SD) in mm, which was measured at the fifth internode from the base of the stalk to the apex, measured with a caliper on a randomly sampled stalk from each tussock; internode length (IL), which was measured with a graduated ruler at the same internode as where the SD was obtained; the number of tillers (NT), which was obtained by counting the number of tillers on each tussock in the usable area of the plot; the number of green leaves (NGL), which was obtained by counting the number of green leaves on each plant that had been selected to measure the SD; and stalk dry matter (SDM), which was the total mass of the plot (stalks without leaves, cut close to the soil). The technological variables evaluated were TRS, degrees Brix (Brix), tons of pol (apparent sucrose content) per hectare (TPH), juice purity, fiber, juice pol, and tons of sugarcane per hectare.

Initially, analysis of variance was performed for each environment (collection time) separately, and the homogeneity of the residual variance was checked. Later, a combined analysis of variance was performed that included progeny and environment as random terms, and subsequently, genotypic and phenotypic correlations were estimated.

The degree of multicollinearity of a X'X correlation matrix was established based on its condition number (CN), which is the ratio between the highest and lowest eigenvalue of the matrix (Montgomery and Peck, 1981) and indicates weak multicollinearity between explanatory variables if the ratio between the highest and lowest eigenvalue is equal to or lower than 100. If the CN is 100-1000, multicollinearity is considered moderate to severe, and if CN $\geq$ 1000, it is considered severe (Moura et al., 2012).

After the establishment of the basic path analysis equations, the resolution in matrix form was obtained according to the equation $X'X\beta = X'Y$, where X'X is a nonsingular matrix of correlations between the primary variables, β is the path coefficient vector column, and X'Y is the column vector of correlations between the explanatory variables and the dependent variable (Santos et al., 2014).

The following conditions were considered for the path analysis: i) SDM was the main variable and the production variables (NGL, NT, SL, IL, and SD) were explanatory variables; ii) TRS was the main variable and the technological variables [Brix, fiber, purity, juice pol, reducing sugar (RS), TPH, and tons of sugarcane per hectare] were explanatory variables. All of the analyses were performed using the GENES software (http://www.ufv.br/dbg/genes/genes.htm).

RESULTS AND DISCUSSION

Preliminary analyses confirmed the normality of the data and homogeneity of the variance of the experimental error, allowing the inclusion of the six periods of evaluation in a combined analysis. Significant differences were found for all of the traits evaluated. Good experimental precision was observed for most traits, with coefficients of variation lower than 20%. The CN values were lower than 100, except at 120 days of age for the production variables, for which a path analysis under multicollinearity was performed. After this procedure, the highest variance inflation factor was 6.11, using a K value of 0.2069. For the other periods evaluated, multicollinearity was classified as weak. Therefore, the path analyses were performed without the need for more complex statistical approaches. In addition, the high coefficients of determination of the path models (greater than 0.83) supported the use of this technique (Tables 2 and 3).

The genotypic correlations among the six independent variables and the dependent or main variable, as well as a decomposition of the genotypic correlations into components of direct and indirect effects on the dependent or main variable (SDM) and the independent explanatory variables for production traits, are described in Table 2 for each timepoint. The strongest genotypic correlations between the explanatory variables and the main variable were obtained for SD, IL, and SL at most of the timepoints. Considering only the correlation coefficients, SD, IL, and SL were the most important production components in the determination of SDM.

Among the explanatory variables, SD was the only trait that exhibited a strongly positive direct effect (greater than the residual effect) on SDM at all timepoints. The direct effect of SD on SDM was also higher than the indirect effect. SD explained most of the variation in the SDM yield.

Table 2. Decomposition of genotypic correlations into components of direct and indirect effects involving the main dependent variable (stalk dry matter, SDM) and independent, production explanatory variables in 12 sugarcane genotypes at six timepoints.

