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Morphology and physiology of Tifton development

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ABSTRACT

Background: The different genotypes of Tifton won economic importance among the forage, the higher dry mass yield, greater response to fertilization, higher digestibility, higher crude protein content and ratio of calcium and phosphorus in relation to other forages of *Cynodon*. The fall of the nutritional value of the function in pastures plant growth is related to the following factors: reduction in protein and phosphorus, with the aging of the plant is increased stem/leaf relationship. The aim of this study was to obtain estimates of the morphological and physiological development of Tifton genotypes, with the use of regression analysis and the breakdown of correlations in direct and indirect effects by path analysis, valued at ten different growing seasons. **Methods:** The study was conducted in Plant Breeding and Plant Production Laboratory, Federal University of Santa Maria, Campus Frederico Westphalen, coordinates (27º23'26 "S latitude), (53º25'43" The longitude) and 461,3m altitude in the crop year 2013. Were used for the experiments three genotypes Tifton (*Cynodon spp*.), they Tifton-68, Tifton-85 and Jiggs. The experimental design was a randomized block design with three replications. Each experimental unit consisted of six plants from pasture production unit at the University, were transplanted to the experimental area so that each plant were obtained from three stolons, two below and above ground. **Results:** By analyzing the regressions (Figure 1), where the Tifton-85 genotype was characterized by red, the Jiggs by green and Tifton-68 by the color blue, it was observed that the plant height (PH) showed linear growth during development culture, being explained by first-degree equations, with the most outstanding genotype the Tifton 85, its highest growth in relation to the others. It is observed also that the regression coefficients for the Tifton-85 genotypes (89.98%), Jiggs (92.37%) and Tifton-68 (92.58%) explained much of the variation between the evaluation seasons. **Conclusions**: To getting the genotypes with greater protein content, is necessary the selection plants with greater stolon diameter, leaf width, leaf length, lower number of tillers, green mass and dry mass. In a general way, the Tifton 85 genotype has greater highlight cultivation aiming greater development, resulting in a higher green mass, dry mass and chlorophyll content, however, when it aims genotypes that have high protein content, should have some caution, since the negative correlation seen between this and the traits green mass and dry mass.

INTRODUCTION

The different genotypes of Tifton won economic importance among the forage, the higher dry mass yield, greater response to fertilization, higher digestibility, higher crude protein content and ratio of calcium and

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phosphorus in relation to other forages of *Cynodon* (Patel *et al*., 2013). These characteristics are very important for animal production, and this group of grasses can be used in various production systems (Carvalho, 2012).

The cumulative production of dry mass increases with the development of culture and decreases the nutritive value when the plant passes from vegetative to reproductive phase (Shi *et al*., 2014). The fall of the nutritional value of the function in pastures plant growth is related to the following factors: reduction in protein and phosphorus, with the aging of the plant is increased stem/leaf relationship (Parthasarathy *et al*., 2015). Because the stem of grass species have higher levels of less fiber and protein and phosphorus in relation to the leaves, as well as increased lignification process and reducing the digestibility of nutrients (Carvalho *et al*., 2005). The ideal time of consumption would be one where the forage was with the greatest balance between quantity and quality of digestible nutrients for animals. The analysis of the development of genotypes in the study of regressions seeks to find a value prediction equation of one or more response variables (dependent variables) through a set of explanatory variables (independent), enabling the analysis of each genotype behavior for each study character (Hair *et al*., 2009). The use of indirect selection may be feasible for the study of simple correlation between the traits, as it allows evaluating the magnitude and direction of relations leading to faster genetic progress in breeding programs (Cruz and Carneiro, 2006). However, this technique does not assess the interrelationships between two groups of variables determined by a larger number of traits.

For a better understanding the association between traits, use the method called path analysis, which unfolds the estimated correlations in direct and indirect effects of each trait on a basic variable (Wright, 1921). This method is based on evaluation of the effect of an independent variable (x) over a dependent variable (y), after removing the influence of all other independent variables (xi) that are included in the analysis. For the path analysis results are useful to plant breeding, it is very important to check the degree of multicollinearity between the explanatory variables path analysis (Cruz and Carneiro, 2006). Because the estimates of the direct and indirect effects may be incorrect if the analysis is carried out with severe multicollinearity. When the values of the estimates in the analysis are larger than one (01) of the module it is recommended to make the path analysis crest, by adding a value k to the diagonal elements of the correlation matrix, because of the high degree of multicolinearity analysis (Carvalho, 1995). The aim of this study was to obtain estimates of the morphological and physiological development of Tifton genotypes, with the use of regression analysis and the breakdown of correlations in direct and indirect effects by path analysis, valued at ten different growing seasons.

