AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.18, n.4, e3003, 2023
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v18i4a3003 - Protocol 3003
Subm. 22/11/2022 • App. 12/09/2023 • Pub. 08/11/2023

Path analysis associated with fungicide use in corn hybrids

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ABSTRACT: The objective of this study was to identify the linear relationships between the morphological variables of corn hybrids, associated with grain yield and fungicide use in subtropical environments. Seven experiments were conducted in three locations: Santa Maria - RS, São Vicente do Sul - RS, and Frederico Westphalen - RS, Brazil. The design used was a randomized block design with subdivided plots and three replications, where the main factor was the use or not of fungicide and the subplots corresponded to six corn hybrids. The following traits were evaluated: leaf angle, stem diameter, ear insertion height, ear length, ear diameter, number of rows, grain length, thousand-grain mass, percentage of damaged grains, and grain yield. The characters were subjected to Pearson correlation analysis and path analysis using R software. It can be concluded that there is a difference in the magnitudes of the linear relationships depending on whether or not fungicides are used on corn hybrids in subtropical environments, with cultural management influencing the results of the linear relationships. The characters ear diameter, grain length, ear length, and thousand-grain mass are positively related, while leaf angle and the percentage of damaged grains are negatively related to grain yield in corn hybrids.

Key words: indirect selection; linear correlation; *Zea mays* L.

Análise de trilha associado ao uso de fungicida em híbridos de milho

RESUMO: O objetivo deste estudo foi identificar as relações lineares entre as variáveis morfológicas de híbridos de milho, associadas a produtividade de grãos e ao uso de fungicida em ambientes subtropicais. Foram conduzidos sete experimentos em três locais: Santa Maria - RS, São Vicente do Sul - RS e Frederico Westphalen – RS, Brasil. O delineamento utilizado foi o de blocos ao acaso com parcelas subdivididas, com três repetições, onde o fator principal foi o uso ou não de fungicida e as subparcelas corresponderam a seis híbridos de milho. Foram avaliados os caracteres: ângulo foliar, diâmetro do colmo, altura de inserção da espiga, comprimento da espiga, diâmetro da espiga, número de fileiras, comprimento do grão, massa de mil grãos, porcentagem de grãos deteriorados e produtividade de grãos. Os caracteres foram submetidos a análise de correlação de Pearson e análise de trilha com auxílio do software R. Conclui-se que existe diferença nas magnitudes das relações lineares em função do uso ou não de fungicidas em híbridos de milho em ambientes subtropicais, com o manejo cultural influenciando nos resultados de relações lineares. Os caracteres diâmetro de espiga, comprimento de grãos, comprimento de espiga e massa de mil grãos relacionam-se positivamente, enquanto o ângulo foliar e a porcentagem de grãos deteriorados relacionam-se negativamente com a produtividade de grãos em híbridos de milho.

Palavras-chave: seleção indireta; correlação linear; *Zea mays* L.

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Associate Editor: Mário de Andrade Lira Júnior

Introduction

The production of corn (*Zea mays* L.) has an important economic role in Brazilian agribusiness, consolidating the country as the world third largest producer of corn grains, with an area of more than 22.4 million hectares. In recent years, the emergence of diseases affecting the corn crop has caused significant losses in grain yields. This is associated with the use of hybrids with lower tolerance to some diseases, climatic conditions, and the management practices adopted (Fraile & Arenal, 2016).

A study carried out by Souza et al. (2015) reports that the use of preventive fungicides at the V6 stage and another application at the pre-sprouting stage of corn plants reduced the severity of diseases, especially cercosporiosis, and could be an auxiliary measure for integrated disease management, since late applications are not possible for corn crops using ground sprays. This management, combined with the choice of hybrids with greater tolerance or genetic resistance to the main diseases, has the potential to increase corn production in Brazil.