NT	Estimate (90 days)	Estimate (120 days)*	Estimate (150 days)	Estimate (180 days)	Estimate (210 days)	Estimate (240 days)
Direct effect on SDM	0.2739	-0.0077	0.3732	1.0942	0.9114	0.2331
Indirect effect via NGL	-0.0051	-0.0024	-0.0711	-0.1029	0.0042	-0.0989
Indirect effect via IL	-0.1669	-0.1395	0.2458	-0.0268	-0.0249	0.0220
Indirect effect via SL	-0.2297	0.0607	0.1017	-0.4969	0.0120	-0.1493
Indirect effect via SD	0.2173	-0.0812	-0.2456	-0.0068	-0.0623	0.0323
Indirect effect via LDM	0.0751	0.2421	-0.1282	-0.0129	-0.2556	0.0254
Total	0.1646	0.0704	0.4164	0.4477	0.5848	0.2049
NGL						
Direct effect on SDM	-0.0155	-0.0112	0.1529	-0.3205	0.7099	-0.1653
Indirect effect via NT	0.0902	-0.0016	0.1735	0.3512	0.0054	0.1394
Indirect effect via IL	-0.2034	0.0261	0.1348	-0.0069	-0.0838	-0.0255
Indirect effect via SL	0.2719	0.0576	-0.0294	0.1466	0.0011	-0.4515
Indirect effect via SD	0.2176	-0.0758	-0.1452	0.0316	-0.1048	0.2730
Indirect effect via LDM	-0.0402	-0.0936	-0.0294	-0.0225	0.0097	-0.0094
Total	0.3205	-0.1009	0.2572	0.1795	0.5375	-0.3386
IL						
Direct effect on SDM	-0.3870	0.3807	-0.6522	-0.2071	-0.3346	0.1964
Indirect effect via NT	0.1181	-0.0028	-0.1406	0.1419	0.0680	0.0261
Indirect effect via NGL	-0.0082	-0.0007	-0.0316	-0.0107	0.1777	0.0215
Indirect effect via SL	0.3214	0.1529	-0.0899	0.2727	0.0009	-0.1302
Indirect effect via SD	0.8525	0.4250	0.4953	0.2980	-0.0836	0.4437
Indirect effect via LDM	-0.0423	-0.1974	0.2090	0.0187	-0.0225	0.1006
Total	0.8545	0.8420	-0.2100	0.5135	-0.1941	0.6861
SL						
Direct effect on SDM	1.1961	0.3608	-0.3384	1.0506	-0.0403	-0.5592
Indirect effect via NT	-0.0526	-0.0013	-0.1105	-0.5175	-0.2721	0.0622
Indirect effect via NGL	-0.0035	-0.0018	0.0132	-0.0447	-0.0197	-0.1335
Indirect effect via IL	-0.1040	0.1614	-0.1734	-0.0538	0.0074	0.0457
Indirect effect via SD	-0.2088	-0.0101	0.2744	0.0180	-0.0209	0.4533
Indirect effect via LDM	-0.2197	0.0261	0.2568	0.01269	0.0997	0.0002
Total	0.6075	0.6097	-0.0778	0.4653	-0.2459	-0.4676
SD						
Direct effect on SDM	0.9630	0.4681	0.6818	0.3888	0.3935	0.6341
Indirect effect via NT	0.062	0.0013	-0.1344	-0.0192	-0.2933	0.0119
Indirect effect via NGL	-0.0035	0.0018	-0.0325	-0.0260	0.2841	-0.0711
Indirect effect via IL	-0.3426	0.3457	-0.4738	-0.1587	-0.1444	0.1474
Indirect effect via SL	-0.2593	-0.0077	-0.1362	0.04874	-0.0043	-0.3998
Indirect effect via LDM	0.0643	-0.0934	0.3632	-0.0078	0.1836	0.0063
Total	0.4839	0.8127	0.2681	0.2258	0.3686	0.7002
LDM						
Direct effect on SDM	-0.3555	0.4224	0.4572	-0.1016	-0.4268	0.0335
Indirect effect via NT	-0.0578	-0.0044	-0.1046	0.1396	0.5458	0.1771
Indirect effect via NGL	-0.0018	0.0025	-0.0098	-0.0709	-0.0161	0.0464
Indirect effect via IL	-0.0461	-0.1780	-0.2982	0.0381	-0.0177	0.0619
Indirect effect via SL	0.7396	0.0223	-0.1901	-0.1312	0.0094	-0.0031
Indirect effect via SD	-0.1751	-0.1035	0.5417	0.0298	0.0153	0.1196
Total	0.1034	0.2488	0.3962	-0.0963	0.1099	0.4555
Coefficient of determination	0.8653	1.0026	0.8324	0.9124	0.8711	0.9593
Residual effect	0.3670	0	0.4093	0.2959	0.3589	0.2016

NT, number of tillers; NGL, number of green leaves; IL, internode length; SL, stalk length; SD, stalk diameter; LDM, leaf dry matter. *Path analysis with multicollinearity (highest variance inflation factor = 6.11; K value = 0.2069).

NT was second in importance, as it had strongly positive, direct effects on SDM at most timepoints (90, 120, 150, and 180 days). This suggests that significant gains in SDM can be obtained through indirect selection for SDM via SD, or SD and NT. These results corroborate those obtained by Kang et al. (1983), who decomposed genotypic correlation coefficients and reported a large contribution by SD, followed by the number of stalks, on sugarcane yield per hectare. The authors stated that genotypic path coefficients are important in choosing an effective selection criterion (Kang et al., 1983).

Table 3. Decomposition of genotypic correlations into components of direct and indirect effects involving the main dependent variable (total recoverable sugar, TRS) and independent, technological explanatory variables in 12 sugarcane genotypes at harvest*.

