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**RESEARCH ARTICLE**

# Canonical interrelationships in morphological characters, yield and nutritional components of corn

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### **ABSTRACT**

The increase in the world population, the need to increase food production, both in quantity and quality, becomes increasingly prominent. The objective of this work was to identify the canonical correlations between yield components, morphological characters, micronutrients, bioactive compounds and amino acids in corn. The experimental design used was a randomized block containing 11 treatments arranged in three replications. The treatments consisted of 11 Top Crosses hybrid genotypes, these being made through crosses directed between a narrow genetic base tester hybrid for specific combining ability with 11 S5 inbred lines. It is inferred that groups considered yield components, secondary traits, bioactive compounds, micronutrients and amino acids are dependent. Promising characters are identified for the corn breeding for high yields, nutritional and energetic quality of corn grains. The indirect selection of grains with additions in essential amino acids can be directed to plants with superiority in height, mass and width of grains, phenols, flavonoids, soluble solids and zinc content.

**Keywords**: *Zea mays*, nutracenthic compounds, metabolomics, biofortification, nutritional properties, uses of corn.

#### **INTRODUCTION**

Corn (*Zea mays*) stands out as one of the most important crops in world agribusiness. Its importance is linked to its great diversity of use, mainly in human food, being raw material for the elaboration of starches, flours, canjica (dessert), breads, drinks and porridge, and animal, mainly in the production of silage and elaboration of rations and concentrates.

Brazil is the third largest corn producer in the world, behind only the United States of America and China. In the 2020/2021 crop season, the Brazilian area sown with corn surpassed 19 million hectares with a productivity of 4,371 kg ha<sup>-1</sup> of grains, representing the most produced cereal in the country. The South Region is the second largest corn producer, accounting for about 28% of total corn production nationwide (Companhia Nacional de Abastecimento [CONAB], 2021).

With the increase in the world population, the need to increase food production, both in quantity and quality, becomes increasingly prominent. In this sense, the plant breeding has a fundamental role regarding the identification of genotypes that are more productive and with nutritional properties that meet people's basic needs. The identification of superior genotypes is based on direct and indirect selection strategies. In view of this, the use of linear and canonical relationships makes it possible to facilitate the understanding of the degree and tendency of association of pairs or groups of characters.

Indirect selection is an important tool for increasing the efficiency of plant breeding programs. These biometric approaches allow the selection of characteristics with low heritability, highly influenced by the environment, difficult and costly to measure. In this context, reveals that this method allows to analyze the interrelationships of groups with varying number of characters, where the associations are explainable in a simple way through a few scenarios and associations. Based on the above, the objective of this work was to identify the canonical correlations between yield components, morphological characters, micronutrients, bioactive compounds and amino acids in corn.

#### **MATERIAL AND METHODS**

The experiment was carried out in 2018 at the Federal University of Pelotas. The seeds were produced in the 2017/2018 crop season in Capão do Leão, RS, Brazil, under the geographical coordinates 31º47'58''S and 52º31'02''W, with an altitude of 13.2 meters. The soil is classified as dystrophic red yellow Argisol (Ultisol) and the climate is characterized by Köppen as subtropical Cfa.

The experimental design used was a randomized block containing 11 treatments arranged in three replications. The treatments consisted of 11 Top Crosses hybrid genotypes (IRC001, IRC002, IRC003, IRC004, IRC005, IRC006, IRC007, IRC008, IRC009, IRC010 and IRC011), these being made through crosses directed between a narrow genetic base tester hybrid for specific combining ability with 11 S5 inbred lines.

The plots contained 10 meters in length and four sowing lines spaced by 0.5 meters, with all genotypes arranged in the population of 80 thousand plants, for all treatments, basic fertilization with 350 kg per hectare of NPK was used in the 10-20- 20 formulation and for topdressing, 250 kg per hectare of urea applied at the phenological stage V4. To measure the yield to grains and morphological components, 10 corn ears per experimental unit were selected. The control of diseases, insect pests and weeds was performed preventively in order to minimize the external effects on the results of the experiment.

