










New insights on the influence of the quality of tomato seedlings on production of fruits cultivated in substracts

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ABSTRACT: *This study evaluated the relationships between the biometric characters of tomato seedlings with the production characters obtained at the end of the crop cycle, to determine how the quality of seedlings interferes with tomato productivity. An experiment was carried out in two growing seasons, in a completely randomized design, with four replications and the experimental unit consisting of five plants. The treatments consisted of seedlings with different ages, differentiated by the days after sowing (DAS): (15, 20, 25, 30, 35, and 40 DAS). After each evaluation of the seedlings, they were transplanted to the definitive production site, being evaluated until the end of the cycle. Biometric characters of seedlings and characters of fruit production were evaluated. Pearson correlation analysis and principal components (PCA) were performed to determine the relationships between the characters, path analysis to determine the direct and indirect effects, and analysis of canonical correlations between the groups of characters. In all analyses performed, weak correlation between the biometric characters of the seedlings (which evaluate the quality) with the characters of tomato fruit production in substrate cultivation was identified.*

Key words: *canonical correlations, path analysis, PCA analysis, Pearson, seedling quality, Solanum lycopersicum.*

Novas descobertas sobre a influência da qualidade de mudas de tomate na produção de frutos cultivados em substrato

RESUMO: *O objetivo deste trabalho foi avaliar as relações entre os caracteres biométricos de mudas de tomate com os caracteres de produção obtidos ao final do ciclo da cultura, para determinar como a qualidade das mudas interfere na produtividade do tomate. O experimento foi conduzido em duas épocas de cultivo, em delineamento inteiramente casualizado, com quatro repetições e a unidade experimental composta por cinco plantas. Os tratamentos foram constituídos por mudas com diferentes idades, diferenciadas pelos dias após a semeadura (DAS): (15, 20, 25, 30, 35 e 40 DAS). Após cada avaliação das mudas, eram transplantadas para o local de produção definitivo, sendo avaliadas até o final do ciclo. Avaliaram-se os caracteres biométricos das mudas e os caracteres da produção de frutos. Análise de correlação de Pearson e componentes principais (PCA) foram realizados para determinar as relações entre os caracteres, análise de trilha para determinar os efeitos diretos e indiretos e análise de correlações canônicas entre os grupos de caracteres. Em todas as análises realizadas, foi identificada correlação fraca entre os caracteres biométricos das mudas (que avaliam a qualidade) com os caracteres da produção de frutos de tomate em cultivo em substrato.*

Palavras-chave: *análise PCA, análise de trilha, correlações canônicas, qualidade de mudas, Pearson.*

INTRODUCTION

Seedling quality is a prerequisite for successful performance in the field and therefore influences yield (YILMAZ et al., 2017; ZHOU et al., 2019). This statement is widespread and recommended among all seedling and tomato producers, being a fundamental requirement when implementing a production crop. However, studies that assess the correlations between the biometric characters of the seedlings and their productivity in

the field are scarce. If necessary, clarify how much more vigorous seedling will impact fruit productivity.

Seedling quality is studied in all corners of the world. For tomato (*Solanum lycopersicum*), these studies are essential, since it is a crop of great economic importance and is susceptible to several biotic and abiotic factors that can lead to failure of cultivation. Among the factors that influence the success of a tomato planting, are the temperature, solar radiation, rainfall, seedling quality, insect pressure, and diseases, among others (KONG

et al., 2021; PLOEG & HEUVELINK, 2005; YILMAZ et al., 2017; ZHOU et al., 2019). High quality seedlings increase production, while the malformed ones can cause damage to the producer (GUIMARÃES et al., 2002).

Tomato is a crop that has been used in soilless cultivation - substrate. Among soilless cropping systems there are two major groups: hydroponics and substrate cultivation (SAVVAS et al., 2013). Substrate cultivation can be closed or open, and allows plants to grow and develop faster, because there is a balanced supply of air, water and nutrients (SUVO et al., 2016).