Brix	Estimate	RS	Estimate
Direct effect on TRS	0.5172	Direct effect on TRS	-0.1125
Indirect effect via fiber	-0.0553	Indirect effect via Brix	-0.4088
Indirect effect via purity	0.0868	Indirect effect via fiber	0.0667
Indirect effect via juice pol	0.2867	Indirect effect via purity	-0.1098
Indirect effect via RS	0.0889	Indirect effect via juice pol	-0.2977
Indirect effect via t pol ha^{-1}	0.0316	Indirect effect via t pol ha^{-1}	-0.0373
Indirect effect via t sugarcane ha^{-1}	-0.0109	Indirect effect via t sugarcane ha^{-1}	0.0158
Total	0.9742	Total	-0.8897
Fiber		t. pol ha^{-1}	
Direct effect on TRS	-0.1078	Direct effect on TRS	0.0485
Indirect effect via Brix	0.2651	Indirect effect via Brix	0.3368
Indirect effect via purity	0.0678	Indirect effect via fiber	-0.0303
Indirect effect via juice pol	0.1852	Indirect effect via purity	0.0844
Indirect effect via RS	0.0695	Indirect effect via juice pol	0.2343
Indirect effect via t pol ha^{-1}	0.0136	Indirect effect via RS	0.0864
Indirect effect via t sugarcane ha^{-1}	-0.0039	Indirect effect via t sugarcane ha^{-1}	-0.0239
Total	0.4834	Total	0.7388
Purity		t sugarcane ha^{-1}	
Direct effect on TRS	0.1098	Direct effect on TRS	-0.0283
Indirect effect via Brix	0.4089	Indirect effect via Brix	0.1989
Indirect effect via fiber	-0.0667	Indirect effect via fiber	-0.0150
Indirect effect via juice pol	0.2978	Indirect effect via purity	0.0613
Indirect effect via RS	0.1125	Indirect effect via juice pol	0.1570
Indirect effect via t pol ha^{-1}	0.0373	Indirect effect via RS	0.0628
Indirect effect via t sugarcane ha^{-1}	-0.0158	Indirect effect via t pol ha^{-1}	0.0410
Total	0.8900	Total	0.4761
Juice pol			
Direct effect on TRS	0.3094	Coefficient of determination	0.9745
Indirect effect via Brix	0.4793	Residual effect	0.1598
Indirect effect via fiber	-0.0645	K value used in the analysis	0.0056
Indirect effect via purity	0.1057		
Indirect effect via RS	0.1082		
Indirect effect via t pol ha^{-1}	0.0367		
Indirect effect via t sugarcane ha^{-1}	-0.0144		
Total	0.9778		

RS, reducing sugar. t, tonne. *Path analysis with multicollinearity (highest variance inflation factor =14.41; K value = 0.005638).

Unlike SD and NT, the other variables did not have strong direct effects of stalk dry matter, at most timepoints, which, in addition to being weak, were mainly negative. According to Cruz et al. (2006), in this situation, the independent trait is not the main determinant of alterations in the main variable; other factors can also have a large impact in terms of genetic gain in selection. Given these biometric data results, we suggest the development of a selection index for sugarcane genotypes using SD and NT.

Interestingly, despite its weak and negative direct effects at most timepoints, IL had a strongly positive indirect effect on SDM via SD at most timepoints (Table 2). Therefore, SD may be used to indirectly select for sugarcane yield. Sugarcane yield is a complex trait that is influenced by several inter-related traits. In this regard, path analysis is an important statistical tool in identifying components that have a large effect (Esposito et al., 2012). SD, a variable that is easily measured, was the component that contributed the most to sugarcane yield among the production variables, indicating the possibility of obtaining significant gains through indirect selection for SDM via SD.

As shown in Table 3, strong, positive genotypic correlations were obtained between the technological variables and the main variable (TRS), except for RS (-0.8897). However, only for Brix and juice pol did these significant genotypic correlations result in direct effects (greater than the residual effect) on the TRS. Working with technological variables in sweet sorghum, Lombardi et al. (2015) reported strongly positive direct effects of total Brix per hectare on ethanol production per hectare, and concluded that Brix was the variable that most contributed to ethanol production.

There were positive, indirect effects (greater than the residual effects) of fiber, purity, juice pol, and TPH via Brix on the dependent variable. The exception was the indirect effect of RS via Brix (-0.4088), which was strong but negative. These results confirm the importance of the Brix variable as the main component that increases sugarcane TRS; degrees Brix, followed by juice pol, were the traits that most contributed to TRS. Indirect selection for TRS via Brix, or via Brix and juice pol, could provide significant gains in sugarcane yield.

CONCLUSIONS

SD contributed the most to sugarcane SDM. Significant gains can be obtained through indirect selection for SDM via SD, or via SD and NT. Of the technological variables, degrees Brix, followed by juice pol, most contributed to sugarcane TRS production. These variables could provide significant gains in sugarcane yield through indirect selection for TRS via Brix, or via Brix and juice pol.

Conflicts of interest

The authors declare no conflict of interest.

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