MATERIAL AND METHODS

The study was conducted in Plant Breeding and Plant Production Laboratory, Federal University of Santa Maria, Campus Frederico Westphalen, coordinates (27º23'26 "S latitude), (53º25'43" The longitude) and 461,3m altitude in the crop year 2013. Were used for the experiments three genotypes Tifton (*Cynodon spp*.), they Tifton-68, Tifton-85 and Jiggs. The experimental design was a randomized block design with three replications. Each experimental unit consisted of six plants from pasture production unit at the University, were transplanted to the experimental area so that each plant were obtained from three stolons, two below and above ground.

In advance planting were carried cultivation to eliminate weeds and disease control. Fertilization was performed after planting with the use of NPK fertilizer (10-25-20) at a dose of 150 kg ha^{-1}. After the first winter, the plants dried Tifton, due to frost, and in its first year regrowth were performed assessments every ten days of development, totaling ten times and 100-day evaluation. The traits evaluated were:

- Plant height (PH): it was assessed the full extent of the main stolon of each plant parcel for each evaluation season, with the length given in centimeters (cm).
- Number of tillers (NT): it was evaluated in each plant with the count of all issued tillers at each evaluation season, given in units (u).
- Internode lenght (IL): was evaluated with a pachymeter accuracy by measuring the length between the first and second node above the ground level, the main stolon of each plant in each evaluation season, given in millimeters (mm).
- Stolon diameter (SD): it was evaluated with a pachymeter accuracy by measuring the diameter of the main stolon in each evaluation season, given in millimeters (mm).
- Number of nodes (NN): it was evaluated by counting all nodes formed in the main stolon of each plant in each evaluation season, given in units (u).
- Number of leaves (NL): it was evaluated by counting all the leaves contained in the main stolon and tillers issued by each plant in each evaluation season, given in units (u).
- Leaf widht (LW): it was evaluated with a pachymeter accuracy, by measuring the width of five leaves of each plant assessed per plot in each evaluation season, given in millimeters (mm).
- Leaf lenght (LL): it was evaluated by measuring tape to measure the length of five leaves of each plant assessed at each season evaluation, given in millimeters (mm).

– Chlorophyll content (CC): was evaluated with chlorophyll manual, by measuring the chlorophyll content in three leaves of the middle part of each plant evaluated in each experimental unit for each evaluation season. After the average for each experimental unit given in milliliters per liter (ml L^{-1}) was calculated.

– Green mass (GM): green mass production was measured by taking samples from an area of 0.250 m² per experimental unit, weighed on analytical balance only for the last time evaluation. After the productions were calculated for one hectare in area (10,000 m²), with the final production given in kilograms per hectare (kg ha^{-1}).

– Dry mass (DM): after measuring the green mass production, each sample was brought to a drying oven at a temperature of 45 ° C for a period necessary to reached the constant weight. The mass was measured on analytical balance given in kilograms per hectare (kg ha^{-1}) only for the last evaluation season.

– Protein content (PC): determined by acid digestion of the vegetable material with presence of catalysts, the total nitrogen present in the sample is obtained through the vapor dragging with subsequent acid titration, the values are translated to protein, as described by Nogueira and Souza (2005), using the formula:

$$PC = (TN \times 6{,}25) \times 100$$

Where PC is the protein content (%) and TN is the total nitrogen content.

Each sample used for evaluation of green and dry mass production underwent a grinding and homogenization. Protein levels were obtained in nitrogen still all in triplicate and then calculated the averages, percentage data (%) only for the last time evaluation.

Regression analysis was performed in order to obtain genetic information useful in plant breeding by the dispersion of the varieties used, assessing the growth of value and importance of each variable used in the statistical analyses, which were carried out with the assistance of the GENES program (Cruz, 2006). Path analysis was performed from the matrix of phenotypic correlations, considering the protein content (PC) trait as the dependent variable, and the other as explanatory variables GENES (Cruz, 2006). Identified the presence of high-grade multicollinearity for the three treatments individually proceeded path analysis under multicollinearity (path analysis Crest), an increase of a value *k* to the elements of the correlation matrix diagonal (Cruz and Carneiro, 2006).

