Effect of reduced spacing on relationship of physiological, morphological and productive traits of corn yield

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Abstract

Increasing corn grain production without devastating new forest areas is a viable alternative to controlling deforestation. However, increasing plant density in the area may alter plant morphophysiological and productive traits. The objective of this study was to characterize relationships between physiological, morphological and yield traits of corn plants, as well as the cause, effect and relationship of the traits on grain yield. The experiment was carried out in randomized complete block design with four replications. The corn hybrids (2B655PW, AG7088PRO3 and P4285YHR) were grown with row spacing of 40 cm, 60 cm, 80 cm, 95 cm. The evaluated traits physiological were: net photosynthesis (P_N), stomatal conductance (G_s), intercellular concentration of CO_2 (Ci), leaf transpiration (E), water use efficiency (WUE) and carboxylation efficiency (CE). The morphological were: plant height (PH) and ear insertion height (EIH), stem diameter (SD), and leaf area (LA) and the productive traits were the total number of ear per hectare (NE), number of grains per ear (NGE), grain mass per ear (GME), 100 grain weight (100GW) and grain yield (GY). The characteristics of maize hybrids cultivated in environment with reduced spacing (40cm, 60cm, 80cm, 95cm) of the 2016/2017 crop were investigated through the multicollinearity path analysis. The physiological, morphological and productive traits are considered sources of variation of cause and effect of corn yield in reduced spaced. This traits are essential for observations in maize breeding programs to obtain high yielding varieties in reduced spacing. In conclusion, the physiological (P_N, CE, WUE, Ci, Gs), morphological (EIH, SD, LA) and productive (NE, GME) traits provide gains in maize grain yield via indirect selection when the crop is subjected to 40 cm row spacing.

Keywords: Path Analysis; biometry; correlations; *Zea mays.*
Abbreviations: P_N_net photosynthesis; G_s_stomatal conductance; Ci_intercellular concentration of CO_2; E_leaf transpiration; WUE_water use efficiency; CE_carboxylation efficiency; PH_plant height; EIH_ear insertion height; SD_stem diameter; LA_leaf area; NE_total number of ear per hectare; NGE_number of grains per ear; GME_grain mass per ear; 100GW_100 grain weight; GY_grain yield.

Introduction

Maize crop (*Zea mays* L.) has significant importance in the world market, being crucial for global food security (Anjum et al., 2016). Meeting the needs of the consumer market requires large extensions of agricultural lands. However, environmental concerns about preserving native forests have led to greater control of this crops expansion. Thus, increasing grain yield by techniques that optimize cultivation in smaller areas is of interest to modern science (Saath and Fachinello, 2018). The growing need for grain leads to continuous research focused on genetic improvement and management technologies, aiming to increase productivity in cropping systems (Nardino et al. 2016). However, the selection of superior genotypes is quite complex because grain yield characteristics have quantitative action on genes

and must be considered simultaneously in grain yield (Ribeiro et al., 2014). In maize breeding programs, one of the basic objectives is to obtain genotypes with higher productive capacity (Olivoto et al., 2018). This can be achieved with management practices such as reducing row spacing capable of increasing maize productivity in small agricultural land (Nascimento et al., 2019). In contrast, with variations in row spacing, excessive amounts of plants per area may result in competition for survival components such as light, water and nutrients, interfering with corn physiology, growth and development (Pellizzaro et al., 2019). Since corn crop yield is a function of the interaction of environment with genotype, the expression and association of physiological, morphological and productive components

can expose the contribution of each trait to grain yield (Vian et al., 2016; Yin et al., 2018). Thus, the knowledge of the degree of this association by correlation study allows identifying traits that can be used in indirect selection for yield (Santos et al., 2018a), as well as modifying the selection dynamics in maize breeding programs (Carvalho et al., 2018).

Among the correlation techniques, the path analysis has been used in studies to evaluate the direct and indirect effects of phenological, morphological and productive characters on grain yield in modern agriculture (Toebe and Cargnelutti Filho, 2013; Milander et al., 2017; Guimarães et al., 2019). In addition to the components related to productivity, it is interesting to know the processes related to plant growth and development associated with photosynthetic activity to expose physiological performance in certain situations (Santos et al., 2018b).