Seedling production is evaluated for substrate composition (AKPO et al., 2014; YILMAZ et al., 2017), quantity and spectra of light (CLAYPOOL & LIETH, 2020), under salinity (PARRA et al., 2007), seed quality (PEÑALOZA & DURÁN, 2015), rootstock type (ZEIST et al., 2020), environmental variables (ZHOU et al., 2019), among others, but none of them takes into account the evaluation of these seedlings in the field until the end of the crop cycle. This is important for evaluating the relationships between the biometric characters that are responsible for the concept of quality seedlings with the production characters at the end of the cycle, so that you can say that a seedling with a smaller root size will have less fruit production, for example.

To have these results and affirm based on scientific data the existence or not of these relationships, in addition to evaluating the seedlings in the field until the end of the cultivation cycle, multivariate analysis is an important tool for evaluating this type of experiment. Linear relationships between biometric characters of seedlings with productivity, can answer important questions for the management of tomato crop in the seedling production phase. For this, Pearson correlation and principal components (PCA) analyzes can be used to interpret the linear relationships between variables. PCA was substantiated by Hotelling (1936) and summarize the data and allow you to view important information in multivariate data expressed by new variables that are called principal components and correspond to a linear combination of the original variables, and reveal the total variation that the data set contains (KASSAMBARA, 2017).

Determining correlations between variables is important, but interpretations of Pearson's correlation in isolation can cause distortions in the results obtained. So in 1921, WRIGHT (2017) defined the method of path analysis, which allows the correlations to be broken down into direct and

indirect effects of variables on a basic characteristic. Also, analysis of canonical correlations allows the relationships between groups of variables to be determined, thus allowing the identification of the existence of dependence between these groups (MINGOTI, 2005).

This study evaluated the relationships between the biometric characters of tomato seedlings with the production characters obtained at the end of the cycle to determine mathematically how the quality of seedlings interferes with tomato productivity in a soilless cultivation system.

MATERIALS AND METHODS

Site of cultivation and plant material

The experiment was carried out in a greenhouse, with an automated opening, in the Phytotechnics Department of the Universidade Federal de Santa Maria (29° 43' 23''S, 95 m altitude). The climate of the region, according to the Köppen climate classification, is of the Cfa type, humid subtropical, presenting characteristics of rainy temperate, with average annual precipitation of 1800mm and well distributed throughout the year (ALVARES et al., 2013).

The seedlings were produced from tomato seeds cv. BRS Nagai, sown in 128-cell polyethylene trays, washed with 10% sodium hypochlorite, containing Carolina® commercial substrate. Sowing was carried out at the rate of one seed per cell. For season 1 sowing occurred on 02/27/2020, and for season 2 sowing occurred on 08/26/2020. After sowing, the trays were placed on benches with sprinkler irrigation so that the seeds had ideal humidity for germination. After the seeds had germinated, the trays were placed in a "floating" type system.

Experimental design

A completely randomized design (RLD) was used, with four replications and the experimental unit composed of five plants. The treatments consisted of seedlings with different ages, differentiated by the days after sowing (DAS): T1: 15 DAS T2: 20 DAS, T3: 25 DAS, T4: 30 DAS, T5: 35 DAS, and T6: 40 DAS.

The evaluations of the biometric characters of the seedlings started at 15 DAS and afterward, they were evaluated every 5 days, until 40 DAS. After each evaluation of the biometric characters, the seedlings were transplanted into 10-liter pots, containing substrate, where they remained until the end of the cycle. Irrigation and fertigation were

carried out using a drip system, located on top of the pots, consisting of drip tubes spaced 0.50 meters apart. Fertigation was carried out in accordance with the requirements of the crop (CQFS -COMISSÃO DE QUÍMICA E FERTILIDADE DO SOLO, 2016). All treatments were carried out under the same conditions of cultivation, environment, humidity, and fertilization, the only variation being the age of the seedling.