The use of fungicides is not used in all corn crops, and their use can influence the morphological development of hybrids, including the corn plants ability to carry out photosynthesis. This, in turn, may be associated with characters related to grain yield, such as the number of grains per ear and grain mass. Other characters, such as leaf angle, may also be related to higher grain yields, since the canopy structure of higher yielding corn hybrids, which are more efficient in the use of resources (Liu et al., 2022), is characterized by a smaller leaf angle (Li et al., 2021). More upright leaves, i.e. with a smaller leaf angle, can improve the interception of solar radiation and photosynthetic efficiency.

Pearson correlation coefficient is used to assess the relationship between plant characters and grain yield, which has been widely applied in studies on corn (Bonea & Bonciu, 2019; Crevelari et al., 2020; Silva et al., 2021; Ruiz et al., 2022). Using this coefficient, it is possible to identify the measure of association between two variables with a linear relationship. Pearson correlation coefficient ranges from -1 to 1 and can be classified as perfect linear correlation, positive or negative, or null when $\hat{p} = 0$ (Cruz et al., 2004).

Path analysis was proposed in the early decades of the 20th century by Sewall Wright and continues to be a current and widely applied methodology in the study of linear relationships in corn crops (Crevelari et al., 2020; Fadhli et al., 2020; Silva et al., 2021). It can be used to perform a joint analysis of data, allowing the study of the direct and indirect effects of independent variables on a dependent variable. This methodology is multivariate and deals specifically with the relationship between a set of variables. In this analysis, Pearson linear correlation is broken down into direct and indirect effects, making it possible to determine the cause and effect relationship between the variables. It is also possible, using this analysis, to study the indirect influence of characters that interfere with grain yield (Olivoto et al., 2017).

For the corn crop, can the use of fungicides interfere with evaluations of linear relationships, such as Pearson and path analysis? These are important gaps in the literature that deserve to be highlighted. It is therefore important to study the relationship between these crop management methods and the plant characteristics of modern hybrids. The objective of this study was to identify the linear relationships between the morphological variables of corn hybrids, associated with grain yield and the use of fungicides in subtropical environments.

Materials and Methods

For this study, seven experiments were carried out in a randomized complete block design in subdivided plots, with three replications, where the main factor corresponded to the use or not of fungicide and the subplot corresponded to the cultivar. The experiment was conducted in three locations in the state of Rio Grande do Sul, Brazil: Santa Maria (SM, 29° 43' 28" S and 53° 43' 41" O, at an altitude of 95 m), Frederico Westphalen (FW, 27° 23' 42" S and 53° 25' 43" O, at an altitude of 480 m), and São Vicente do Sul (SVS, 29° 42' 27" S and 54° 41' 34" O, at an altitude of 129 m), in the 2020/2021 agricultural year.

According to the Köppen classification, the climate of the three sites is characterized as humid subtropical with no defined dry season (Cfa) (Alvares et al., 2013). During the experiment period, meteorological data was collected from the Instituto Nacional de Meteorologia automatic weather stations (codes A854 for FW, A803 for SM, and A889 for SVS), located less than 1,000 m apart at the three experiment sites. The soils of the sites are classified as 'Argissolo Vermelho Distrófico arênico' in SM and SVS, and 'Latossolo Vermelho Distrófico típico' in FW.

The experiments were sown at two sowing times in the three locations. Season 1 was between the dates of 20/08 and 20/09/2020, and season 2 was between the dates of 20/10 and 20/11/2020. A third experiment was conducted in SVS, sown in season 1. Three experiments were carried out at SVS: two with the use of supplementary irrigation by means of a center pivot and one experiment under rainfed conditions, where only sprinkler irrigation was used in two critical periods of water limitation, close to flowering, with the objective of not compromising plant development, as was done in the SM and FW experiments. This makes a total of seven experiments, four of which were sown in season 1 and three in season 2.

The treatments consisted of six corn hybrids chosen on the basis of their representative use by farmers in the regions, with and without fungicide application. The main plot consisted of two levels of the fungicide factor (with and without), while the subplot consisted of six levels of the corn hybrid factor (AG9025, AS1730, P3016, MG300, DKB230, and FEROZ). All hybrids show moderate to high resistance to most of the main corn crop diseases.

