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CAUSE AND EFFECT RATIO OF GRAIN CHEMICAL COMPONENTS ON SOYBEAN YIELD

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Abstract–The chemical composition of the grain is strongly influenced by genetic and environmental effects, so studies involving correlations and analyses can be useful in breeding programs. Therefore, the present work was carried out aiming at the study of cause and effect and correlation of chemical composition on grain yield, in eight trials represented by the combination of crops (years)/sites/sowing times, four of which were carried out in the 2014/15 crop and four in the 2015/16 crop. The experimental design used in each assay was randomized blocks with three replications and eight treatments, represented by the cultivars: 8473RSF RR, 8576 RSF, 8579RSF IPRO, ST 820 RR, TMG 132 RR, 9086RSF IPRO, M8644 IPRO, and M9144 RR. The chemical characteristics of the grains evaluated were; oil content, protein, carbohydrates, neutral digestive fiber - Fdn and acid digestive fiber - FDA. Variance analysis of each crop (year) and sowing time was performed and, subsequently, track and correlation analyses were performed to quantify the contribution and relationship of each chemical component of the grain over the final yield. The time and year influenced the magnitude of the correlations and the direct and indirect effects on grain yield.

INTRODUCTION

Soy (*Glycine max* (L.) Merrill), has an important role in the Brazilian economy, due to its broad and diversified use, based on grain, and its two main derivatives; protein bran and crude oil (Faria *et al*., 2018).

In the Tocantins, the state where it occupies the first place in terms of participation in the gross domestic product, soybean was cultivated over an area of approximately 1.08 million hectares of planting, producing about 3 million tons (around 50% of all volume produced in the North region) in the harvest 2019/20 (CONAB, 2020).

The choice of cultivar, season and sowing site are preponderant factors that influence both grain yield and its chemical and productivity components (Faria *et al*., 2018).

The soybean cultivar is directly influenced by the different environmental conditions to which it is subjected, i.e., cultivars may present variations in the productive performance and/or chemical composition of the grains, as the dates and sowing sites vary (Zuffo *et al*., 2018).

Thus, both knowledge about the genetic variability available in the species and understanding and certain variables are related to the formation of genotypes with desirable components, which are fundamental prerequisites for the success of breeding programs (Oliveira *et al.*, 2010).

To increase efficiency in selecting a character, you can use indirect selection through the use of correlated characters. Correlation analyses between productive variables and productivity components are essential in determining selection criteria (Oliveira *et al*., 2010). These correlations indicate the existence or not of association, intensity, and meaning of linear associations, between two characteristics, but are not sufficient to clarify the relationships between the variables studied, because they do not consider the influence of other characters on the association (Zuffo *et al*., 2018).

The path analysis, proposed by Wright (1921), allows the partitioning of the correlation coefficient between direct and indirect effects, generating more accurate estimates of cause and effect, which makes it easier to select plants through the indirect and positive effect of other characters on productivity (Azevedo *et al*., 2016; Cabral *et al*., 2016; Oliveira *et al*., 2021; Pinheiro *et al*., 2021; Ribeiro *et al*., 2016; Santos *et al*., 2018; Silva *et al*., 2016; Silva *et al*., 2021; Teodoro *et al*., 2016; Vian *et al*., 2016).

Because of the above, this study aims to analyze the cause and effect relationship of the chemical components of the grain on soybean yield in the State of Tocantins.

MATERIALS AND METHODS

Eight competition trials of cultivars were carried out, four in the 2014/15 crop and four in the 2015/16 crop. In each harvest, two trials were conducted in the municipality of Porto Nacional - TO (Site 1 - Serra Azul Farm, 234 m al titude, 10°422 27" S and 48°24251"W) and two in Santa Rosa - TO (Local 2 - Fazenda Mariana, 288m altitude, 11°26′31′′S and 48°7′2′′W).