Despite the efforts, it is noteworthy that information on the direct and indirect influence of reduced spacing on physiological characteristics, growth and yield on grain yield of cultivated corn remains unknown. Therefore, the objective of this research was to determine the association of physiological, morphological and productive components, as well as the cause and effect relationship on corn grain yield under reduced spacing by trajectory analysis.

Results and discussion

Phenotypic correlation

The physiological, morphological and productive components of corn presented positive and negative correlation on grain yield, which explains the variation of corn grain production in environments with reduced spacing (Table 1). In general, phenotypic correlation is used to verify the linear association between two directly observed characteristics (You et al., 2016). Thus, the positive effect between the traits shows that the increases are proportional, while the negative coefficients indicate inversely proportional associations (Alves and Cargnelutti Filho, 2017).

The characteristics of high positive correlation with GY were SD > EIH > GME > CE > PH > WUE, with greater total effect, respectively. On the other hand, the characteristics LA > NE > Ci > Gs > E, presented a high negative coefficient on GY. The P_N, NGE and 100GW showed no phenotypic association in GY of the hybrids cultivated of 95 cm and 40 cm row spacing. Probably, the effect of additional factor acted on this relationship and prevented any association between these characters.

Because the components are interrelated and the plant develops in environments with high plant density, phenotypic correlations may not provide clear importance of each component in determining grain yield, as the correlation between two traits may be due to a third factor that is environmental or genetic (Xu et al., 2017). Therefore, because corn grain yield is a quantitative trait, path analysis can more accurately assess the true cause and effect relationships between the characters studied simultaneously on grain yield (Reddy and Jabeen, 2016).

The direct and indirect effects of physiological traits on GY

Path analysis under multicollinearity with the addition of the constant k = 0.05 was efficient to estimate the direct and indirect effects of physiological traits on GY (Table 2). The value of the coefficient of determination (R2 = 0.98) and the low effect of the residual variable (U = 0.15) showed that P_N, Ci, Gs, E, CE and WUE are 98% of the total variation of yield grains of hybrids cultivated of 95 cm and 40 cm row spacing. According to Zuffo et al. (2018) the characteristics that present direct and indirect effect trail coefficient below the residual variable do not influence the main variable. Thus, the physiological components with the greatest positive direct effect on grain yield were P_N (r = 0.48), CE (r = 0.34) and WUE (r = 0.19), while Ci (r = -0.27) and Gs (r = -0.17) had the greatest negative direct effect on the main variable (GY). Analyzing the indirect effect found that leaf transpiration (E) via P_N (r = 0.29) and CE (r = -0.31) were the traits with the highest positive and negative magnitude on grain yield, respectively. This shows the importance of these characteristics in the cause and effect relationship of grain yield. The main reason that maize has high physiological ability to intercept sunlight and convert CO_2 into assimilates, especially carbohydrates (Mansfield and Mumm, 2014) is the efficiency of radiant energy conversion in dry matter due to the C4 photosynthetic process, in which carbon dioxide is concentrated in the leaf vascular sheath cells and the carbohydrates are translocated to regions where they will be stored, providing higher grain yield (Renato et al., 2018).

These results suggest that high liquid photosynthesis, as well as carboxylation efficiency and water use efficiency can directly promote increase in grain yield, while CO_2 concentration and stomatal conductance present low magnitude. Such observation corroborates with the reports of Santos et al. (2018b), when they pointed out that the physiological trits are interrelated with the photosynthetic efficiency, so that high photosynthesis rates combined with low internal CO_2 concentrations provide greater carboxylation efficiency, and consequently, increases the production of photoassimilates.

The direct and indirect effects of morphological traits on GY

The analysis of the path under multicolinarity showed good adjustment of the constant k (0.05) for the morphological traits. The cause and effect coefficients of GY, presented coefficient of determination of R2 = 0.98 (Table 3) and the U effect was 0.14. Thus, the high percentage of variation observed for grain production can be explained by characteristics of plant and ear height, stem diameter and leaf area.