Biometric characters

The evaluations consisted of measurements of biometric characters of the seedlings, being: plant height (H, cm), stem diameter (D, cm), number of leaves (NL), longest root length (RL, cm), total green seedling mass (TGSM, g) measured immediately after removal from the tray, dry root mass (DRM, g), shoot dry mass (SDM, g), total plant dry mass (TPDM, g), Dickson's quality index (DQI) calculated from the total dry mass of the plant (TPDM, g), the ratio of plant height and stem diameter (RHD) and the dry mass ratio of the aerial part and dry mass of the root (RMS), as: $DQI = TPDM / (RHD + RMS)$ (DICKSON et al., 1960), and production characters: mass of commercial fruits (MCF, g) and number of commercial fruits (NCF).

Statistical Analysis

To assess the relationships between variables, Pearson's linear correlation coefficients were estimated, and from this, the principal component analysis (PCA) of the original variables was performed and the interpretation was performed based on linear relationships. The importance of each variable to explain the variability between the treatments evaluated was determined.

Direct and indirect effects between variables were determined, using Pearson's correlation, defining MCF as the main dependent variable and the variables H, RHD, DQI, RL, NCF as explanatory variables. The other variables were excluded from the analysis because they showed high multicollinearity. The diagnosis of multicollinearity between the explanatory variables was carried out by analyzing the number of conditions $NC = \frac{\lambda_{\max}}{\lambda_{\min}}$, which represents the ratio between the highest and lowest eigenvalue of the correlation matrix and the variance inflation factor $VIF = \frac{1}{1 - R_j^2}$, Where R_j^2 is the coefficient of determination.

The path coefficients were obtained by the methodology proposed by (CRUZ et al., 2012) by the equation $Y = P_{o1}X_1 + P_{o2}X_2 + \dots + P_{on}X_n + P_u$, where Y is the coefficient of the dependent variable;

P_{o} is the direct effect coefficient; X is the explanatory independent variable; P_u is the residual effect and the standardization variable.

Canonical correlation analyzes were performed to identify relationships between groups of variables. The estimation of the maximum correlation between linear combinations of characters in groups I and II, with X1 and Y1 being the linear combinations of characters in groups I and II respectively, has to be: $X_1 = a_1x_1 + a_2x_2 + \dots + a_px_p$ and $Y_1 = b_1y_1 + b_2y_2 + \dots + b_qy_q$ on what: $a' = [a_1a_2\dots a_p]$ = 1xp vector of weights of the group I and $b' = [b_1b_2\dots b_q]$ = 1xq vector of weights of II characters. The first canonical correlation will be the one that maximizes the relationship between X1 and Y1. The functions X1 and Y1 form the first canonical pair associated with that canonical correlation expressed

by: $r_1 = \frac{C\hat{ov}(X_1, Y_1)}{\sqrt{\hat{V}(X_1)\hat{V}(Y_1)}}$, being $C\hat{ov}(X_1, Y_1) = a'S_{12}b$, $\hat{V}(X_1) = a'S_{11}a$ and $\hat{V}(Y_1) = b'S_{22}b$ being: S_{11} = pxq matrix of covariance between characters in group I; S_{22} = qxq matrix of covariance between characters in the group II; S_{12} = pxq matrix of covariance between characters in group I and II (CRUZ et al., 2012).

The groups formed were production variables (MCF and NCF) and biometric variables of the seedling (RL, RMS, DC, DQI, and H). The diagnosis of multicollinearity was carried out within each group of variables by VIF and NC and when multicollinearity was high, the variable that caused multicollinearity was excluded. For all groups formed, multicollinearity was low.

Statistical analyzes were performed at 5% significance and with the aid of packages Hmisc (FRANK & HARRELL Jr, 2021), FactoMineR (LE et al., 2008), metan (OLIVOTO & LÚCIO, 2020), biotools (SILVA et al., 2017), yacca (BUTTS, 2018) available in the R program (R Development Core Team, 2021).

RESULTS

Results of the analyzes revealed a weak relationship between the biometric characters measured in the seedlings and the production characters. Pearson's correlation, in both growing seasons, revealed that the RL variable is not related to any other variable, inferring that, for the season of seedlings and in the cultivation system used, the size of the root does not interfere in the growth and development because the nutrients are supplied via fertigation. The correlated variables in the two growing seasons are H, NL, DC, TGSM, SDM,

DRM, TPDM, and DQI. MCF has a strong correlation with NCF, but they do not correlate with any other evaluated variables (Figure 1 A and B).