The measured characters followed the methodology proposed by Carvalho et al. (2015) and these being: plant height (PH), results in centimeters (cm); ear diameter (ED), results in millimeters (mm); ear length (EL), results in cm; number of grain rows of the ear (NRG), results in units; number of grains per ear row (NGR); ear mass (EM), results in g; ear grain mass (EGM), results in g; thousand grain mass (TGM), results in g; seed length (SL), expressed in millimeters; seed width (SW), expressed in millimeters; seed thickness (ST), expressed in millimeters; and grain yield (GY), results in kg ha $^{-1}$ .

The post-milling characters measured were: total phenols (PHE) in  $\mu$ g g<sup>-1</sup>; total flavonoids (FLAV) in μg g<sup>-1</sup>; total carotenoids (CAR) in μg g<sup>-1</sup> (Aoac, 2005); antioxidant potential by the radical DPPH (DPPH) in percentage of inhibition; antioxidant potential by the radical ABTS (ABTS) in percentage of inhibition; soluble solids (SS) in ° Brix; and seed color (HUE) at Hue angle; The micronutrients: copper (Cu), zinc (Zn), sodium (Na), manganese (Mn) and iron (Fe) were expressed in mg  $kg<sup>-1</sup>$ .

Subsequently, the *LC-ESI-qToF-MS* method of mass spectrometry was used to quantify the intensities of the amino acids contained in corn seeds, which are: alanine (ALA), arginine (ARG), asparagine (ASP), glycine (GLYC), proline (PROL), serine (SER), threonine (THR), tryptophan (TRYP) and methionine (MET). The collected data were subjected to the assumptions of the statistical model, normality by Shapiro Wilk, homogeneity of residual variances by Bartlett and additivity of the statistical model, after which the analysis of variance at 5% probability was performed. Subsequently, a multicollinearity analysis was performed.

Thus, for the characters that showed variability, the canonical correlations were performed through the groups of characters focused on yield components (EM, EGM, TGM, SL, SW, ST and GY), secondary characters (PH, ED, EL, NRG and NGR), bioactive compounds (PHE, FLAV, CAR, DPPH, ABTS, SS and HUE), micronutrients (Fe, Cu, Zn, Na and Mn) and amino acids (ALA, ARG, ASP, GLYC, PROL, SER, THR, TRYP and MET). The significance of the canonical pairs was based on the likelihood rate at 5% probability (LRT). After multiple linear regressions were performed to select determinant characters for the construction of the causal diagram of amino acids, first a multicharacter was built through the linear combination of the nine amino acids, as follows: multi-character index of amino acids (MIA), yi: observation at the experimental unit level: sample standard deviation of the character of interest (Table 1).

$$
\sum MA = \left[\frac{yi (ALA)}{s(ALA)} + \frac{yi (ARG)}{s(ARG)} + \frac{yi (ASP)}{s(ASP)} + \frac{yi (GLYC)}{s(GLYC)} + \frac{yi (PROL)}{s(PROL)} + \frac{yi (SER)}{s(SER)} + \frac{yi (THR)}{s(TRYP)} + \frac{yi (TRYP)}{s(TRYP)} + \frac{yi (MET)}{s(MET)} + \right]
$$

Subsequently, a predictive model was developed based on the multiple regression of Step wise considering the statistical model:

$$
\hat{Y} = A + B_{(x, y \dots z)} + C_{(x, y \dots z)} + D_{(x, y \dots z \text{ F4})} + E_{(x, y \dots z)} + F_{(x, y \dots z)}
$$

Where: Ŷ: dependent character (multi-character index of amino acids (MIA), ALA, ARG, ASP, GLYC, PROL, SER, THR, TRYP and MET); A: data source point (intercept); B, C, D, E and F: multiple slope coefficients; x, y ... z: PH, ED, EL, NRG, NGR, EM, EGM, TGM, SL, SW, ST, GY, PHE, FLAV, CAR, DPPH, ABTS, SS, HUE, Cu, Zn, Na, Mn and Fe were considered as explanatory characters of the stochastic model. For the analysis, the R Software was used (R CORE TEAM, 2015).