The ear insertion height (LA) and the stem diameter (SD) showed the greatest positive direct effect. These traits can contribute proportionally to the increase of grain yield, indicating that plants with higher ear insertion height and high stem diameter are likely to increase grain yield. Therefore, high plant density increases competition for light and stimulates apical dominance and increases ear height by decreasing stem diameter. However, modifications are needed where the ratio of plant to ear height is lower and increases the stem diameter, so that the plants do not break and reach higher yield (Leolato et al., 2017). On the other hand, leaf area showed negative direct association on GY (r = -0.43), indicating that the larger the leaf area, the plant tends to decrease grain yield. Similarly, Mansfield and Mumm (2014), evaluated maize hybrids at different planting densities and observed that leaf area was negatively correlated (r = -0.62) with grain yield. In general, continuous growing maize plants begin to use their nutritional reserves to expand their leaves, which reduces the production of

Table 1. Phenotypic correlation of physiological, morphological and productive components on grain yield of maize hybrids cultivated in reduced spacing.

Associated characters	Phenotypic coefficient
P_N x GY	0.19
Ci x GY	-0.88
Gs x GY	-0.80
E x GY	-0.66
CE x GY	0.92
WUE x GY	0.83
PH x GY	0.88
EIH x GY	0.98
SD x GY	0.99
LA x GY	-0.99
NE x GY	-0.86
NGE x GY	0.27
GME x GY	0.93
100GW x GY	0.02

Note: P_N_net photosynthesis; Ci_CO_2 internal concentration; Gs_stomatal conductance; E_perspiration; EC_Carboxylation efficiency; WUE_water use efficiency; PH_plant height; EIH_Ear insertion height; SD_stem diameter; LA_leaf area; NE_number of ear; NGE_Number of grains per ear; GME_Grain mass per ear; 100GW_100 grain weight; GY_Grain yield.

Table 2. The phenotypic correlation of physiological components in direct and indirect effect on the dependent trait main grain yield (GY).

Characters P_N	Path coeficiente	Characters E	Path coeficiente
Direct effect on GY	0.48	Direct effect on GY	-0.04
Indirect effect via Ci	-0.07	Indirect effect via P_N	0.29
Indirect effect via Gs	-0.07	Indirect effect via Ci	-0.25
Indirect effect via E	-0.02	Indirect effect via Gs	-0.17
Indirect effect via CE	-0.06	Indirect effect via CE	-0.31
Indirect effect via WUE	-0.07	Indirect effect via WUE	-0.18
Total	0.19	Total	-0.66
Ci		CE	
Direct effect on GY	-0.27	Direct effect on GY	0.34
Indirect effect via P_N	0.13	Indirect effect via P_N	-0.09
Indirect effect via Gs	-0.17	Indirect effect via Ci	0.27
Indirect effect via E	-0.04	Indirect effect via Gs	0.16
Indirect effect via CE	-0.34	Indirect effect via E	0.04
Indirect effect via WUE	-0.19	Indirect effect via WUE	0.19
Total	-0.88	Total	0.92
Gs		WUE	
Direct effect on GY	-0.17	Direct effect on GY	0.19
Indirect effect via P_N	0.20	Indirect effect via P_N	-0.18
Indirect effect via Ci	-0.26	Indirect effect via Ci	0.27
Indirect effect via E	-0.05	Indirect effect via Gs	0.17
Indirect effect via CE	-0.33	Indirect effect via E	0.04
Indirect effect via WUE	-0.19	Indirect effect via CE	0.34
Total	-0.80	Total	0.83
Determination coefficient (R^2)			0.98
K value			0.05
Residual trait (U)			0.15