Each subplot consisted of six sowing rows spaced 0.5 m apart and 5 m long. Fertilization was carried out based on the recommendations in the fertilization and liming manual, for an expected grain yield of 12 t ha^{-1}. Base fertilization was carried out using a seeder with furrowing rods, spaced 0.50 m apart. After basic fertilization, the cultivars were sown by hand, with the sowing density adjusted to 70,000 corn plants per hectare. The fungicide treatments were carried out in the vegetative period, when the plants had eight true leaves (V8), and in pre-flowering (VT), using fungicides composed of the active ingredients Propiconazole, Picoxystrobin, and Cyproconazole. Application was carried out using a manual backpacking machine, with a spray flow rate of 200 L ha^{-1}, following the technical indications for the crop.

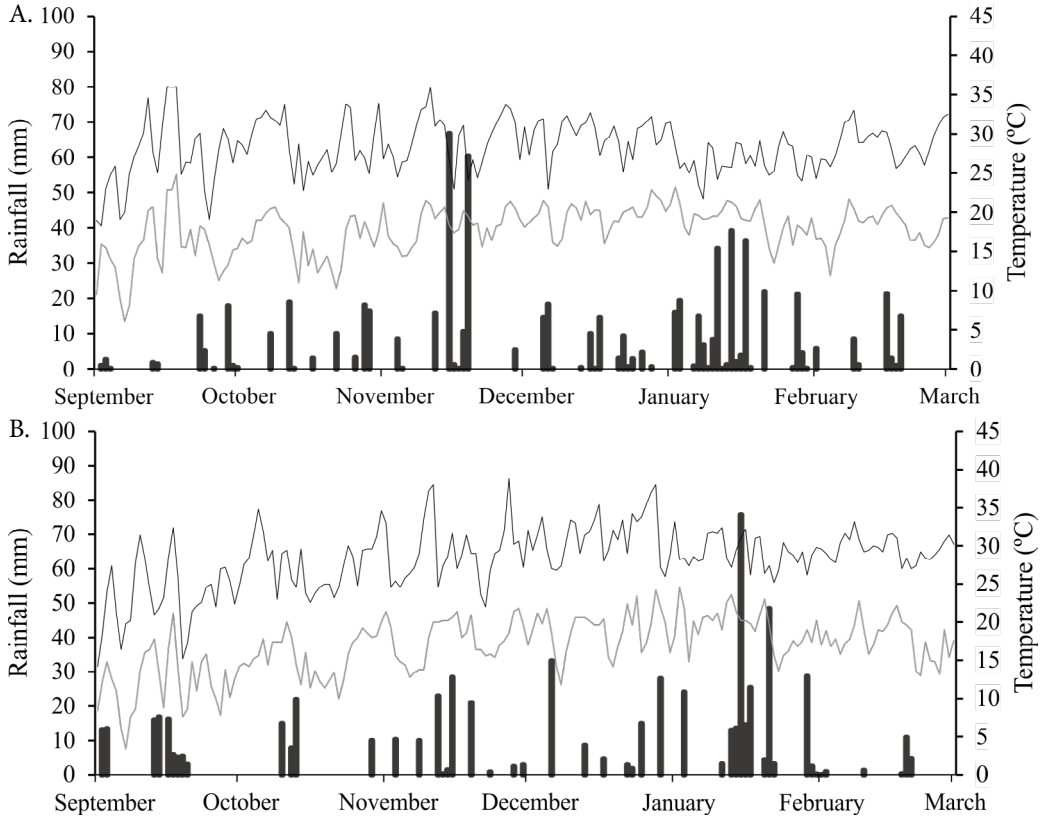
Eight random plants were evaluated in each subplot in the seven experiments, totaling 2016 corn plants evaluated. The variables measured were: plant height (PH, in cm), ear insertion height (EIH, in cm), leaf angle (LA, in degrees), stem diameter (SD, in cm), stuffing note (SN, from 0 to 10), number of ears (NE), ear length (EL, in cm), number of rows (NR), number of grains per row (NGR), ear diameter (ED, in cm), cob diameter (CD, in cm), grain length (GL, in cm), and percentage of damaged grains (PDG), totaling 13 variables. To better represent the control of fungal diseases with the use of fungicide, the grain yield values from the sub-plot were used. A total of 252 subplots with a useful area of 8 m^2 (4 central rows × 4 m central rows) were evaluated to measure grain yield (GY in kg ha^{-1}, corrected to 13% moisture).