RESULTS AND DISCUSSION

By analyzing the regressions (Figure 1), where the Tifton-85 genotype was characterized by red, the Jiggs by green and Tifton-68 by the color blue, it was observed that the plant height (PH) showed linear growth during development culture, being explained by first-degree equations, with the most outstanding genotype the Tifton 85, its highest growth in relation to the others. It is observed also that the regression coefficients for the Tifton-85 genotypes (89.98%), Jiggs (92.37%) and Tifton-68 (92.58%) explained much of the variation between the evaluation seasons.

For the number of tillers, it is observed that Jiggs and Tifton-85 genotypes showed quadratic behavior with regression coefficients of 59.05% and 85.77% respectively, with a decrease from the second season (20 days) and growth the issuance of tillers from the fourth season evaluation (40 days). For Tifton 65 genotype, a linear model with a regression coefficient 83.86%.The highlight of genotype on the number of tillers was Jiggs, with a final number more than 18 tillers per plant. Management practices aimed at maintaining or even increasing the population density of tillers in Tifton-85 pastures during the fall and winter, allow the occurrence of high tiller appearance rates during the summer, especially at the end of this (Carvalho *et al*., 2000).

The stolon diameter trait showed a linear behavior for Tifton 68 and Jiggs genotypes, with regression coefficients of 58.72% and 78.29%. Tifton 85 genotype showed quadratic behavior, with 88.98% regression coefficient. All three genotypes growth of stolon diameter due to the development of the plants. Highlighted the Tifton 85 genotype, which began the experiment with lower average and the last evaluation season (100 days) had the highest average of the experiment

For the trait internode length, all genotypes showed linear behavior, with regression coefficients of 87.75% (Tifton 85), 79.69% (Jiggs) and 69.80% (Tifton 68). The most prominent genotype was the Tifton 85 that the first evaluation had average close to 30 cm and when evaluated last season had averaged more than 120 cm. As for the genotypes of Tifton, works with grass Esmeralda, San Carlos and Tifton, 419 showed similar results for the internode length and number of tillers, where the higher the number of nodes, the larger the leaves forming potential, tillers and new stolons (Kojoroski Silva *et al*., 2010).

As for the internode length, the number of nodes showed linear behavior trait for all genotypes, with regression coefficients of the 91.02% (Tifton 85), 78.29% (Jiggs) and 88.98% (Tifton 65), explain much of the variation between the evaluation seasons. Again, the highlight of genotype was the Tifton 85, with the development of two to 20 nodes during the experiment. Nevertheless, the Jiggs genotype also was highlighted with a mean end more than 18 nodes. The number of leaves showed a quadratic behavior for Jiggs and Tifton 85 genotypes, with regression coefficients of 83.15% and 78.92%, respectively. Tifton 68 genotype showed a linear behavior, with 88.78% regression coefficient. For this trait the Jiggs genotype presented the highest average of the experiment, however the maximum number of leaves has been reached between the seventh (70 days) and the ninth (90 days) evaluation season. The larger number of leaves on plants with greater height, explained by the longer time required the plant to reach heights of upper sections, where the total number of leaves per tiller was represented by a linear function of the accumulation of degree-days during the period (Carvalho, 2012). Therefore, the higher the age of regrowth, greater will also be the number of emerged leaves per tiller.

Contrasting results between genotypes were observed for the trait leaf width. It is observed that the Tifton 85 genotype showed a linear behavior, with averages higher than other genotypes. However, the regression coefficient is low, 24.86%, do not satisfactorily explain the development of this genotype. The Tifton 68 and Jiggs genotypes presented quadratic behavior. However, it is observed that the Jiggs genotype showed a slight growth until the fifth evaluation season (50 days) and subsequent reduction in the width of the leaves with a regression coefficient of 37.10%. Differently from Tifton 68 genotype showed a large increase until the eighth evaluation season (80 days) and subsequent fall, with regression coefficient of 87,83%. The most outstanding genotype for wider leaves was the Tifton 85.