**Table 1.** Predictive models for the selection of the determinant characters and load of the multiple slope coefficients of the regression.





<sup>1</sup>Plant height (PH), ear diameter (ED), ear length (EL), number of ear grain rows (NRG), number of grains per ear row (NGR), ear mass (EM), ear grain mass (EGM), thousand grain mass (TGM), seed length (SL), seed width (SW), seed thickness (ST), grain yield (GY), total phenols (PHE) , total flavonoids (FLAV), total carotenoids (CAR), antioxidant potential by the DPPH radical (DPPH), antioxidant potential by the ABTS radical (ABTS), soluble solids (SS), seed coloring (HUE), copper (Cu), zinc (Zn), sodium (Na), manganese (Mn) and iron (Fe).

#### **RESULTS AND DISCUSSION**

The estimates of canonical pair correlation coefficients established by the chisquare test between the groups were estimated and discussed with references to the greater canonical load of the second group in reference to the characteristics of the first group. Estimates of correlations between secondary traits and yield components, secondary traits and amino acids, secondary and bioactive traits, secondary and micronutrient traits, yield and amino acid components, yield and bioactive components, yield components and micronutrients, amino acids and bioactive, amino acids and micronutrients, bioactive and micronutrients, were explained in II, III, III, V, III, V, II, V, V and IV canonical pairs, respectively. Similar magnitude results were observed Alves, Cargnelutti-Filho, Burin and Toebe (2016), Nascimento et al. (2008) and Crevelari et al*.* (2019).

In the secondary traits (Figure 1), the condition of increases in ear diameter (ED) in canonical pair I and number of grains per row of ear (NGR) in canonical pair II, in addition to the reduction of the latter in III canonical pair, elevate the yield components: ear mass (EM), ear grain mass (EGM) and grain yield (GY) in the three canonical pairs described. The selection of plants with higher height, stem diameter, dry weight and shorter ear height resulted in an increase in the components of primary production (dry ear weight, ear length and hundred grain mass), allowing the targeting of corn breeding (Ceccon, Santos, & Teodoro, 2016). Zanatto et al*.* (2016) employing canonical correlations to identify characters related to productivity in grain sorghum hybrids, found that lower grades attributed to plant height increased crop yield levels. As found by Carvalho et al. (2015) when observing that the stem diameter of the sorghum tillers was directly related to the grain mass per ear.



Figure 1. Estimates of correlations and canonical pairs between secondary traits: plant height (PH), ear diameter (ED), ear length (EL), number of rows per ear (NRG), number of grains per ear row (NGR) and yield components: ear mass (EM), ear grain mass (EGM), thousand grain mass (TGM), seed length (SL), seed width (SW), seed thickness (ST), and grain yield (GY), in 11 corn genotypes.

The highest loads present in the canonical pairs of the secondary lines were observed in number of rows per ear (NRG), ear diameter (ED) and plant height (PH) with values of 0.84, 0.63 and 0.33, for I, II and III canonical pairs, respectively. Such positive charges contributed to the elevation of the amino acid proline (PROL) (Figure 2) in all related canonical pairs. Alves et al. (2016) found a positive phenotypic correlation between phenological and nutritional characters (protein and energy) in corn genotypes. For Nascimento et al. (2018), significant correlations between bromatological and agronomic variables show that, the higher the final state of the plant and the greater production of marketable ears, the greater dry mass and the higher crude protein content can be verified, the results show that, by indirect selection, based on the final state of the plant and the production of marketable tips, the authors can select corn genotypes for forage production.



**Figure 2.** Estimates of correlations and canonical pairs between secondary traits: plant height (PH), ear diameter (ED), ear length (EL), number of rows per ear (NRG), number of grains per ear row (NGR) and amino acids: alanine (ALA), arginine (ARG), asparagine (ASP), glycine (GLYC), proline (PROL), serine (SER), threonine (THR), tryptophan (TRYP) and methionine (MET), in 11 corn genotypes.

