(DOI): 10.5935/PAeT.V7.N2.05

This article is presented in English with abstracts in Spanish and Portuguese

Brazilian Journal of Applied Technology for Agricultural Science, Guarapuava-PR, v.7, n.2, p.41-47, 2014

Cientific Paper

Path analysis in characteristics of sweet potato clones aiming ethanol yield

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Abstract

Developing new products is an indispensable practice for companies, which makes it essential studies that seek to find new ways of using raw materials in the biofuels market. The sweet potato is a vegetable of great energetic potential, and can be used directly for producing ethanol. With the objective of estimating the genotypic, phenotypic and environmental correlations and direct and indirect effects of path coefficients on ethanol yield, we conducted an experiment in field conditions at the Experimental Station of the Federal University of Tocantins, in Palmas. The experimental design utilized was entirely randomized, represented by 100 treatments (sweet potato clones) in three repetitions. The studied characteristics were the dry matter, productivity, starch content and ethanol production. The genotypic correlations presented equal sign and, in most cases, superior values to its correspondent phenotypic correlations, indicating that the phenotypic expression is decreased under the environment influences. The selection of plants with high dry matter content would enable the indirect improvement for materials with high ethanol production.

Keywords: *Ipomoea batatas*; bioenergy; genetic correlation; ethanol production

El análisis de trilla en caracteres de clones de patata dulce visando a la producción de etanol

Resumen

El desarrollo de nuevos productos es un imperativo para las empresas, por lo que aumenta la necesidad de estudios que tratan de encontrar nuevas formas de utilizar las materias primas dentro de la práctica de mercado de los biocombustibles. La patata dulce es un vegetal de gran potencial energético, y se puede utilizar directamente para la producción de etanol. Se realizó un experimento en condiciones de campo en la Estación Experimental de la Universidad Federal de Tocantins en la Ciudad de Palmas, con el objetivo de estimar las correlaciones genotípicas, fenotípicas, ambientales y efectos directos e indirectos de coeficientes de trilla en el rendimiento de etanol. El diseño experimental fue completamente al azar representado por 100 tratamientos (clones de patata dulce) en tres repeticiones. Las características estudiadas fueron el rendimiento de materia seca, productividad, almidón y la producción de etanol. Las correlaciones genotípicas mostraron el mismo signo, y en la mayoría de los casos, valores correspondientes a las correlaciones fenotípicas, lo que indica que la expresión fenotípica se reduce frente a las influencias ambientales. La selección de plantas con altos niveles de materia seca posibilitaría el mejoramiento indirecto para los materiales con mayor producción de etanol.

Palabras clave: *Ipomoea batatas*; bioenergía; correlación genética; producción de alcohol

Análise de trilha em caracteres de clones de batata-doce visando rendimento em etanol

Resumo

Desenvolver novos produtos é uma prática imprescindível para as empresas, o que torna indispensáveis estudos que busquem encontrar novas formas de uso de matérias primas dentro do mercado dos biocombustíveis. A batata-doce é

Recived at: 18/12/13. Aceppted for publication: 21/07/14.

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uma hortaliça de grande potencial energético, e pode ser utilizada diretamente para a produção de etanol. Foi conduzido um experimento em condições de campo na Estação Experimental da Universidade Federal do Tocantins em Palmas, com o objetivo de estimar as correlações genotípicas, fenotípicas, ambientais e efeitos diretos e indiretos de coeficientes de trilha no rendimento de etanol. O delineamento experimental utilizado foi inteiramente casualizado representado por 100 tratamentos (clones de batata-doce) em três repetições. As características estudadas foram matéria seca, produtividade, teor de amido e produção de etanol. As correlações genotípicas apresentaram igual sinal e, na maior parte dos casos, valores superiores às suas correspondentes correlações fenotípicas, indicando que a expressão fenotípica é diminuída ante as influências do ambiente. A seleção de plantas com elevados índices de matéria seca possibilitaria o melhoramento indireto para materiais com elevada produção em etanol.

Palavras-chave: *Ipomoea batatas*; bioenergias; correlação genética; produção de álcool

Introduction

The bioenergy or biofuel are products derived from renewable biomass that can substitute, partially or totally, fuels derived from petroleum and natural gas, in combustion engines or in other types of energy generation.

According to KOHLHEPP (2010), Brazil shows decades of experience in the production of biofuel using the sugarcane as its main raw material. Although this culture is hegemonic on ethanol production, a series of bottlenecks are intrinsic to its cultivation (LOPES et al., 2006; SOUZA et al., 2010).