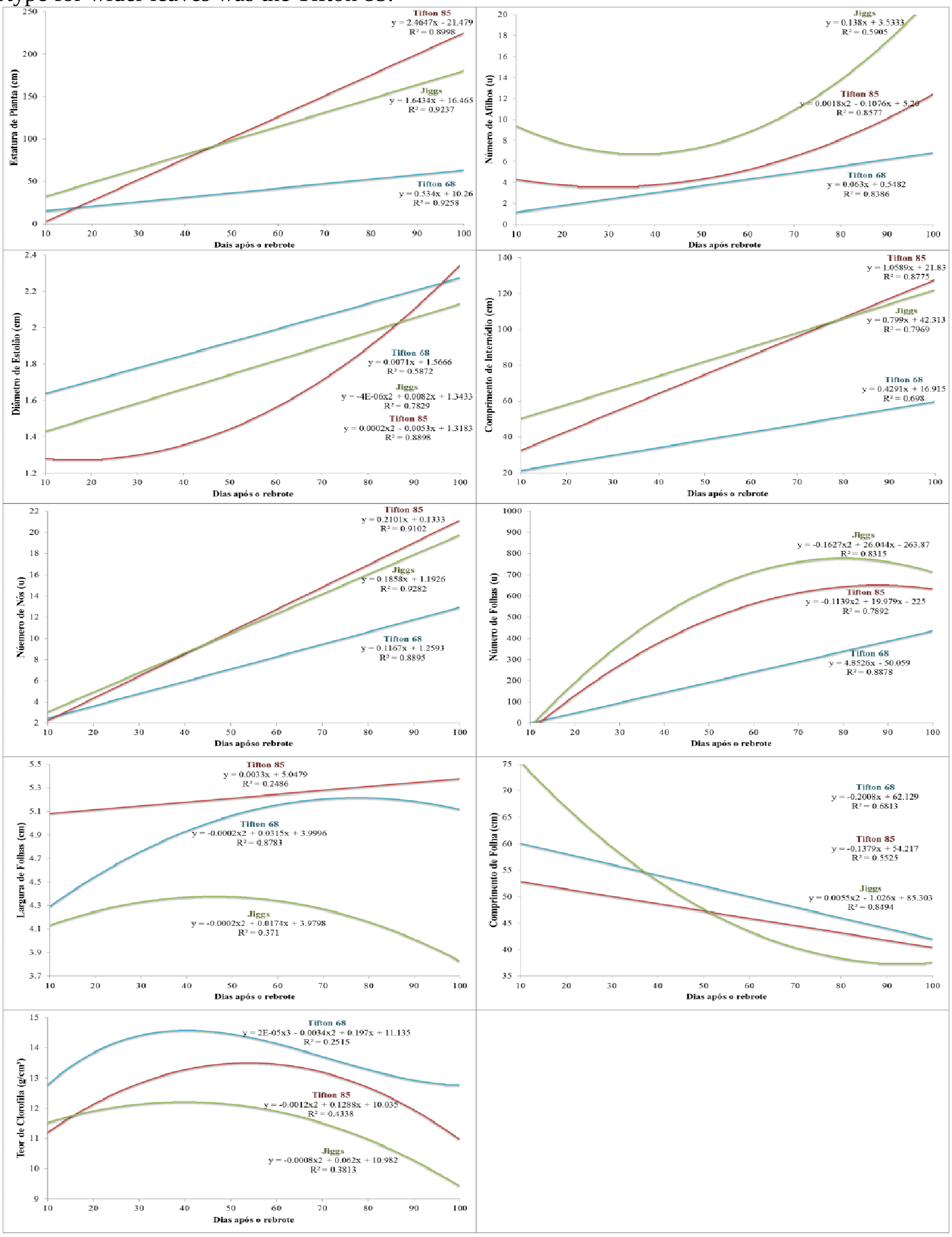

Fig. 1: Regression analysis for traits evaluated in ten different times of development on Tifton 68, Tifton 85 and Jiggs genotypes.

For the leaf length, it is observed that Tifton 68 and Tifton 85 genotypes showed a linear behavior, with a reduction in all the experiments and regression coefficients of 68.13% and 55.25%, respectively. The Jiggs genotype also showed a decrease in the experiment. However, his behavior was quadratic way with regression coefficient of 84.94%. The highlight of genotype to greater length leaf was Tifton 68. With progressive reduction of plant growth speed, the canopy needs more days to reach the level of critical interception, after which the stem accumulation and senescence are, dramatically intensified (Oliveira *et al*., 2010).