The correlations were tested for multicollinearity according to the criteria of Montgomery et al. (2013), using the eigenvalues of the correlation matrix and the condition number (CN) as an indicator. The CN was estimated by dividing the highest eigenvalue by the lowest eigenvalue. If the condition is less than 100, multicollinearity is considered weak; if it is between 100 and 1,000, it is considered moderate to strong; and, if it is above 1,000, it is classified as severe, according to the criteria of Montgomery et al. (2013). The diagnosis of multicollinearity and the extent to which the variance of the coefficient is "inflated" were checked using the variance inflation factor test.

Based on the absence of multicollinearity, the variables used for Pearson correlation and path analysis were: EIH, LA, SD, EL, NR, ED, GL, TGM, PDG, and GY. Statistical analyses were carried out using Microsoft Office Excel® 2017 and R software (R Core Team, 2019). The metan package (Olivoto & Lúcio, 2020) was used to apply Pearson correlation and path analysis, and the ggplot2 package (Wickham, 2011) was used to generate the figures.

Results and Discussion

With regard to accumulated rainfall, the daily maximum and minimum temperatures during the experiments at the three sites are shown in Figure 1. The air temperature remained within the appropriate range for crop development. The amount of rainfall fluctuated during the period in which the experiments were conducted, but grain yields were 50% above the state average this year, producing 8,259 kg ha^{-1}, while the state average reported by official bodies was 5,470



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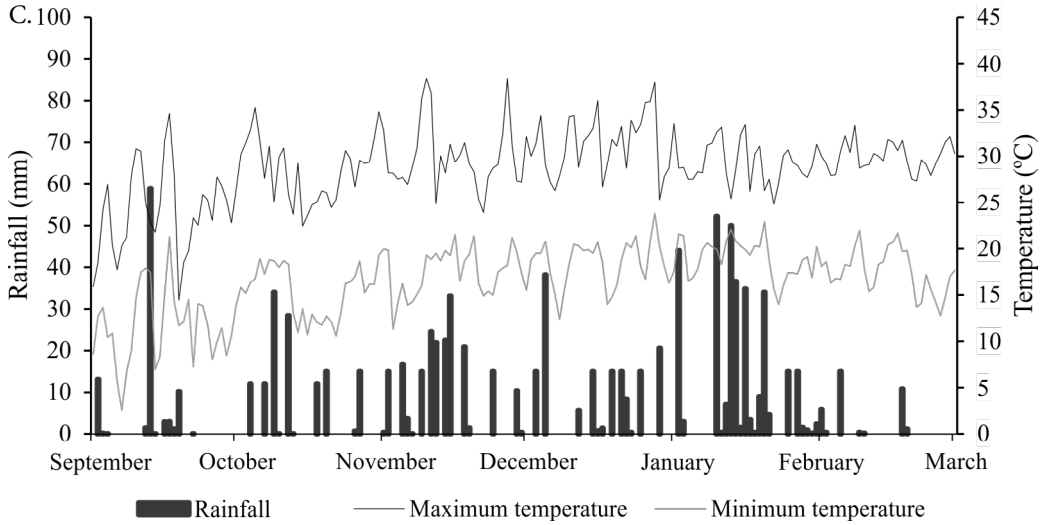

Figure 1. Rainfall, maximum and minimum temperature during the experiments in Frederico Westphalen (A), Santa Maria (B), and São Vicente do Sul (C), in the state of Rio Grande do Sul, Brazil, from September/2020 to March/2021, Santa Maria - RS, Brazil, 2022.

kg ha^{-1}, indicating adequate management practices during the conduct of the experiments.