In each location, the sowings were carried out in two seasons, the first on November 3 in Porto Nacional and on November 15 in Santa Rosa, which were considered as season 1. On the other hand, the second season occurred 15 days after the first planting, being November 18 in Porto Nacional and November 30 in Santa Rosa, considered as season 2, always respecting the planting window of the two sites.

The experimental design used in each environment was randomized blocks with three replications and eight treatments, represented by the cultivars: 8473RSF RR, 8576 RSF, 8579RSF IPRO, ST 820 RR, TMG 132 RR, 9086RSF IPRO, M8644 IPRO, and M9144 RR.

The experimental plot consisted of four rows of 5.0 m in length, spaced by 0.45 m. At harvest, plants from the two central rows were used, excluding 0.5 m from the ends of each row.

Rainfall temperature and precipitation data, recorded in the agricultural year 2014/2015 and 2015/2016, were obtained through the monthly collection at the test site (Table 1).

The grains from each cultivar in each year/place/season, from the useful area of each plot, were harvested, identified, and weighed (transformed into kg $ha^{-1).}$ Then, part of the grains was crushed and stored in the form of soybean meal in a cold chamber, at the Federal University of Tocantins – Gurupi Campus, under temperature and controlled humidity, aiming at maintaining the chemical quality of the grain.

Subsequently, these samples were submitted to physicochemical analyses for in the laboratory of industrial raw material of the Federal University of Tocantins - Campus de Palmas, where the grain compositions of each cultivar were determined to the oil contents (Bligh-Dyer method), protein content (Kjeldhal method), carbohydrate content (Fehling reagent method) and fiber content (gravimetric enzymatic method – where the values for Fdn (neutral detergent fiber, consisting of cellulose, were calculated, hemicellulose and lignin, through the treatment of the sample with neutral pH detergent solution and subsequent filtration) and Fda (acid detergent fiber, composed of cellulose and lignin, through the use of the residue obtained in the enzymatic Fdn method, which was added of acid detergent solution and subsequently filtered).

After obtaining the data of productivity and chemical components of the grains, variance analysis was performed for each crop (year) (Crop 2014/15 and crop 2015/16) and each sowing season (season 1 and season 2) and then Pearson correlation coefficients were estimated between the grain yield characters. Correlations with values of $r \geq 0.6$ or $r \leq -0.6$ were adopted as significant, derived from the methodology proposed by Dancey and Reidy (2005), where r above 0.6 is considered moderate to strong.

Subsequently, the diagnosis of multicollinearity was made through the analysis of the number of conditions (NC), which represents the ratio between the highest and lowest self-value of the correlation matrix. Thus, when the number of conditions is less than 100, the multicollinearity is weak and does not cause a problem for the analysis; when between 100 and 1,000, multilinearity is moderate to strong; and when it is greater than 1,000, multilinearity is severe (Montgomery *et al.*, 2006). Severe multicollinearity overestimates both the values of the simple correlation coefficients and the direct effects on the grain yield character estimated through the track analysis (Coimbra *et al.*, 2005) if it is necessary to correct this factor.

With the finding of moderate to strong multicollinearity, except for Season 1, for the other environments, the trail analysis was performed through crest regression, that is, under multicollinearity, proposed by Carvalho (1995), and the correlations were unfolded in direct and indirect effects of the chemical components of soybean grain (independent variables) on grain yield (basic variable) (Wright, 1921).

The analyses were performed using the Computational Genes program.

RESULTS AND DISCUSSION

The values of phenotypic correlation coefficients for chemical components and grain yield in the 2014/15 and 2015/16 harvests are found in Table 2.

For the 2014/15 harvest, grain yield showed a negative correlation with protein (-0.812) and a positive correlation with carbohydrates (0.785), indicating that increases in grain yield are associated with increases in carbohydrate content and reduction in protein content. In addition, the protein showed a negative correlation with carbohydrates (-0.606). In this harvest, no significant associations of oil, FDN, and FDA contents were detected with any of the characters.