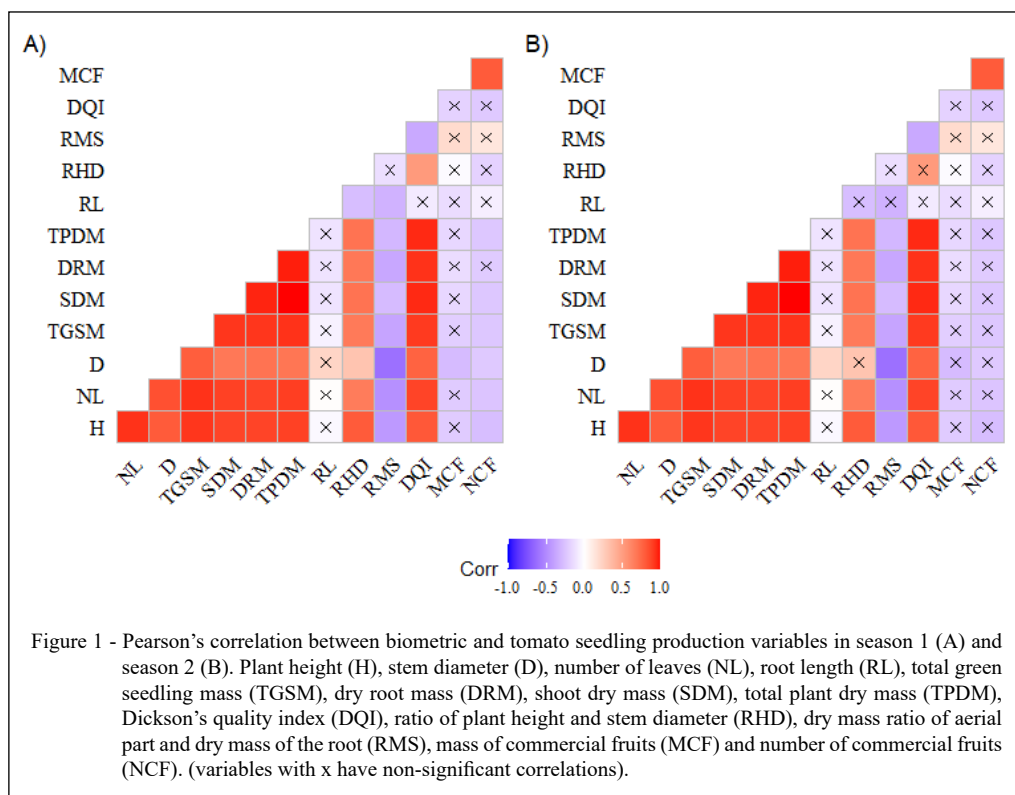
Results of the PCA are explained by the variables MCF, NCF, DQI, TGSM, H, and NL, which have a higher quality of the variables in the factor map, with \cos^2 above 0.98 in season 1 and 0.85 in season 2 (Figure 2 A and B). In the two growing seasons, the variable H is the most perfectly represented, as it presented a value closer to 1, in both seasons, the one that represents the least is the NCF variable.