Note: P_N_Liquid Photosynthesis; Ci_Internal concentration of CO_2; Gs_stomatal conductance; E_leaf transpiration; CE_Carboxylation efficiency; WUE_Water use efficiency.
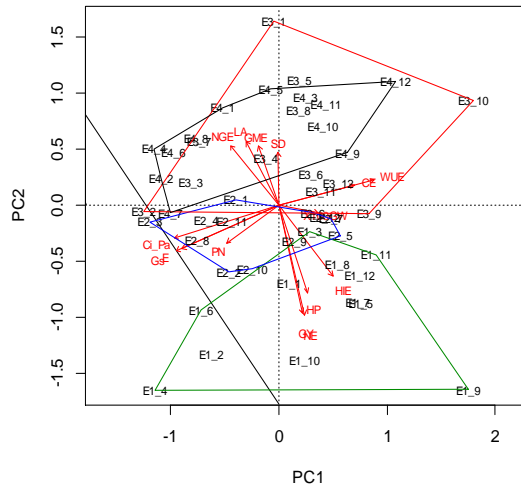
Fig 1. Principal component analysis (PCA) of physiological, morphological and productive traits of hybrid corn in reduced spacing.
PN_net photosynthesis; Gs_stomatal conductance; Ci_intercellular concentration of CO_2; E_leaf transpiration; WUE_water use efficiency; CE_carboxylation efficiency; PH_plant height; EIH_ear insertion height; SD_stem diameter; LA_leaf area; NE_total number of ear; NGE_number of grains per ear; GME_grain mass per ear; 100GW_100 grain weight; GY_grain yield; E1_40 cm spacing; E2_60 cm spacing; E3_80 cm spacing; E4_95 cm spacing; Green Polygon_40 cm spacing; Blue Polygon_60 cm spacing; Red Polygon_80 cm spacing; Black Polygon_ 95 cm spacing.

Table 3. The phenotypic correlation of morphological components in direct and indirect effect on trait dependent main grain yield (GY).

Characters PH	Path coefficient	Characters SD	Path coefficient
Direct effect on GY	-0.03	Direct effect on GY	0.35
Indirect effect via EIH	0.22	Indirect effect via PH	-0.02
Indirect effect via SD	0.32	Indirect effect via EIH	0.23
Indirect effect via LA	0.37	Indirect effect via LA	0.43
Total	0.88	Total	0.99
EIH		LA	
Direct effect on GY	0.23	Direct effect on GY	-0.43
Indirect effect via PH	-0.02	Indirect effect via PH	0.02
Indirect effect via SD	0.35	Indirect effect via EIH	-0.23
Indirect effect via LA	0.42	Indirect effect via SD	-0.35
Total	0.98	Total	-0.99
Determination coefficient (R^2)			0.98
K value			0.05
Residual trait (U)			0.14

Note: PH_plant height; EIH_ear insertion height; SD_stem diameter; LA_leaf area.

Table 4. The phenotypic correlation of productive components in direct and indirect effect on trait dependent main grain yield (GY).

Characters NE	Path coeficiente	Characters GME	Path coefficient
Direct effect on GY	-0.51	Direct effect on GY	0.53
Indirect effect via NGE	0.03	Indirect effect via NE	0.33
Indirect effect via GME	-0.35	Indirect effect via NGE	0.08
Indirect effect via 100GW	-0.03	Indirect effect via 100GW	-0.01
Total	-0.86	Total	0.93
NGE		100GW	
Direct effect on GY	0.13	Direct effect on GY	0.04
Indirect effect via NE	-0.12	Indirect effect via NE	0.27
Indirect effect via GME	0.30	Indirect effect via NGE	-0.13
Indirect effect via 100GW	-0.04	Indirect effect via GME	-0.16
Total	0.27	Total	0.02
Determination coefficient (R^2)			0.97
K value			0.05
Residual trait (U)			0.17

Note: NE_ear number; NGE_number of grains per ear; GME_grain mass per ear; 100GW_100 grain mass.

photoassimilates intended to fill the grain of the cobs, resulting in lower yields (Guerra et al., 2019).

The influence of leaf area on grain yield may be affected by the cultivation method. It was experimentally observed that higher plant density in the area provided negative relationship of leaf area on grain yield, which can be reinforced by Mansfield and Mumm (2014). In contrast to these results, Adesoji et al. (2015) evaluated the influence of green manure on maize hybrids germplasm. They found that the leaf area showed a direct positive relationship (7.11%) with grain yield. Therefore, the contribution of explanatory traits on a quantitative character needs to be carefully analyzed and should not be generalized to increase productivity in different planting systems.

The smallest direct effect of plant height on grain yield (-0.03) was due to the high indirect effects of stem diameter (r = 0.32) and leaf area (r = 0.37). However, the use of plant height as a trait for indirect selection or explanatory of grain yield is not recommended in crop system with high plant densities per hectare. As plant height is directly related to the number of ears produced per individual, larger plants allow emission of more than one ear per plant, contributing to the reduction of grain mass (Testa et al., 2016).