The only trait that presented cubic behavior was the chlorophyll content, for Tifton 68 genotype, with regression coefficient of 25.15%. The Tifton 85 and Jiggs genotypes presented quadratic behavior with regression coefficients of 43.38% and 38.13%. For the three genotypes behavior in relation to the chlorophyll content has not been satisfactorily explained by the evaluation seasons. However, it notes that the maximum chlorophyll content in plants, occurs between the fourth (40 days) and sixth (60 days) evaluation season. The highlight of genotype for greater chlorophyll content in the leaves was the Tifton 68. Depending on the plant growth occurs decrease the nutritional value of pastures, related to the reduction of protein and phosphorus. That occurs due to the aging plant with increased respect stem/leaf, because the stems of grasses has higher fiber content and lower of protein and phosphorus in relation to the leaves; increased lignification process and reducing the digestibility of nutrients (Carvalho *et al*., 2005).

Through path analysis (Table 1) it is observed that the PC trait has high magnitude and positive correlation with SD (0.94), LW (0.99), LL (0.92) traits, and negatively with characters NT (0.87) and DM (-0.82). It is also observed that the determination coefficient were very high (0.9911), the k value used in the analysis was low (0.0507) as well as the effect of the residual variable (0.0943) and also the determining the value of the correlation matrix between the eigen values (6,48E-11). This indicates that the path analysis crest was effective to estimate the effects of traits (CRUZ and CARNEIRO, 2006). Although not presented significant correlation, note to the PH trait that the main positive effects are indirect via LL, GM and DM and negative via NT, SD, NL and CC. The other variables were negative and low magnitude. The direct effect of PH on PC was not pronounced for this analysis.

We observe to the NT that all the traits had negative indirect effect, except the PH. Negative direct effect pronounced between the NT and PC was observed, as well as negative indirect effect via SD, NL, LW, LL, CC and DM, resulting in negative correlation (rP = -0.87). Although the trait number of tillers be quite complex as to their best advantage to plant breeding in many species, is the complexity of the genetic control and the processes involved in their phenotypic expression, as its differential response to environmental conditions and management systems (Kuraparthy *et al*., 2007). These results indicate that the selection of plants with increased number of tillers, promote the reduction of protein content of plants tifton.

Table 2: Estimates of path analysis under multicolinearity to morphological and physiological traits evaluated in Tifton genotypes, using as the dependent variable the protein content (PC) and the traits, plant height (PH), number of tillers (NT), stolon diameter (SD), internode length (IL), number of nodes (NN), number of leaves (NL), leaf width (LW), leaf length (LL), chlorophyll content (CC), green Mass (GM) and dry mass (DM) as independent variables.

Direct Effect				Indirect effetct via traits								Total
	[1]PC	[2]PH	[2]NT	[2]SD	[2]IL	[2]NN	[2]NL	[2]LW	[2]LL	[2]CC	[2]GM	[2]DS	[3]*(rP) PC*
	(%)	(cm)	(u)	(mm)	(mm)	(u)	(u)	(mm)	(mm)	(ml L^{-1})	(Kg ha^{-1})	(Kg ha^{-1})	(%)
PH	0.03		-0.07	-0.06	-0.04	-0.02	-0.07	0.00	0.07	-0.07	0.11	0.09	-0.02
NT	-0.14	0.01		-0.15	-0.03	-0.01	-0.09	-0.14	-0.11	-0.10	-0.02	-0.07	-0.87*
SD	0.16	-0.01	0.14		0.03	0.01	0.09	0.15	0.13	0.10	0.05	0.09	0.94**
IL	-0.04	0.03	-0.11	-0.11		-0.02	-0.09	-0.06	0.00	-0.09	0.07	0.03	-0.39
NN	-0.02	0.03	-0.10	-0.09	-0.04		-0.09	-0.04	0.02	-0.09	0.09	0.05	-0.27
NL	-0.10	0.02	-0.13	-0.14	-0.04	-0.02		-0.11	-0.06	-0.10	0.02	-0.02	-0.67
LW	0.17	0.00	0.12	0.14	0.01	0.00	0.06		0.17	0.08	0.09	0.14	0.99**
LL	0.18	0.01	0.08	0.11	0.00	0.00	0.03	0.16		0.05	0.13	0.16	0.92**
CC	0.10	-0.02	0.14	0.15	0.03	0.01	0.10	0.12	0.08		0.00	0.04	0.77
GM	-0.15	-0.02	-0.02	-0.05	0.02	0.01	0.02	-0.11	-0.16	0.00		-0.16	-0.62
DM	-0.16	-0.02	-0.06	-0.09	0.01	0.01	-0.01	-0.14	-0.18	-0.03	-0.14		-0.82*
Determination coefficient												0.99111	
k value used in the analysis												0.05066	
Effect of residual variable												0.09428	
Determinant of correlation matrix												6.48E-11	