The sweet potato can be an alternative raw material to the sugarcane in the agro-energy scenario. Despite its rusticity, the cultivation requires cultural handling that must be followed rigorously when the planting is motivated by business interests, as, for example, the ethanol production (BOMFORD and SILVERNAIL, 2010).

On the State of Tocantins, the cultivation of sweet potato can occur during the whole year, as long as irrigation is used. The minimum temperature of 22ºC and the maximum of 38ºC, associated with annual precipitations of 1500 mm, showed highly favorable for the obtaining of high productivity, fulfilling the edaphoclimatic requirements of the sweet potato (SILVEIRA et al., 2008). For its versatility, the most adequate material can be chosen for the soil, climate and the socioeconomic needs (DAM et al., 2010), enabling its cultivation in small rural communities.

However, it is known that the efficiency on the mechanisms for the choosing of cultivars propitious to the industry finds itself in parameters hard to overcome without the utilization of studies that determine parameters and specific characteristics for improvement programs.

According to OLIVEIRA et al., (2002), little information are available regarding genotypes most adapted to particular conditions of producers from a certain region, in face of the scarcity of inherent researches. On the State of Tocantins, there is a great genetic diversity of sweet potatoes, derived from asexual and sexual segregation, apart from plants from other regions.

In this sense, the genetic diversity analysis in collections of germplasm can facilitate the classification and identification of access groups with superior characteristics to be used for improvement means (MOHAMMADI and PRASANNA, 2003). According to VENCOVSKY and BARRIGA (1992), it is noteworthy that the correlation studies between characters do not allow us to take conclusions about relations of cause and effect, being necessary its unfolding, done by the path analysis.

The application of this method in sweet potato cultures is recorded by MIRANDA et al., (1988), who evaluated the effect of physic-chemical characteristics over the average weight of tuberous roots. SILVA (2010) conducted the evaluation and selection of sweet potato clones regarding productivity and bioconversion of starch into ethanol, using correlation and path analysis to the variables. Currently, few are the studies to use these methodologies for sweet potato cultivation.

In the present study, we aimed to estimate the phenotypic, genotypic and environmental correlations, in addition to the direct and indirect effects of path coefficients on the ethanol yield for the sweet potato cultivation, aiming to provide subsidies for selection means to be adopted in a future program for the institution's improvement.

Material e Methods

We conducted a competition of sweet potato clones on the experimental area of the Federal University of Tocantins, in Palmas, (220 m altitude, 10º45′ S e 47º14′ W), in Dystrophic Red-Yellow Latosol.

An essay of competition of sweet potato clones was conducted using 100 clones of sweet potato with the following origins: BD-106; IZABELA; LÍVIA; AMANDA; ANA CLARA; DUDA; BD-115; BD-04; BD-02; PALMAS; BD-78.

The experimental design used was entirely randomized with three repetitions and 100 clones. The parcels were constituted of a 4.0 m length row, with 0.80 m spacing and 0.30 height. The spacing between plants was of 0.40 m, totaling 10 plants per parcel, with useful area of 3.2 m².

Previous to the implantation of the culture, we conducted a soil analysis. Due to the absence of criteria for fertilization recommendations specific to soils of Tocantins State, the fertilization was conducted according to the established parameters by the 5º approximation of Minas Gerais State. The control of pests, diseases and weeds was performed during the whole experiment.

We evaluated the following characteristics: 1 – root productivity (P): refers to the weight in $t.ha^{-1}$ of the roots, performed by a precision scale 0.01g; 2 – dry matter level of the roots: refers to the percentage of dry matter contained in the roots. After washed, the roots were peeled and grated. Subsequently, we placed it in a greenhouse with air circulation and renovation at 55ºC, during 24 hours. The potato was transformed into flour through grinding on multiprocessor.

For the determination of the dry matter we used 5 g of the grated sweet potato sample. The containers with the potatoes were again placed in a greenhouse of air circulation at the temperature of 105ºC during 8 hours. The samples were weighed until the weight became constant. The percentage of dry matter was obtained through the calculation of the plate with the dry sample minus the weight of the empty plate and divided by the weight of the dry sample without the plate; 3 – starch content: the determination of the starch content proceeded to the parameters of the spectrophotometry, using the spectrophotometer of proximate infrared, NIR 900 PLS. The device was calibrated for reading using 156 reference samples. The samples were tested by the reference technique on the bench. The difference between the value predicted by the NIR and the real, obtained by the reference analysis, was used to calculate the standard error of prediction.