The direct and indirect effects of productive components on GY

Through the path analysis under multicollinearity of traits on grain yield, the coefficient of determination (R^2 = 0.97) and the low effect of the residual variable (U = 0.17) indicated optimal representation of the characters NE, NGE, GME and 100GW on grain yield expression (Table 4).

Grain mass (GME) was the component with the highest positive direct effect (r = 0.53), whereas NE was of high negative direct effect (r = -0.51) on grain yield. However, NGE had influence on GY only by the indirect effect via GME (r = 0.30), because its direct effect was smaller than the residual variable. The yield characteristics, such as NE and GME, clarified the cause and effect relationship on GY, indicating that the lower number of ears per plant causes the higher grain mass, regardless of the number of grains.

According to Assefa et al. (2018), maize grain yield is result of the sum of the effects of traits number of ears per plant, number of beans per ear and grain mass. In addition, traits that do not make up this sum, but have a high direct and indirect effect, allows grain yield gains and may be useful in selecting traits in the maize breeding program (Begum et al., 2016).

It is noteworthy that some trait, even with direct correlation with the dependent variable, may not be the main cause of variations in the characteristic of interest, with an indirect effect being the largest contributor to the basic variable (Crevelari et al., 2018). Among the productive components, we observed that the 100GW presented the lowest direct effect coefficient (r = 0.04) in the GY. However, its major influence on corn grain yield showed indirect effect via NE (r = 0.27), indicating that 100GW can only positively affect on grain yield, when the number of ear per area is increased.

Similar results to 100GW were observed by Pandey et al. (2017), who verified direct effect of smaller magnitude of 100GW on grain yield (r = 0.0047). Higher numbers of grains in the ear and intraspecific competition for resources in crops with high plant density may decrease grain mass (Kopper et al., 2017; Williams, 2016). In this case, the larger amount of grains and ears per area in high densifiy planting system can compensate for the increase of corn grain yield (Testa et al., 2016).

Principal component analysis (PCA)

In the multidimensional space of the main components (Figure 1), the data matrix variance is distributed to PC1 (26%), PC2 (24%) and PC3 (21%) presenting 71% of the total variation ratio (Fig 1). A useful interpretation of the PCA is that the total percentage change in data is explained by PCs (Jolliffe and Cadima, 2016). However, all components are not normally used because most variations and patterns in the data are limited to PC1 and PC2, which can be summarized in scatter plots (Lever et al., 2017). In this study, we observed in PCA that the traces fit into three grouping patterns as a function of the spacing used.

The set of variables with NGE, AF, GME, SD, CE, WUE and 100GW were more prominent in 95 cm and 80 cm spacing between lines (black and red polygon, respectively). While PN, Ci, Gs, E and 100GW were the source of greatest variation in 60 cm spacing (blue polygon), while in 40 cm spacing (green polygon) GY, NE, HP and HIE are the traits most relevant in the data variation (Figure 1). The productive potential of corn crop, as well as the physiological and morphological traits, depend on the environment in which it is inserted so that the smaller or larger row spacing influences the expression of these characteristics (Greveniotis et al., 2019).

Based on the obtained results, the path analysis reflected the importance of the physiological (P_N, CE, EUA, Ci, Gs), morphological (EIH, SD, LA) and productive (NE, GME) traits as main sources of cause and effect in the variation of grain yield of corn cultivated in reduced spacing. Thus, traits with high path coefficients are essential for observations in maize breeding programs to obtain high grain yield varieties (Santos et al., 2018a).

Materials and methods

Plant materials and experimental design of field trials

This work was conducted in the 2016/2017 agricultural year in Senador Guiomard, Acre, Brazil (09º 50.9'S 67º 26.4 'W in WGS84 datum). Maize hybrids were cultivated in reduced spaced environment and physiological, morphological and productive were evaluated. The experiment was carried out in randomized complete block design with four replications. The treatments were corn hybrids (2B655PW, AG7088PRO3 and P4285YHR) which grown with row spacing of 40 cm, 60 cm, 80 cm, 95 cm.