When analyzing the response of GY to the application of fungicides on corn crops, Wise et al. (2019) reported that, regardless of the time of application or the active ingredient of the fungicide, higher GY occurs when foliar fungicides are used. This result may be associated with the plants greater ability to express the traits responsible for production potential when fungicide is used on the corn crop.

Evaluating the linear associations between the characters related to the treatments without fungicide application, described on the upper diagonal (Figure 2), it can be seen that the correlations are of a lower magnitude. Eighteen of these associations were not considered significant (p-value > 0.05). In the lower part, with the application of fungicides, this number was lower, with 15 associations not being significant. In addition, correlations such as ED, GL, and TGM have a stronger relationship with GY, indicating that in seven environments, the use of fungicide leads to better associations in the corn crop.

Management with fungicides can interfere with the evaluation of linear relationships. This result is in line with the study by Barbosa et al. (2016), who, when studying phenotypic effects and genetic dissimilarity in corn, concluded that ED and TGM are characters with a great response to GY. The linear association of LA is also greater with the use of fungicide, but there is a negative relationship with GY, ED, and TGM. Smaller LA, i.e. more upright leaves, is among the characteristics selected during plant genetic improvement (Wang et al., 2020). Corn hybrids with high yield potential depend heavily on canopy structure, which is determined by plant architecture (Liu et al., 2022), with the angle of leaf insertion with the stalk being one of the factors responsible for the greatest population increase in

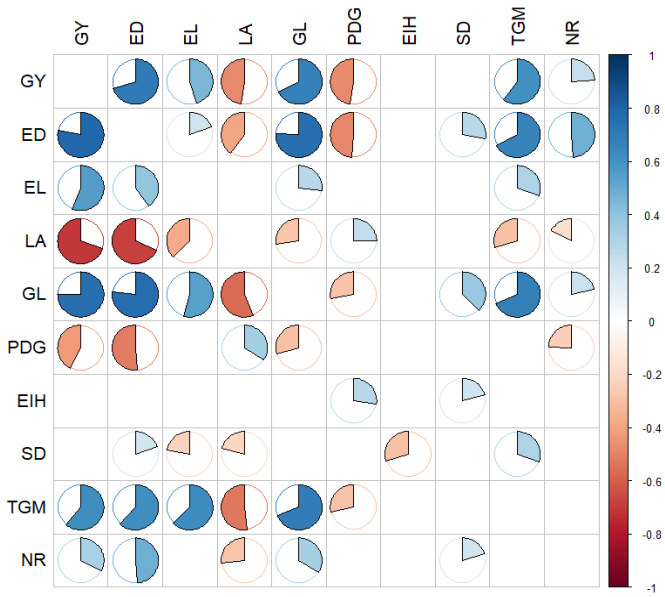

Figure 2. Pearson linear correlation between corn plant traits with and without fungicide application. The upper diagonal shows the values without fungicide application and the lower diagonal shows the values with fungicide application. The characters are: grain yield (GY), ear diameter (ED), ear length (EL), leaf angle (LA), grain length (GL), percentage of damaged grains (PDG), ear insertion height (EIH), stem diameter (SD), thousand-grain mass (TGM), and number of rows (NR). The values used are the averages per experimental plot, totaling 252 samples from seven experiments carried out in the municipalities of Santa Maria - RS, Frederico Westphalen - RS, and São Vicente do Sul - RS, Brazil. * Significant correlations are represented in blue (positive) and red (negative) according to the t-test ($p < 0.05$).

the vegetative canopy and for boosting GY. The smaller LA allows for better interception of solar radiation in the canopy, improving photosynthetic efficiency, leaf area duration,

resistance to stresses and, consequently, higher GY (Cao et al., 2022; Liu et al., 2022).