High and positive loads of the ear diameter (ED) in the I canonical pair, number of rows per ear (NRG) in the II canonical pair, and negative in the ear length (EL), justified the increase in the levels of total bioactive carotenoids (CAR) for all canonical pairs (Figure 3). Corroborating with Alves, Cargnelutti-Filho, Burin and Toebe (2017) that through the analysis of canonical correlation showed that morphological versus energetic-nutritional characteristics in early maturation genotypes, and phenological, morphological and productive versus energetic-nutritional characteristics in super early maturation genotypes are dependent, indicating a linear relationship between the studied characteristics. These estimates showed an inheritable association between characteristics. Therefore, they can be used in indirect selection processes in breeding programs (Crevelari et al., 2019).



**Figure 3.** Estimates of correlations and canonical pairs between secondary traits: plant height (PH), ear diameter (ED), ear length (EL), number of rows per ear (NRG), number of grains per ear row (NGR) and bioactive: total phenols (PHE), total flavonoids (FLAV), total carotenoids (CAR), antioxidant potential by the radical DPPH (DPPH), antioxidant potential by the radical ABTS (ABTS), soluble solids (SS) and coloring of the seeds (HUE), from 11 corn genotypes.

The levels of the micronutrient Manganese (Mn) (Figure 4) increased in all the canonical pairs analyzed in the sequence, with the most relevant conditions for increasing the ear length (EL), number of rows per ear (NRG), number of grains per ear row (NGR), plant height (PH) and together in the last canonical pair with the variables of ear length (EL) and number of rows per ear (NRG). Rigo et al. (2018) points out that the magnitudes of micronutrients are dependent on the genetic constitutions of soybean cultivars, where variations in the content of boron, copper, manganese, zinc, molybdenum, sodium and aluminum are expressed, while iron is the most stable micronutrient in soybean seeds.

Alves et al. (2017), verifying the linear dependence between the phenological, morphological and productive and energetic-nutritional characters in the corn genotypes, identified that the significant canonical pair showed that taller plants have lower levels of amylose in the grains and less apparent metabolizable energy corrected by nitrogen. Jaradat and Goldstein (2018) identified that the micronutrients Fe and Zn were responsible for explaining the genetic variation found among corn genotypes.



**Figure 4.** Estimates of correlations and canonical pairs between secondary traits: plant height (PH), ear diameter (ED), ear length (EL), number of rows per ear (NRG), number of grains per ear row (NGR) and micronutrients: iron (Fe), copper (Cu), zinc (Zn), sodium (Na) and manganese (Mn), in 11 corn genotypes.

The canonical correlations between the variables Yield components and Amino acids (Figure 5) in the first canonical pair explained cause and effect of positive dependence, where the reduction of the amino acids alanine (ALA) and arginine (ARG), are linked to the increase of all yield components.

The second canonical pair explains that the increase in the content of the amino acids Alanine (ALA), Arginine (ARG), Asparagine (ASP), Proline (PROL), Serine (SER), Tryptophan (TRYP) and Methionine (MET), are correlated to reduction of the seed length (SL) and seed thickness (ST) components. This situation can be explained by nitrogen deficiency, given that this element is a component of the structure of amino acids, as well as, emphasizes Alzaben, Fraser and Swanton (2019), by stating that nitrogen is one of the most important nutrients to limit corn yield, and supported by Cates and Ruark (2017) with decreases in the yield of Poaceae due to the reduction of the organic matter content of the soil and consequently the supply of nitrogen.



**Figure 5.** Estimates of correlations and canonical pairs between yield components: ear mass (EM), ear grain mass (EGM), thousand grain mass (TGM), seed length (SL), seed width (SW), seed thickness (ST) and amino acids: alanine (ALA), arginine (ARG), asparagine (ASP), glycine (GLYC), proline (PROL), serine (SER), threonine (THR), tryptophan (TRYP) and methionine (MET), in 11 corn genotypes.