In minimum cropping system, sowing was carried manually on November 19, 2016. Fertilization was based on soil chemical characteristics. Weeds were controlled as needed for cultivation using the systemic herbicide glyphosate at a dose of 1,920 g ha^{-1} of the active ingredient.

Field methods for assessment of traits

The sets of physiological traits analyzed were: net photosynthesis (P_N); stomatal conductance (G_s); intercellular concentration of CO_2 (Ci) and leaf transpiration (E). Water use efficiency (WUE) and carboxylation efficiency (CE) were also calculated by the relationship between assimilated CO_2 and transpiration, and the assimilated CO_2 with intercellular CO_2, respectively, in the leaves of maize hybrids.

Measurements of liquid photosynthesis and gas exchange were carried with IR-IRGA model LI-6400XT (Li-Cor Inc., Lincoln, USA). The evaluations took place at the VT full bloom stage, between 10:00 am and 12:00 pm on the same day, in the median region of the fully expanded and healthy leaf. The analyzes were performed in one plant for each treatment in the four blocks, totaling 48 individuals sampled. The photosynthetically active photon flux density (PPFD) was maintained at 1000 $\mu mol.m^{-2}.s^{-1}$ and the reference CO_2 at 380 $\mu mol.mol^{-1}$, compatible with the ambient CO_2 concentration.

At the end of the male flowering period, the morphological traits of the hybrids were evaluated in 10 plants of the useful area: plant height (PH) and ear insertion height (EIH), stem diameter (SD), and leaf area (LA) according to Nascimento et al. (2019).

For the set of traits of the production components all ears of the useful area were harvested and subsequently extrapolated to the total number of ear per hectare (NE). Ten ears were randomly taken, multiplying the number of rows of grains by the number of grains in the row to obtain the number of grains per ear (NGE). After threshing of the ears, the grain mass per ear (GME) was determined and the 100 grain weight (100GW) was estimated with 13% humidity. Grain yield (GY) was calculated from threshing and weighing of grains from all ears harvested from the useful area corrected to 13% humidity and estimated for hectare.

Path analysis

Estimates of phenotypic correlation coefficients were split into direct and indirect effects of sets of physiological, morphological variables and yield components (explanatory trait) separately on grain yield (main trait), by means of the path analysis, as shown described by Wright (1921).

First, the phenotypic correlation matrix X'X was obtained for each trait set. Then, the degree of multicollinearity of the matrix X'X of each set of trait was verified by the condition numbers (CN), which is the ratio between the highest and lowest eigenvalues of these matrices (Montgomery and Peck, 1981).

Under severe multicollinearity (CN > 1000) for the set of physiological (CN = 295421.99), morphological (CN = 777061.86) and productive (CN = 66569.26) trait, the multicolinarity path analysis method was chosen according to Cruz and Carneiro (2006). Such method uses a constant k on the main diagonal of the X'X matrix of each trait set to reduce the variance associated with the path analysis least squares estimator and to stabilize the path coefficients. Thus, the system of normal equations, $X'X\, \hat{\beta} = X'Y$, became $(X'X + k)\, \hat{\beta} = X'Y$, where $\hat{\beta}$ are the direct effect estimators of the explanatory trait Xn over the main trait (Y), and X'Y is the matrix of correlations of the explanatory variables with grain yield.

Phenotypic correlation matrices of physiological, morphological traits and production components, and other analyzes and tests related to multicollinearity and path analysis were performed with the aid of the GENES program (Cruz, 2016).

Principal component analysis (PCA)

To detect clustering and relationship between traits, principal component analysis (PCA) using the prcomp command of the R statistical software (R Core Team, 2015).

Conclusion

Liquid photosynthesis, carboxylation efficiency, water use efficiency, internal CO_2 concentration and stomatal conductance of maize plants cultivated in 40 cm reduced spacing are the physiological traits with the highest direct and indirect relationship on corn grain yield. The ear height, stem diameter, and leaf area constitute the main morphological trait of cause and effect on the grain yield of maize plants cultivated in 40 cm row spacing. Ear number and grain mass become the main characteristics of direct and indirect effect on corn yield. Therefore, physiological, morphological and productive traits can provide grain yield gains via indirect selection when the crop is subjected to reduced of 40 cm row spacing.

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