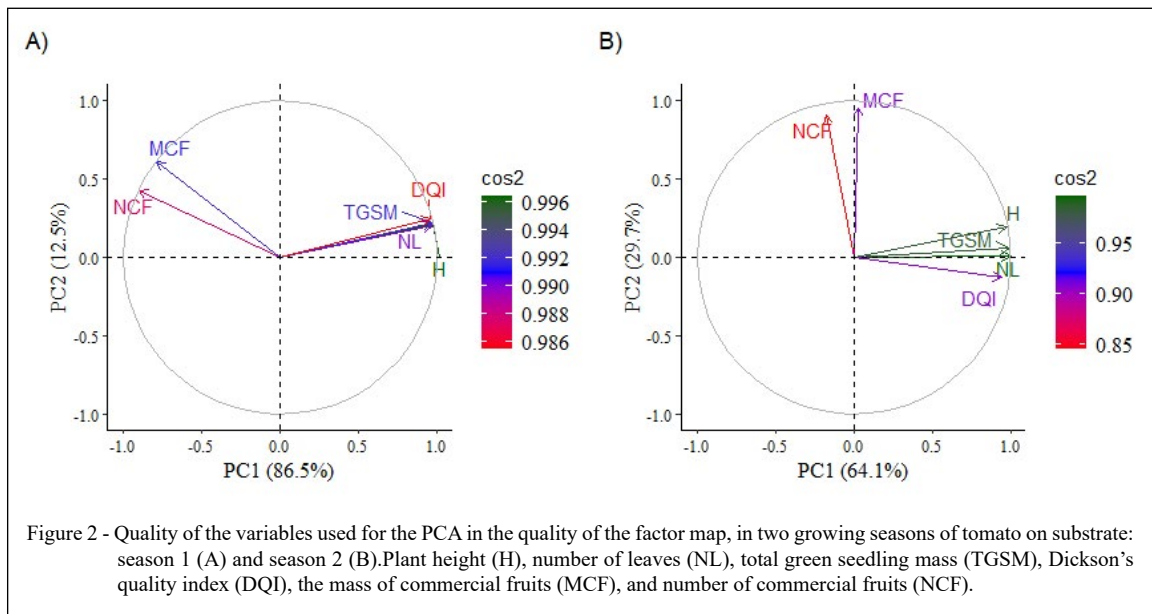
In the PCA analysis, in season 1 the variances contained in the original variables can be explained by the first two main components, being 86.5 and 12.5% for PC1 and PC2, respectively, totaling over 99% of the variability. For Season 2 PC1 and PC2 totaled 93.8% of the variance contained in the original variables, confirming the results of Pearson's correlation. Briefly, in the two growing seasons, the PCA reveals that the variables DQI, TGSM, NL, and H are positively correlated with each other, but they are not related to the variables MCF and NCF, which are correlated (Figure 3). The results

reveal that the biometric variables of the seedlings are not related to fruit production.

Even with the magnitudes of the estimates of the correlation coefficients being low with the production variables, we opted for their consequences in direct and indirect effects on the main dependent character MCF. The selection of variables H, RHD, DQI, RL, and NCF as explanatory variables for indirect selection of MCF in the path analysis caused low condition number (CN) and variance inflation factor (VIF) in the two growing seasons (Table 1 and 2). Likewise, the decomposition of linear correlations into direct and indirect effects showed a coefficient of determination of 68% and 50% and residual effect of 0.56 and 0.7 for periods 1 and 2 respectively, proving that the variables do not explain much of the observed variation (Tables 1 and 2).

For season 1, the RHD and DQI variables have no cause and effect relationship with the MCF variable, since the correlations are negligible and, so prove the PCA results. The variable NCF have a direct effect with the variable MCF because they are equal in magnitude and sign, The variables RL and H has an indirect effect with MCF. The results indicated that





taller plants with a greater number of fruits can increase in the mass of commercial fruits in tomatoes (Table 1).

For season 2, the RHD and DQI variables, as well as in season 1, have no cause and effect relationship with the variable MCF, as the correlations

are negligible. The variable H has an indirect effect with the variable MCF, and the variables RL and NCF have a direct effect with the variable MCF since the direct effect and Pearson's correlation are equal in magnitude and sign (Table 1).