The negative correlation of grain yield with protein content and positive carbohydrate content and negative correlation of carbohydrate-protein content occurs as a function of competition for carbon skeletons available for carbohydrate and protein yield.

It is emphasized that in this harvest there was a lower precipitation index in January and February (Table 1), in the grain filling phase (February), which may have further favored the increase in carbohydrate content and the reduction in grain protein content.

Under conditions of low water availability, there is a reduction in protein content, due to a higher activity of the enzyme protease and a lower supply

Table 1. Monthly temperature and precipitation data in two agricultural years (Harvests) 2014/15 and 2015/16, in the municipalities of Porto Nacional and Santa Rosa.

Period	Climate data			
	Crop 2014/15		Crop 2015/16	
	Average temperature (°C)	Precipitation (mm)	Average temperature (°C)	Precipitation (mm)
October	28.0	152	27.0	90
November	27.5	206	27.5	176
December	26.5	241	29.5	133
January	26.0	261	26.0	485
February	26.5	165	28.0	72
March	27.0	142	26.5	260
Epoch 1		178		203
Epoch 2		187		212
Average crop	26.9	194	27.4	204
Full crop		1166		1215

Table 2. Pearson phenotypic correlation coefficients between grain yield and five chemical components of the grain (oil, protein, carbohydrate, neutral digestive fiber - Fdn and acid digestive fiber - Fda) of eight soybean cultivars in two agricultural years (2014/15 crop and 2015/16 crop) in the State of Tocantins.

Components	Oil	Protein	Carbohydrate	FDN	FDA	Grain production
Oil	1	-0,570	-0,217	0,031	0,293	0,313
Protein	-0,371	1	-0,606*	-0,465	-0,448	-0,812*
Carbohydrate	-0,762*	-0,149	1	0,238	0,457	0,785*
Fdn	0,647*	0,102	-0,771*	1	0,113	0,052
Fda	-0,570	-0,226	0,629*	-0,652*	1	0,356
Grain yield	0,315	-0,475	0,064	0,453	-0,257	1

Values for Year 1 (Harvest 2014/15) are presented in the upper diagonal of the table; Values for Year 2 (Crop 2015/16) are presented in the lower diagonal 1/ Fdn: cellulose, hemicellulose, and lignin; Fda: cellulose and lignin.

of N, as a result of low modulation and fall in the symbiotic fixation process (Albrecht *et al*., 2008). In addition, there are increases in the biosynthesis of sucrose, which is translocated via phloem and stored as a source of carbohydrates (Toller *et al*., 2018).

Carbohydrates play a key role in maintaining osmotic adjustment in soybean plants. Under conditions of low water availability, there is usually an increase in carbohydrate content, related to the translocation rate of osmosolutes into the storage drains, aiming at restoring osmotic adjustment (Toller *et al*., 2018).

In the 2015/16 crop (year 2), concerning the other environments, a greater number of significant correlations were observed between the characters, except for grain yield and protein content, which did not present significant associations. In this harvest, the carbohydrate content showed a negative correlation with oil (-0.762), neutral digestive fiber (Fdn) (-0.771), and positive acid digestible fiber (Fda) (0.629), indicating that increases in carbohydrate content result in a fall in oil and Fdn contents and an increase in The Fda content. In addition, Fdn showed a positive association with oil (0.647) and a negative association with Fda (-0.652).

The negative correlation between the characters comes from the competition between these characters for the carbon skeletons of photosynthesis during the grain filling phase (Albrecht *et al*., 2008).

The positive and significant correlation of carbohydrate with Fda and also of the oil content with Fdn may be related to the fact that Components of FDA and Fdn, such as lignin, when present in higher concentration in the grain, contribute to greater thickness and resistance of its integument, reducing the hydration speed of seeds (França Neto *et al*., 2007), the respiratory process and lipid peroxidation of cell membranes (Ávila & Albrecht, 2010), thus minimizing losses due to deterioration, even in extreme stress conditions from high temperatures associated with low water availability during the grain filling phase (Faria *et al*., 2018).