For the validation of the results we used 156 data; 4 – ethanol yield: for the conduction of the ethanol yield evaluation, we proceeded with the starch hydrolysis. The hydrolysis process was conducted in Erlenmeyer with capacity of 250 mL volume. We weighed 20g of potato flour previously dried, being provided in the Erlenmeyers along with 132 mL of distilled water for hydration, remaining immersed during 30 minutes. After the hydration, the Erlenmeyer was directed to water bath at the temperature of 90ºC and added 0.60 µl of the enzyme **α-**amylase Termamyl 120 L, staying 90 minutes under stirring.

Subsequently, the material was cooled at 68ºC for the addition of the second enzyme, Amyloglucosidase AMG 300 L. After the inoculation, the water bath maintained the temperature at 68ºC and the Erlenmeyer remained under stirring during 1 hour. After, the material was cooled at room temperature, for later inoculation of the yeast responsible for the fermentation process. The fermentation essay was conducted in conical flasks equipped with fermentation measurer which enabled us to follow the fermentation process through the weight loss, observed by the CO_2 detachment. Such recipient has a device that directs the passage of CO_2 through a small volume of water, so that the losses by evaporation or by gas drag that detaches were minimized. The ethanol production was obtained based on the stoichiometry, considering the detached CO_2 during the fermentation process, and was calculated by the following equation:

Where: Petanol = Ethanol production (g) and Lco2 = CO_2 release during the fermentation process (g). The ethanol yield was found according to the equation of Gay Lussac, whereas, from 100g of glucose, it is obtained, in average, 51.11g of alcohol. The ethanol yield at the end of the process was determined by the following equation:

Where: □ = Ethanol yield (%) or conversion efficiency of glucose in ethanol; F = represents determination at the end of the essay; 1 = represents determination at the beginning of the essay; 0.511 – Theoretical yield (stoichiometric) of the glucose conversion in ethanol.

We estimated the phenotypic (rp), genotypic (rg) and environmental (re) correlations (FALCONER 1987). Subsequently, we conducted the unfolding of these correlations with direct and indirect effects of the three characteristics over the ethanol yield, through path analysis, described by CRUZ and CARNEIRO (2003).

The level of multicollinearity of the singular matrix X'X was established by the product of the respective diagonal element of X'X by the residual variance component. The variance shall be as long as the magnitude of this diagonal element, named variance inflation factor (FIV), as found by MARQUARDT (1970). According to NETER et al., (1974), the existence of only one value of FIV superior to 10 is an indicative that the regression coefficients associated to these values have estimates influenced by the multicollinearity. All analyses were conducted using the statistical and computational app Genes (CRUZ, 2006).

Results and Discussion

The variance analysis summary for root productivity, root dry matter, starch level and theoretical ethanol yield is found on Table 1.

Significant differences occurred between the clones for all evaluated characteristics, indicating the existence of genetic variability. However, the variation coefficients were low, indicating the good precision of the experiment.

On Table 2 are presented the estimating coefficient of genotypic (G), phenotypic (F) and environmental (A) correlations, among the four variables.

The genotypic correlations presented equal sign and, in most cases, superior values to its corresponding phenotypic correlations, indicating that the phenotypic expression is decreased in face of the environment influences. These results match the evaluations conducted by SILVA (2010) and by CAVALCANTI et al., (2006), in sweet potato clones.

The environmental correlation between the characteristics with differences in magnitude and signal, in relation to the respective genotypic correlation, revealed that the environment favored one characteristic over another and that the causes of genetic and environmental variations presented different physiological mechanisms, hindering the indirect selection. Among these, we quote the correlation between the root productivity characteristics (P) and starch content (A).

According to LOPES et al. (2002), there is a tendency among the plant breeders to place greater value on the signal (positive and negative) and the value magnitude on the interpretation applied to the correlations, preferring the estimates below

Table 1. Variance analysis for the evaluated characteristics in 100 sweet potato clones.

F.V	GL	Productivity (t ha^{-1})	Dry Matter (%)	Starch Content (%)	Ethanol Yield (L t^{-1})
Clones	99	90.416**	16.013**	28.225**	753.601**
Residue	200	6.756	3.567	16.669	164.096
Average		17.350	33.590	75.450	195.560
CV (%)		14.98	5.62	5.41	6.55

***significant at 5% of probability by the F test*

Table 2. Estimating coefficient of phenotypic (F), genotypic (G) and environmental (A) correlations among four studied characteristics of sweet potato.