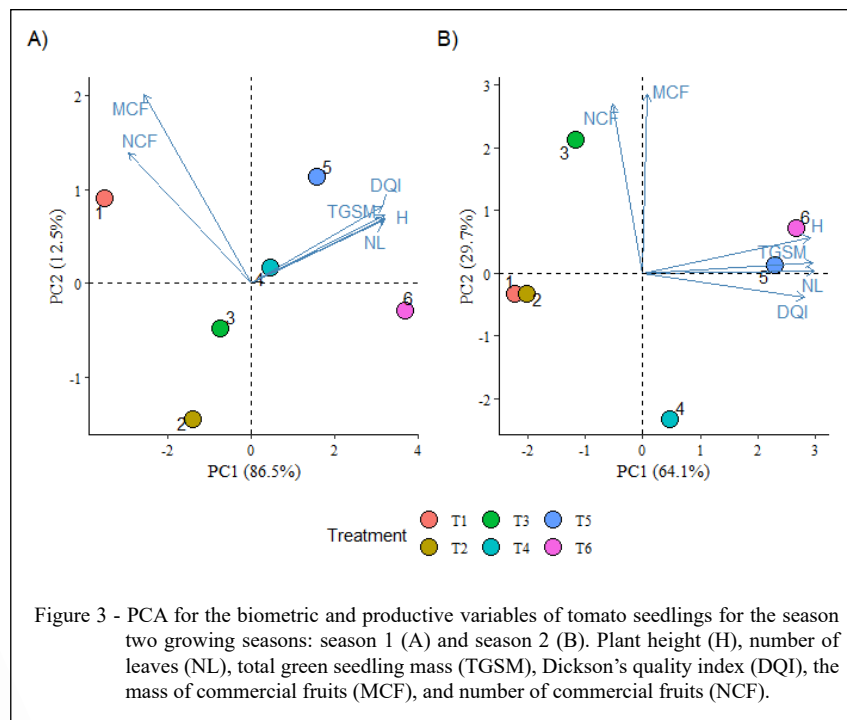


Table 1 - Path analysis between the main dependent variable - direct effect (diagonal) total fruit mass (MCF) and the explanatory (indirect effects) H, RHD, DQI, RL and NCF of tomatoes grown at season 1. Plant height (H), root length (RL), Dickson's quality index (DQI), ratio of plant height and stem diameter (RHD) mass of commercial fruits (MCF) and number of commercial fruits (NCF).

Characters	H	RHD	DQI	RL	NCF	r
H	-0.385	0.30037	0.08461	-0.0002	-0.2194	-0.2196
RHD	-0.3072	0.37637	0.05482	-0.0025	-0.1451	-0.0236
DQI	-0.3109	0.19694	0.10477	-0.0008	-0.179	-0.189
RL	0.01059	-0.1071	-0.0093	0.00877	-0.0564	-0.1534
NCF	0.10787	-0.0697	-0.024	-0.0006	0.78294	0.79647
R ²	0.68					
Residual effect	0.56					
VIF	8.23	4.20	3.59	1.36	1.09	
Condition number	35.11					
Determinant X'X	0.07					

The canonical correlation analysis performed between the groups of production variables and biometric variables showed low multicollinearity. In both growing seasons, the canonical correlations were low and had no significant effect, so they are independent and suggested that it is not possible to select productive characteristics based on the biometric characters of tomato seedlings (Table 3).

DISCUSSION

The quality of vegetable seedlings, in general, is widely discussed. When talking about tomato seedlings, the importance of this issue stands

out, since it is a crop susceptible to various weather conditions in the field after transplanting the seedlings and when they show fragility when they leave the nursery, they can be easily affected by factors that cause low performance in their development. These factors range from the climatic conditions where the seedlings will be transplanted to the diseases and insects that may come to attack them. Therefore, when the seedlings are well developed, the setting and growth from transplantation are a strategy for the success of the planting.

The relationship between the quality of tomato seedlings has been discussed over time (RODRIGUES et al., 2010; SOUZA et al., 2013;

Table 2 - Path analysis between the main dependent variable - direct effect (diagonal) total fruit mass (MCF) and the explanatory (indirect effects) H, RHD, DQI, RL and NCF of tomatoes grown in season 2. Plant height (H), root length (RL), Dickson's quality index (DQI), ratio of plant height and stem diameter (RHD) mass of commercial fruits (MCF) and number of commercial fruits (NCF).

Characters	H	RHD	DQI	RL	NCF	r
H	0.41787	-0.0407	-0.2817	-5.78E-05	0.0096	0.105
RHD	0.23652	-0.0719	-0.0502	-7.54E-04	-0.017	0.09676
DQI	0.31839	-0.0098	-0.3697	6.20E-04	0.00865	-0.0518
RL	-0.007	0.01567	-0.0663	3.46E-03	0.01661	-0.0376
NCF	0.00604	0.00183	-0.0048	8.64E-05	0.66448	0.66762
R ²	0.50					
Residual effect	0.70					
VIF	5.01	2.15	3.57	1.11	1.00	
Condition number	18.15					
Determinant X'X	0.17					

Table 3 - Canonical correlations and crossed canonical loads for groups of production variables and biometric variables of tomato seedlings in two growing seasons. Number of commercial fruits (NCF), mass of commercial fruits (MCF), root length (RL), dry mass of the root (RMS), stem diameter (D), Dickson's quality index (DQI) and plant height (H).