According to France Neto *et al*. (2007), the increase in lignin content in soybean seed integument confers less permeability to the tegument, which reduces the effect of environmental moisture fluctuations on the seed, especially in the maturation and pre-harvest phases, making the seeds less susceptible to deterioration. In addition, the permeability of the integument may reflect greater mechanical strength, also resulting in higher storage potential and a lower rate of occurrence of mechanical damage.

Phenotypic correlations for chemical components and grain yield in the two sowing seasons (season 1 and season 2) are found in Table 3.

In the first sowing season, grain yield showed a negative and significant correlation with protein content (-0.724) and positive with oil content (0.615), indicating that increases in grain yield result in a decrease in protein content and increases in oil content in grains. In addition, a negative and significant correlation was observed between oil and protein contents (-0.634). For the other characters, there was no significant association.

According to Albrecht *et al.* (2008), the negative correlation between oil and protein levels in grain can be explained by the competition between them by carbonic skeletons of photosynthesis. These results are in agreement with those obtained by Finoto *et al.* (2017) and Faria *et al.* (2018) who also found a negative correlation between oil and protein contents.

In the second sowing season, significant

Table 3. Pearson phenotypic correlation coefficients between grain yield and five chemical components of the grain (oil, protein, carbohydrate, neutral digestive fiber - Fdn and acid digestive fiber - Fda) of eight soybean cultivars in two sowing seasons in the State of Tocantins.

	Oil	Protein	Carbohydrate	Fdn	Fda	Grain yield
Oil	1	- 0.634*	-0.144	-0.357	0.038	0.615*
Protein	-0.140	1	-0.565	0.505	-0.175	-0.724*
Carbohydrate	-0.084	-0.767*	1	-0.573	0.189	0.317
Fdn	0.068	-0.488	-0.067	1	-0.395	-0.235
Fda	-0.281	-0.406	0.751*	-0.466	1	-0.092
Grain yield	0.525	-0.412	0.476	0.023	0.335	1

Values for Season 1 are displayed on the upper diagonal of the table; Values for Season 2 are displayed on the lower diagonal. Season 1 (Planting carried out between 03 and 15 November); Season 2 (Planting carried out between 18 and 30 November). 1/ Fdn: cellulose, hemicellulose, and lignin; Fda: cellulose and lignin.

correlations were observed only between the carbohydrate contents with protein (-0.767) and with Fda (0.751), demonstrating that, by increasing the carbohydrate content in the grains, there was a reduction in protein content and an increase in the content of neutral digestive fibers (Fda). These results agree with those obtained by Moraes *et al.* (2006), which biochemically characterized two high protein soybean isolines, verified that the increase in protein content was accompanied by a reduction in total carbohydrate content.

A probable explanation for the change in the correlations between characters in soybean as a function of crop seasons and sowing times was due to the interaction genotypes x environments. In this case, the environmental factor can be attributed to climatic variations resulting from rain and precipitation in the years and sowing times (Table 1), influencing the behavior of genotypes and the chemical composition of the grains. Characters that correlate positively with some and negatively with others should be observed with greater attention and care because when selected, this type of character can promote undesirable changes in others (Coimbra *et al.*, 2005). For this and other reasons, track analysis becomes an important strategy for breeding programs; allowing researchers to prioritize characters with a higher degree of association (of greater relevance) to obtain cultivars with higher grain yield in a short period.

The estimates of the direct and indirect effects of the chemical components of soybean grain on grain yield in both seasons (2014/15 and 2015/16) and two sowing seasons are presented in Table 4, and the interpretation of the effects of track coefficients is based on Pearson correlation coefficients (r) and direct effect for each character studied.