Positive correlation of the amino acids alanine (ALA), asparagine (ASP), serine (SER), tryptophan (TRYP) and methionine (MET), was verified with the components of the yield ear mass (EM), ear grain mass (EGM), seed thickness (ST) and grain yield (GY), in the third canonical pair. Alves et al. (2016), found that the amino acids of greatest interest for animal nutrition (tryptophan and lysine) have a negative and significant correlation with grain yield, not allowing to select plants with high productivity and high levels of tryptophan and lysine.

The estimates of the correlations and canonical pairs between Yield components and Bioactive (Figure 6) revealed the pairs of greater magnitude in the increase of the thousand grain mass and reduction of the total flavonoids (FLAV) in the canonical pair I. For the canonical pair II, the reduction of the seed width (SW) provided greater seed coloration (HUE), increased seed length (SL), increased the antioxidant potential by the radical (ABTS). In the last canonical pair, high levels of the antioxidant potential by the radical (DPPH) were obtained with the reduction of seed length (SL). For Alves et al. (2017) the existence of variability between genotypes and the use of phenotypic correlation allow us to infer that it is possible to indirectly identify characteristics indicative of energy-nutritional quality in corn kernels, even in the field.



**Figure 6.** Estimates of correlations and canonical pairs between yield components: ear mass (EM), ear grain mass (EGM), thousand grain mass (TGM), seed length (SL), seed width (SW), seed thickness (ST) and bioactive: total phenols (PHE), total flavonoids (FLAV), total carotenoids (CAR), radical antioxidant potential (DPPH), radical antioxidant potential (ABTS), soluble solids (SS) and seed color (HUE), of 11 corn genotypes.

The correlations between the yield components and micronutrients were positive for all effects in the canonical pair I, as well as in the yield components of the canonical pair II, however with a reduction in the levels of copper (Cu) and zinc (Zn) (Figure 7). The morpho-agronomic characters showed a positive and high-magnitude correlation with most bromatological characteristics, such a result showed that the morpho-agronomic traits influenced bromatological features, since they increased simultaneously (Crevelari et al., 2019). Using only path analysis and canonical correlations allow a clearer interpretation of the association between two or groups of variables and the interference that others have in this association (Nascimento et al., 2018). It is important to note that the correlations in corn can be altered by the environmental conditions exposed to the genotypes. This statement is revealed in Tack and Holt (2016) by providing evidence those spatial correlations practically double in years of good and bad weather compared to normal years in corn yield.



**Figure 7.** Estimates of correlations and canonical pairs between yield components: ear mass (EM), ear grain mass (EGM), thousand grain mass (TGM), seed length (SL), seed width (SW), seed thickness (ST), and grain yield (GY) and micronutrients: iron (Fe), copper (Cu), zinc (Zn), sodium (Na) and manganese (Mn), in 11 corn genotypes.

The correlations between the amino acids and the bioactive ones were reported (Figure 8). It was observed that the amino acids that most contributed to the cause and effect relationship among the canonical pairs were: alanine (ALA) in the canonical pairs I and IV, serine (SER) in canonical pair II, proline (PROL) in canonical pair III and methionine (MET) in the last canonical pair. Factors such as a reduction in total phenols (PHE) and an increase in soluble solids in bioactives for canonical pairs I and II, respectively, can be highlighted. This identification of characteristics is possible because most of the phenotypic correlation is genetic. These data are important in plant breeding programs that aim to identify plants with higher nutritional quality of grains, with a focus on animal nutrition (Alves et al., 2017).