Characters	-----Season 1-----		-----Season 2-----	
	1°	2°	1°	2°
	-----Production-----			
NCF	-0.111	-0.271	0.001	-0.053
MCF	-0.268	-0.155	-0.229	-0.036
	-----Biometric-----			
RL	0.174	0.012	0.073	-0.025
RMS	-0.172	-0.078	-0.048	0.036
D	0.249	0.157	0.023	-0.010
DQI	0.090	0.210	0.081	-0.013
H	0.092	0.265	-0.128	-0.015
Canonical correlation	0.318	0.289	0.308	0.053
χ^2	12.991	5.850	6.873	0.192
df	10.000	4.000	130.000	66.000
P-value	0.225	0.211	0.738	0.996

SANTOS et al. 2017; GESHNIZJANI et al., 2019). Research covering this subject should focus on how much a more vigorous seedling will impact the final production there in the field. In the present research, the characters measured in the plant during the seedling phase did not show any correlation with the production. This fact may be related to the type of plant conduction system, where all nutritional demands are met via fertigation, providing the plants to recover quickly after transplanting and throughout the production cycle. In addition, this fact may also be linked to technological improvement in the production of seedlings and to the characteristics of the genotype.

Between the variables H and MCF, an inverse relationship was observed between them, where it could be interpreted that higher plant heights lead to a lower mass of commercial fruits; however, this statement cannot be made, since these correlations are very weak and not significant. The Person correlation graphs presented in the results showed that the correlation of MCF and NCF with the other variables is very weak, close to zero (shown by the gray color) (Figure 1), and not significant at season 2 (x in the graph). Numerically, the highest correlation between MCF and H was -0.21 (significant at season 1, but very weak), and it cannot be said to have an effect on each other with this value. This significance can be attributed to the size of the database, which is large and ends up generating a very low tabulated t, which leads to significance.

Relationships between plant height and fruit mass would be an interesting answer, but maybe because our study was carried out in a protected environment, with nutrients in optimal amounts via fertigation, the height of the seedlings has not had such a significant effect on productivity. This may have been due to, after transplanting the seedlings to the final cultivation site, smaller seedlings may have recovered quickly, because in substrate cultivation, nutrition is more efficient than in soil cultivation. The relationship considering plant height has been studied by several authors such as SOUZA et al., (2013), SANTOS et al., (2017) and RODRIGUES et al., (2016); however, no studies on its association with the final characters of the cycle, which searches are still incipient.

Much is said about how important root structure is for plant development, in the present study in both growing seasons the character RL is not related to any other variable, and so we could conclude that the size of the root in the seedling phase does not interfere with the growth and development of the tomato. However, in the substrate cultivation system, where all the plant's nutritional requirements are supplied via fertigation, promotes root growth and increases the efficiency of water and nutrient use (HU et al., 2021), causing a seedling with smaller root system can recover quickly when transplanted to the substrate culture system.

The RL is a variable that can be related to the increase in crop yield, a study where the comparison

between autumn-winter growth seasons was carried out and winter-spring there was significant in dry root weight and tomato yield; however, the correlation performed was with the root size of the adult plant (HU et al., 2021), unlike our study, where we sought to relate the biometric characters of the seedlings with the characters of fruit production.