The coefficients of determination found were considered as high and the effects of the residual variable were low, that is, the latter was always inferior to the main direct and indirect effects for all environments, even for those in which they were carried out under multilinearity (Crop 2014/15, Crop 2015/16 and Epoch 2), indicating that the set of seven variables explains the variation found in grain yield.

The track analysis recorded some values of coefficients higher than the unit that, according to Grace and Bollen (2005), can be explained by the fact that the trail coefficients were estimated based on standardized data being, therefore, obtained in the same way as the regression coefficients.

When the values of correlation (r) and direct effect are similar in magnitude and signal, the correlation explains well the association between the variables; if the r was positive and the direct effect is low and/or negative, the correlation that exists is due to indirect effects, indicating that the truncated selection in the auxiliary variable can provide satisfactory gains in the main variable. In this case, the best strategy is the simultaneous selection of variables, with emphasis also on those whose indirect effects are significant; when the r-value is low and/or negative and the direct effect was positive and high, the lack of correlation is caused by indirect effects; and if the r was negative and the direct effect was positive and high, the indirect effects were ignored and only direct effects were considered (Zuffo *et al.*, 2018).

The highest values of direct effects on grain yield (PG) for all characters were obtained in the second sowing season (2^a epoch), with protein content (1.382) and Fdn (1.131) being the characters with the highest direct effects. However, all correlations with the response variable (PG) were of low magnitude, due to the indirect effects, i.e., in the opposite direction, of the explanatory variables on the response variable.

At this time, the indirect effect via % protein was responsible for the low correlation of all characters with PG. In addition, the indirect effects resulting from the oil and Fda content also contributed to the low correlation of the other characters with PG. In this case, increased protein, oil, or Fda may cause a decrease in other explanatory variables, even though they have a direct effect on grain yield. According to Coimbra *et al.* (2005), it is expected that the truncated selection on the auxiliary character may not provide satisfactory gains in the basic variable (productivity). In these cases, the best strategy will be the simultaneous selection of characters, with emphasis also on characters whose indirect effects are significant (Cruz and Carneiro, 2003).

Because of the above, the carbohydrate content, despite the low correlation with grain yield (0.476), showed an indirect effect via the Fda favorable to the correlation with PG. Similarly, the Fda presented a low correlation with PG (0.335) but had the indirect effect via carbohydrate as favorable to the correlation with the basic variable. Thus, at this time, the most favorable situation for indirect selection aiming to obtain more productive genotypes would be using carbohydrate and Fda characters.

In the 2014/15 crop (year 1), the greatest direct effect on grain yield was derived from the carbohydrate content (0.702), which also presented a high correlation (0.783), indicating that the correlation explains well the association between the characters and, that the carbohydrate content, can be used in indirect selection for grain yield. The protein content showed a high correlation (-0.812) and low direct effect (-0.423), with the indirect effect via carbohydrate % (-0.425) being the main responsible for the high correlation, which confirms the

Table 4. Estimation of the direct and indirect effects of the chemical components of soybean grain on grain yield of eight soybean cultivars sowed in two crops (2014/15 and 2015/16) and two sowing seasons, in the municipalities of Porto Nacional and Santa Rosa -TO.