Bernhard & Below (2020) report that characters related to the capture of sunlight, such as angle, width, length, and leaf area, are important predictors of the productivity of corn plants. It can be seen that in the treatments in which no fungicide applications were made, the LA was lower (-0.48). However, in the fungicide treatments, this magnitude is higher (-0.70), which may be associated with a higher GY in the plants that were managed with fungicide. From this correlation, it can be inferred that modern hybrids, combined with fungicide management, have a lower LA, i.e. the positioning of hybrid cultivars with a low LA and adequate phytosanitary management leads to a higher GY.

When studying the cause and effect relationships between the variables (Figure 3), the LA character was the most negatively associated with GY in the treatments in which fungicides were applied (-0.70), indicating that greater LA is negatively related to GY. Cargnelutti Filho et al. (2020), when studying plant architecture and corn GY, concluded that the leaves close to the corn ear have a smaller LA and a larger leaf area, and these characteristics are responsible for a higher GY. However, in this study, management practices such as the use of fungicides were not associated.

There is a direct negative effect of PDG on GY, with the greatest magnitude in treatments without fungicide application (-0.47). PDG is a negative factor for grain yield and can be associated with fungal diseases that start on corn kernels such as *Diplodia maydis, Fusarium graminearum*, and *Fusarium sporotrichioides*, and is considered one of the main problems of corn grain quality (Mielniczuk & Skwaryło-Bednarz, 2020).

The characters GL, ED, EL, and TGM showed positive effects on GY in the treatments with fungicide application (A) (0.75, 0.78, 0.56, and 0.61, respectively). A similar result was obtained with the same direction between treatments, but with a lower magnitude where no fungicides were applied (B) (0.67, 0.70, 0.45, and 0.60), thus demonstrating that fungicide management interferes with linear relationships. Therefore, it is possible to obtain higher GY in corn crops by using fungicide treatment. These results coincide with the conclusions of Bortolini & Gheller (2012) and Wise et al. (2019), on the application of fungicides and effects on GY, where they state that the use of fungicides leads to a significant increase in corn GY. In this way, it is clear that the fungicide management applied to the corn crop influences the relationships between the variables, altering the estimates of Pearson correlation coefficients and, consequently, the direct and indirect effects between the variables obtained in the cause and effect analysis.

Therefore, lower LA (leaf angle) and positive traits associated with GY in corn should be taken into account when positioning cultivars and when plant breeders select them. In this sense, the positioning of hybrid cultivars with low LA and adequate phytosanitary management leads to higher corn yields.