It was already expected that the characters H, NL, DC, TGSM, SDM, DRM, TPDM, and DQI would be correlated, since all of these were plant characters as seedlings, but had no relationship with MCF and NCF. DIEL et al., (2019) evaluated the relationships between productivity components in Italian tomatoes in conventional cultivation, and concluded that H was not related to production, corroborating the results of this study. In many cases, when you have a large number of characters it can make it difficult to interpret the results, and therefore, the use of PCA analysis has the advantages of considering the most informative variables in the data set, providing that individuals are grouped by their greater similarity (HONGYU et al., 2015). Principal component analysis reduces the dimensionality of the data in some new variables called principal components (PC's), which are linear combinations of the original variables. In this case, the variables that most explain the data set were MCF, NCF, DQI, TGSM, H, and NL, and again the PCA indicated that the biometric variables of the seedlings are not related to the fruit yield in tomatoes.

The results continue in the analysis of the trial and the canonical correlations. For these, the assumptions of multicollinearity were met, presenting weak multicollinearity for the explanatory variables in the trail analysis and each group of variables in the canonical correlation analysis (HAIR et al., 1998). The low coefficient of determination and high residual effect in the trail analysis indicates that these results are not satisfactory to explain the cause and effect relationships between the variables (DONAZZOLO et al., 2017; HAIR et al., 1998), similarly, in the analysis of canonical correlations, their non-significance indicates that the groups of morphological and productive variables are independent. In a study where the association between the biometric characteristics of tomato seeds and the growth and development of their seedlings were tested, the results indicated that there was no association between seed size and weight and the subsequent emergence of seedlings, and only weak correlations were found between the dry weight of the radicle and cotyledon and the size of the seed (PEÑALOZA & DURÁN, 2015).

Biometric characters and indexes calculated from these characters are pointed out in many cases as measures that interfere with higher quality seedlings. Dickson's Quality Index, for example, is a relationship that includes morphological parameters of H, diameter, and biomass, being a very efficient quality indicator (COSTA. et al., 2011; SMIRNAKOU et al., 2017). The parameters evaluated to indicate quality seedlings are measured until the time of transplantation in most studies. It is a consensus when it is said that seedlings with greater vigor have advantages for the beginning of the establishment after transplantation. According to JOHKAN et al., (2010), the quality of lettuce seedlings affects their growth and production after transplantation, the production of seedlings from seeds with high germination results in uniform seedlings of high quality, leading to greater crop efficiency, ensuring a higher rate of harvesting and crop yields (JOHKAN et al., 2010), probably caused by the high biomass of the aerial part and the root, high content of photosynthetic pigments and by the high antioxidant activity in the lettuce seedlings before transplantation.

In the literature, it is suggested that seedlings with higher DQI have higher quality (DICKSON et al., 1960; LUIS et al., 2004; SANTOS et al., 2017), but the influence of these parameters are not correlated with the performance of these plants after transplantation, in many cases, the evaluations end at the end of the seedling phase, not following the evaluations and their performance in the field, to assess the real effect on production for example. YILMAZ et al. (2017) evaluated the quality of the tomato seedling in different media in cultivation without soil, however, the evaluations ended at the end of the 45-day experimental period.

The non correlation between the biometric characters of the seedlings with the productive performance in tomato may be related in this case, as previously reported, to the cultivation system, since all the nutritional demand of the plants is supplied via fertigation. When plants are grown in soil, no protected cultivation, the biometric parameters of the seedlings are a differential for the establishment of seedlings in the field, because when they are more fragile they are easily affected by biotic and abiotic factors responsible for the failure of planting (SARMA & GOGOI, 2015; SIVRITEPE et al., 2003; SMIRNAKOU et al., 2017). Conversely, when the conditions for cultivation are more controlled, as in the case of substrate cultivation and protection, the effect of the biometric characters of the seedlings,

which measure the quality of the seedling, has no relation to the fruit production in tomato.

CONCLUSION

The relationships obtained between the biometric characters of tomato seedlings are not sufficient to affirm that they interfere with the production characters at the end of the cycle, that is, the characteristics that evaluate the quality of tomato seedlings do not influence production when grown in a substrate cultivation and protected system.

Research that evaluates the quality of vegetable seedlings should consider experiments after the seedling period to identify interference in productivity.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

Conceptualization: MID and ADL. Data acquisition: MID, FLT, ALT, DML, JAZ and LEM. Design of methodology and data analysis: MID, ADL, FLT and ALT prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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