[1]Column for the direct effect of the line trait on the protein content (PC). [2]Column for the indirect effects on the line trait the protein content (PC). [3] Column for the phenotypic correlation (rP) of the line trait with the protein content (PC).

Direct positive effect was observed for the trait SD on PC, as well as positive direct effect via NT, NL, LW, LL, CC and DM, resulting in positive correlation (rP = 0.94). This indicates that the selection of genotypes with greater stolon diameter result in an increase of plant protein content. In the culture of oilseed radish (*Raphanus sativus* L.), stem diameter has a positive relationship with the green mass and dry mass, and can be used for indirect selection (Cargnelutti-Filho *et al*., 2014).

The IL trait showed no significant correlation with PC. However, it is observed that the NT, SD, NL and CC traits showed high negative indirect effect on the IL. The direct effect of IL on PC was not pronounced for this association.

For NN trait also was no significant correlation. However, pronounced negative indirect effects were observed for the NT, SD, NL and CC traits. There was no direct pronounced effect on PC.

As for the number of nodes, the NF trait showed no significant correlation with PC. However, negative indirect effects were observed via NT, SD, LW and CC, as well as negative direct effect on PC.

Positive direct effect and high magnitude was observed for the LF trait on the PC. Positive indirect effects of high magnitude were observed via NT, SD, LL and DM, resulting in high magnitude correlation between the LW and PC (rP = 0.99). This indicates that the selection of plants with leaf wider, promote increased protein content in the leaves of Tifton.

The greater direct positive effect was observed for the LL trait on the PC. Positive indirect effects were also observed via SD, LW, GM and DM, resulting in high magnitude correlation between LL and PC (rP = 0.92). This indicates that the selection of plants with higher leaf length favor the production of plants with increased protein content.

The CC trait showed no significant correlation with PC. However, the value of the observed correlation can be considered average. Direct positive effect was observed for the relationship of these traits, as well as positive indirect effects via NT, SD, NL and LW. Although no significant correlation with PC, the GM trait showed pronounced negative effects, directly and indirectly via LW, LL and DM.

For DM trait was observed negative direct effect of high magnitude with the PC, as well as indirect and negative effects via LW, LL and GM, resulting in negative correlation between DM and PC (rP = -0.82). This indicates that the selection of plants with higher mass will result in plants with reduced protein content.

In a general way, to increase the protein content in plants Tifton is necessary for the selection of plants with least number of tillers and less dry matter. As well as larger stolon diameter, greater leaf width and greater leaf length.

However, the number of tillers have indirect positive effect of via SD, LW and LL, indicating that a higher number of tillers may be associated with increased stolon diameter, leaf width, the leaf length, and consequently the chlorophyll content. A similar result was observed for DM, as the positive indirect effects via SD, LW and LL. This indicates that plants with larger stolon diameter, greater leaf width and greater leaf length, tend to exhibit greater dry mass. This work presents a great contribution to agricultural and especially the cultivation of Tifton genotypes science, it reveals the phenotypic interrelations and the actions of cause and effects of morphological and productive characters determining the crude protein in the same way, evidence criteria for employment indirect selection of a characters to be used in breeding programs of this species.

Conclusions:

To getting the genotypes with greater protein content, is necessary the selection plants with greater stolon diameter, leaf width, leaf length, lower number of tillers, green mass and dry mass.

In a general way, the Tifton 85 genotype has greater highlight cultivation aiming greater development, resulting in a higher green mass, dry mass and chlorophyll content, however, when it aims genotypes that have high protein content, should have some caution, since the negative correlation seen between this and the traits green mass and dry mass.

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