Higher and positive loads were addressed in the amino acids threonine (THR), serine (SER), arginine (ARG), proline (PROL) and alanine (ALA), in the canonical pairs I, II, III, IV and V correlated with the greater magnitude effects on micronutrients corresponding to the reduction and increase of manganese (Mn) and sodium (Na), as well as the reduction of zinc (Zn) and iron (Fe), respectively (Figure 9). According to Rigo et al. (2018) Molybdenum and copper are characterized as micronutrient determinants for the physiological quality of soybean seeds. Furthermore, the role of micronutrients in soybean correlates with environmental effects such as reducing the effect of water stress on commercial crops (Dimkpa et al., 2017). Associations between groups of variables are also reported by other crops such as soybeans (Pereira et al., 2017), where more productive plants with a higher number of pods and high oil content are associated with seeds with a high percentage of germination and emergence rate. Plants with a reduced maturation cycle are associated with seeds that have a high percentage of are more tolerant to conditions of accelerated aging.



**Figure 8.** Estimates of correlations and canonical pairs between amino acids: alanine (ALA), arginine (ARG), asparagine (ASP), glycine (GLYC), proline (PROL), serine (SER), threonine (THR), tryptophan (TRYP), methionine (MET) and bioactive: total phenols (PHE), total flavonoids (FLAV), total carotenoids (CAR), radical antioxidant potential (DPPH), radical antioxidant potential (ABTS), soluble solids (SS) and seed coloring (HUE), of 11 corn genotypes.

In the cause and effect estimates correlating the bioactive and the micronutrients (Figure 10), it was observed that in the four canonical pairs analyzed all the bioactive showed different behaviors in terms of magnitude and subtraction, or even addition, as well as observed for the micronutrients, except iron (Fe) which has always shown an increase in its levels. Crevelari et al. (2019) also observed high characteristics such as dry matter content, crude protein content, neutral detergent fiber, lignin, crude fat and mineral matter. For Song et al. (2018), the bioactive components are partially responsible for the nutritional and health benefits of soybeans, and these in turn, presented their studies correlations with environmental factors. Still on bioactive, Xu et al. (2017) revealed significant differences between soybean genotypes and an abundance of 25 differential metabolites. This information is useful in promoting the soybean breeding for functional food purposes.



**Figure 9.** Estimates of correlations and canonical pairs between amino acids: alanine (ALA), arginine (ARG), asparagine (ASP), glycine (GLYC), proline (PROL), serine (SER), threonine (THR), tryptophan (TRYP) and methionine (MET) and micronutrients: iron (Fe), copper (Cu), zinc (Zn), sodium (Na) and manganese (Mn), in 11 corn genotypes.

The canonical correlation analysis allows us to observe that only the phenological and nutritional energy characters considered have linear dependence, that is, the groups are dependent (Alves et al., 2016). Based on these results, the study of canonical correlations is important in understanding the relationships



magnitudes of the simple correlations do not always adequately reflect the causeeffect relationship between characters.

**Figure 10.** Estimates of correlations and canonical pairs between bioactive: total phenols (PHE), total flavonoids (FLAV), total carotenoids (CAR), antioxidant potential by the radical (DPPH), antioxidant potential by the radical (ABTS), soluble solids (SS) and seed color (HUE) and micronutrients: iron (Fe), copper (Cu), zinc (Zn), sodium (Na) and manganese (Mn), in 11 corn genotypes.

With the corn crop, the canonical correlation tool also showed expressiveness in the studies of power generation as described by Li, Zhang, Lu and Han (2019) and Liu and Chen (2016). Thus, the use of canonical correlation can simplify and determine which variables are most important (Nobre, Silva, Guimarães, Resende, & Macedo, 2018), however, other elements can be correlated in order to study the nutritional or even food constitution of the corn crop , as reported by Lao and Giusti (2016) when quantifying these components through the content of anthocyanins

#### **CONCLUSIONS**

It is inferred that groups considered yield components, secondary traits, bioactive compounds, micronutrients and amino acids are dependent.

Promising characters are identified for the corn breeding for high yields, nutritional and energetic quality of corn grains.

The indirect selection of grains with additions in essential amino acids can be directed to plants with superiority in height, mass and width of grains, phenols, flavonoids, soluble solids and zinc content.

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