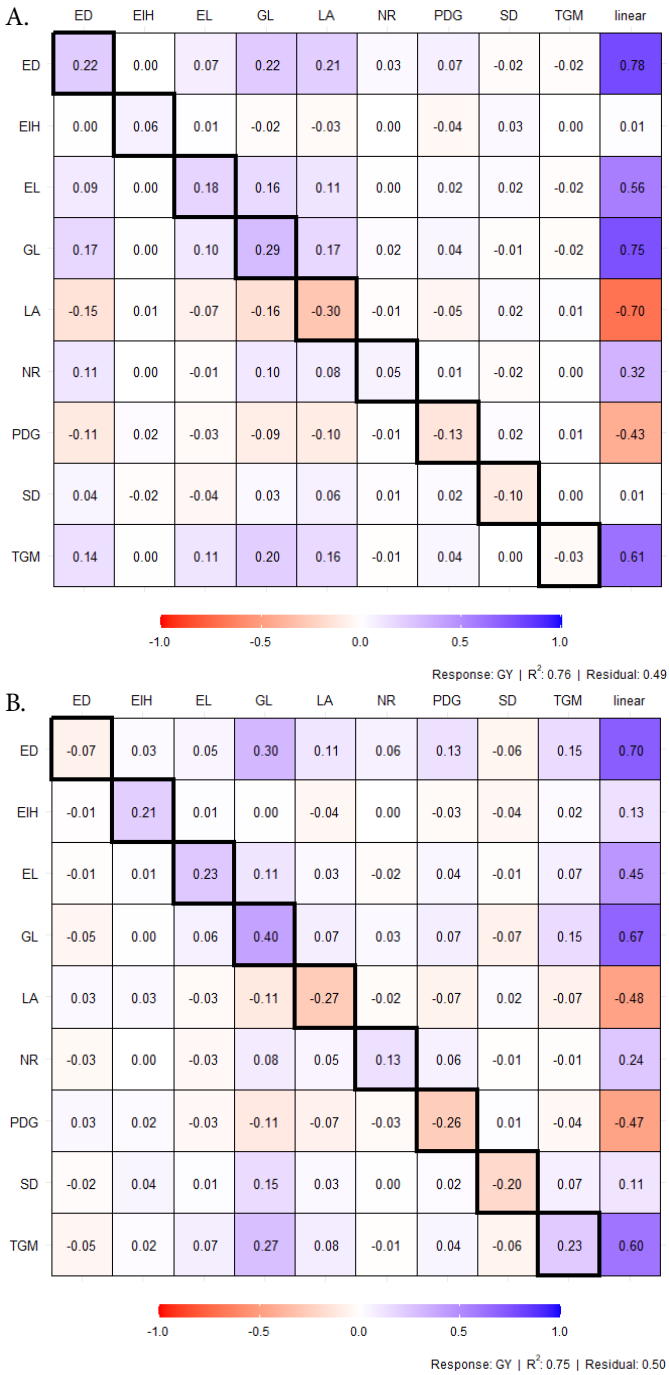

Figure 3. Estimation of the direct effects (diagonal), indirect effects (horizontal lines), and linear correlation (vertical line) of the characters ear diameter (ED), ear length (EL), leaf angle (LA), grain length (GL), percentage of damaged grains (PDG), ear insertion height (EIH), stem diameter (SD), thousand-grain mass (TGM), and number of rows (NR) on total grain yield (GY), for treatments using fungicides (A) and not using fungicides (B), collected from seven experiments conducted in the municipalities of Santa Maria - RS, Frederico Westphalen - RS, and São Vicente do Sul - RS, Brazil.

Conclusions

Therefore, there is a difference in the magnitudes of the linear relationships as a function of the use or not of

fungicides in the evaluation of corn hybrids in subtropical environments in Southern Brazil, with cultural management with fungicide influencing the results of linear relationships.

For subtropical environments in Brazil, the characters ear diameter, grain length, ear length, and thousand-grain mass are characters that are positively related to grain yield in corn hybrids and can be indicated for the indirect selection of corn hybrids in a year with water restriction.

Number of rows, stem diameter, and ear insertion height have a weak association and cause and effect relationship with grain yield in a year with water restriction.

Leaf angle and percentage of damaged grains have a negative cause and effect relationship with grain yield. For higher grain yields, hybrids with a smaller leaf angle and no or low percentage of damaged grains should be selected.

Compliance with Ethical Standards

Author contributions: Conceptualization: DNF, HSE, ADL; Data curation: DNF, HSE, ADL, TO; Investigação: DNF, HSE, ADL; Methodology: DNF, HSE, ADL; Project administration: DNF, HSE; Resources: DNF, ADL, VSM, ICM; Supervision: DNF, ADL, VSM, ICM; Validation: HSE, GBR, FTB; Visualization: DNF, ADL, VSM, ICM, TO, HSE, GBR, FTB; Writing - original draft: DNF, ADL, VSM, ICM, TO, HSE, GBR, FTB; Writing - review & editing: DNF, HSE, GBR.

Conflict of interest: The authors declare that there is no conflict of interest (personal or financial) that can influence the article.

Funding source: The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001 and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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