Route of association	Crop 2014/15	Crop2015/16	1º Epoch	2º Epoch
		Oil Content		
Direct effect on grain yield	0.273	0.090	0.901	0.982
Indirect effect via % Protein	0.241	0.164	-0.007	-0.194
Indirect effect via % Carbohydrate	-0.153	-0.693	-0.106	-0.080
Indirect effect via % of Fdn	-0.009	0.603	-0.168	0.077
Indirect effect via % of Fda	-0.056	0.147	-0.002	-0.274
Full effect (Pearson correlation)	0.313	0.315	0.615*	0.525
		Protein Content		
Direct effect on grain yield	-0.423	-0.440	0.012	1.382
Indirect effect via % Oil	-0.156	-0.034	-0.570	-0.138
Indirect effect via % Carbohydrate	-0.425	-0.136	-0.417	-0.728
Indirect effect via % of Fdn	0.131	0.095	0.239	-0.552
Indirect effect via % of Fda	0.086	0.058	0.013	-0.395
Full effect (Pearson correlation)	-0.812*	-0.475	0.724*	-0.412
		Carbohydrate Content		
Direct effect on grain yield	0.702	0.909	0.739	0.950
Indirect effect via % Oil	-0.059	-0.069	-0.129	-0.083
Indirect effect via % Protein	0.256	0.066	-0.006	-1.059
Indirect effect via % of Fdn	-0.067	-0.719	-0.271	-0.076
Indirect effect via % of Fda	-0.088	-0.162	-0.014	0.730
Full effect (Pearson correlation)	0.785*	0.064	0.317	0.476
		FDN content		
Direct effect on grain yield	-0.282	0.933	0.474	1.131
Indirect effect via % Oil	0.008	0.058	-0.321	0.067
Indirect effect via % Protein	0.197	-0.045	0.006	-0.675
Indirect effect via % Carbohydrate	0.167	-0.701	-0.424	-0.063
Indirect effect via % of Fda	-0.022	0.168	0.030	-0.453
Full effect (Pearson correlation)	0.052	0.453	-0.235	0.023
		FDA Content		
Direct effect on grain yield	-0.191	-0.257	-0.076	0.973
Indirect effect via % Oil	0.080	-0.051	0.034	-0.276
Indirect effect via % Protein	0.189	0.099	-0.002	-0.561
Indirect effect via % Carbohydrate	0.321	0.572	0.140	0.713
Indirect effect via % of Fdn	-0.032	-0.608	-0.187	-0.527
Full effect (Pearson correlation)	0.356	-0.257	-0.092	0.335
Coefficient of determination (R^2)	0.90	0.78	0.70	0.75
K value used in the analysis	0.06	0.04	—	0.01
Effect of residual variable	0.32	0.46	0.57	0.50

*Significant based on the evaluation method proposed by Dancey and Reidy (2005), considering values greater than or equal to 0.6.

importance of the carbohydrate content in the selection process aiming at increasing productivity in this crop.

For the 2015/16 crop (year 2), the carbohydrate (0.909) and Fdn (0.933) contents showed the greatest direct effects with grain yield, but low correlation (0.064 and 0.453, respectively), due, respectively, to indirect effects via Fdn and carbohydrate pathway. In this case, carbohydrate and Fdn contents cannot be neglected for indirect selection for grain yield.

The oil content in the first epoch presented a high and significant correlation (0.615) and high direct effect (0.901), indicating a strong relationship between the variables under study, demonstrating that the correlation alone explained this relationship. The carbohydrate content presented a high direct effect (0.739) and low correlation (0.317), with a low correlation resulting from indirect effects via oil (-0.129) and Fdn (-0.271). Also at this time, the protein content presented a high correlation (0.724) and low direct effect (0.012), where the greatest indirect effects responsible for the high correlation were oil (-0.570) and carbohydrate (-0.417). Thus, at this time, the selection of soybean cultivars with higher oil content for this environment will result in the indirect selection of genotypes with higher grain yields.

The fact that there are changes in the value of correlations between the same characters, between sowing times and/or harvests (years), resulting in changes in direct and indirect effects on grain yield, suggests that the selection of plants based on the chemical composition of the grains will only result in some gain if it is performed in the genotype adaptation environment.

Conflict of Interest

There is no conflict of interest between the authors. All authors contributed directly to the article.

CONCLUSION

Sowing time and year influenced the magnitude of phenotypic and genotypic correlations and direct and indirect effects on grain yield.

The carbohydrate content was the chemical component that most explained variations in grain yield and can be used in indirect selection to increase productivity.

The selection of plants based on the chemical composition of the grains should always be carried out in the adaptation environment of the genotype.

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