Characteristics		MS	A	RE
P	F	-0.0534	-0.0410	0.2610
	G	-0.0523	-0.0762	0.2940
	A	-0.5440	0.0279	0.0083
MS	F		0.2021	0.8890
	G		0.2650	0.8995
	A		0.1449	0.8150
A	F			0.2768
	G			0.4223
	A			0.1055

P = Productivity (t/ha^{-1}); MS = Dry Matter Content (%); A = Starch Content on the dry base (%); RE = Ethanol Yield (L/ton.)w

-0.5 and above 0.5. In this context, the characteristic of dry matter production of the roots (S) would satisfactorily correlate with the ethanol yield (RE), that is, plants with higher levels of starch present greater ethanol yield, agreeing with SILVEIRA et al. (2008). It is noteworthy that the RE characteristic presented positive phenotypic correlation with all other characteristics, although of low magnitude.

The positive correlations show, possibly, the occurrence of pleiotropism or imbalance of genetic linkage between the characteristic pairs and favored the simultaneous selection of two or more characteristics by the selection in only one of those (FALCONER (1987), GOLDEMBERG (1968), JOHNSON et al. (1955)).

For the correlation and path analyses, firstly the diagnosis of the multicollinearity was made, and after, the path analysis, as described by CRUZ and CARNEIRO (2003).

The study of correlations is an association measure and do not allow to conclude about the study of relation between cause and effect. Thus, we proceeded to the path analysis, which investigates the relation between cause and effect (SILVA 2005).

Both the phenotypic and genotypic correlations could be used for estimating the path coefficients (CARVALHO et al., 2004). In this study, as the genotypic correlations presented superior values and of equal sign to its corresponding phenotypic correlations, indicating that the phenotype reflects adequately the genotype, we used the phenotypic correlations to obtain the path coefficients.

The determination coefficient revealed that 79% of the Ethanol Yield can be explained by the effect of the analyzed variables (Table 3). It is worthy pointing out that this value is restricted to the analyzed characteristics, taking into account that the RE is a quantitative characteristic, with great number of alleles of small effect (modifiers) influencing the characteristic and, consequently, low heritability.

P = Productivity (t ha^{-1}); MS = Dry matter content (%); A = Starch content in the dry base (%)

On the path coefficient's unfolding, the characteristic of root dry matter (MS) has high phenotypic correlation and direct effect with the basic variable of ethanol yield (RE) (0.8634 e 0.8809 respectively), revealing the hypothesis of true existence of association, inferring that the selection for dry matter would be efficient in obtaining clones with high potential of ethanol production.

PEIXOTO and MIRANDA (1984), in the Embrapa Hortaliças, selected five cultivars with high level of dry matter (above 39%) and with high productivity, considering these materials proper for the alcohol production. MARTINS (2010), evaluating the phenotypic variability and genetic divergence

Table 3. Estimates of the direct and indirect effects of the path coefficients over the sweet potato ethanol yield.

Characteristics	Association means	Path coefficients		Correlation coefficient
		Direct effect	Indirect effect	
P	Direct effect over RE	0.0748		
	Indirect effect via MS		-0.04438	
	Indirect effect via A		-0.00431	
	TOTAL (direct or indrect)			0.0261
MS	Direct effect over RE	0.8634		
	Indirect effect via P		-0.0038	
	Indirect effect via A		0.0213	
	TOTAL (direct or indrect)			0.8809
A	Direct effect over RE	0.1053		
	Indirect effect via P		-0.0030	
	Indirect effect via MS		0.1745	
	TOTAL (direct or indrect)			0.2768
	Determination coefficient			0.7917
	K value			0.0000
				0.4563

in sweet potato genotypes, found the best ethanol yields for genotypes with higher percentages of Dry Matter. CEREDA et al. (1985), recommended 4 cultivars of cassava for the production of starch or for fermentation, through the high value of starch and quantified dry matter.

Some studies with path analysis in different cultures showed positive values for direct effects of primary components over the basic variable of yield (CARVALHO et al. 1999; KUREKET et al. 2001; BARBOSA et al. 2002), revealing, thus, that the direct effects for several species are good predictors of the genotypic correlation, which evidences the possibility of selection for yielding by its primary components.

Conclusions

We observed a genetic variability among the evaluated clones for the characteristics of root productivity, root dry matter, starch content and ethanol yield.

The genotypic correlations presented equal sign and, in most cases, superior values to its corresponding phenotypic correlations, indicating that the phenotypic expression is decreased in face of the environment influences.

The plant selection with high levels of dry matter would enable the indirect improvement for obtaining sweet potato clones with high ethanol yield.

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