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Full Length Research Paper

Modeling of hypsometric distribution of *Handroanthus heptaphyllus* seedlings in different containers

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Hypsometric information of seedlings allows greater assertiveness of silvicultural decisions in nurseries. This study aimed to evaluate the efficiency of different Probability Density Functions (PDF) to estimate and compare the height distribution of *Handroanthus heptaphyllus* seedlings in different cultivation containers. One hundred and thirty one seedlings were produce in two types of containers; 81 units in tubes and the remaining in plastic bags. The census was realized at 122-day old, measuring total height of the seedlings using millimeter ruler. The seedlings did not present a tender or brittle caulinar system. Data were grouped into biometric classes with regular intervals of 2.5 cm in height. Seven PDF were adjusted using maximum likelihood method and the one with the best predictive performance was selected to identify the statistical equality between the distributions estimated for the seedling size in each container. The order of predictive efficiency of PDF was distinct between recipients. The Weibull of two parameters function can be used to model the height distribution of *H. heptaphyllus* seedlings at 122-day old, produced in plastic bags and tubes. The hypsometric distribution was different between containers.

Key words: Height, probability density functions, ipê-roxo.

INTRODUCTION

Handroanthus heptaphyllus (Vell.) Mattos (Bignoniaceae), commonly known as ipê-roxo and pau-d'arco, is a tree species with records of occurrence in almost all Brazilian territory, from the South to the Northeast (Oliveira et al., 2015; Dullius et al., 2016). It has been widely used for wood purposes, due to its heavy wood (1.07 g cm^{-3}), urban afforestation and restorations of legal reserves and permanent preservation areas (Mori et al., 2012; Oliveira et al., 2015). Regarding seedlings production of native species, the success of a planning depends on quantity and quality of available information. Hypsometric distribution modeling is an important statistical technique

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capable of supporting silvicultural decisions, taking the choice of culture containers as an example.

The choice of containers for seedlings production should take into account various technical and financial criteria. Plastic bags and tubes are the most recommended containers for seedlings production in the country, each one expresses advantages and limitations (Pias et al., 2015). The plastic bags have a low cost of acquisition, but require more production space and labor to handle the seedlings, besides the ease of breaking under inadequate handling (Teixeira et al., 2009). An alternative that facilitates the sequencing of operations in nurseries is the use of reusable polypropylene tubes, which have internal ribs and a lower hole (open bottom) for directing root growth and minimizing problems with folding (Dominguez-Lerena et al., 2006; Ferraz and Engel, 2011).

Containers shape root system, protect the roots from mechanical damage, and provide support for nutrition. In spite of the greater demand for water and fertilizers, voluminous containers provide more space for root development and, in some cases, intensify the growth rate of seedlings (Dominguez-Lerena et al., 2006; Pias et al., 2015). Routinely, voluminous containers are used to seminal propagation of native species, due to the lack of knowledge of the pattern of growth and distribution of the root system.

Height and its ratio to the shoot dry mass are excellent attributes for the qualitative evaluation of seedlings (Gomes et al., 2002). The height is a biometric attribute that allows inferring about the potential performance of seedlings in a fast, objective and non-destructive way. In addition, high seedlings tend to be more successful in establishing and surviving in the field (Landergott et al., 2012; Westfall and McWilliams, 2017).

It is common to represent large datasets by means of generalist measures of position and dispersion, neglecting information related to their distribution. Detailed information on the hypsometric structure of seedlings allows greater precision and assertiveness of silvicultural decisions by individual owners and large forest companies, such as the application of a nutritional and logistics management plan. The adjustment of biometric distribution models allows the estimation of the number of seedlings within intervals or size classes, with a lower and upper limit (Amaral et al., 2009; Rana et al., 2017). The optimization of the input allocation for the growth of certain seedlings with interest size rationalizes the application of correctives and fertilizers.

Modeling of biometric distributions has gained increasing importance due to its contribution to the forest enterprises planning, whose focus is the obtaining of multiproducts. In the specific case of nurseries, the seedlings expedition for different purposes (multiproducts) is a practice that must be analyzed from an operational and logistic point of view in order to maximize yields. A common approach in distribution models is the use of statistical probability functions, known as Probability Density Functions (PDF), to characterize the size structure of a set of plants (Tsogt and Lin, 2014; Diamantopoulou et al., 2015).

Several researchers have devoted themselves to the construction and application of diametric distribution models (Bailey and Dell, 1973; Leite et al., 2010; Binoti et al., 2012; Diamantopoulou et al., 2015; Podlaski et al., 2018) and hypsometric of trees (Koehler et al., 2010; Souza et al., 2013; Westfall and McWilliams, 2017). However, such modeling practice is still incipient or non-existent at the nursery level. The most commonly used functions in the forest area are Weibull, Normal, Log-normal, Gamma, Logistic, Log-logistic, Cauchy, Johnson's SB, Beta, Frechet, Erlang, Rayleigh and Hyperbolic (Leite et al., 2010; Binoti et al., 2012; Campos and Leite, 2017). The PDF are adjusted predominantly by the maximum likelihood method, providing reliable estimates (Campos and Leite, 2017).

Hypsometric distribution of seedlings is based on a histogram of height frequency and is expected to present different forms according to species, age and silvicultural treatments. Despite the intensive use of containers in nurseries, detailed research on their implications in the hypsometric distribution of plants is still lacking. By the aforementioned, the following hypotheses were tested: (i) Is the Weibull function flexible enough to model the height distribution of seedlings? (ii) Are the hypsometric distributions of seedlings cultivated in tubes and plastic bags similar? The objective was to evaluate the efficiency of different PDF to estimate and compare the height distribution of *H. heptaphyllus* seedlings in different culture containers.

MATERIALS AND METHODS

Fruits used in the present work came from routine collections conducted by the Federal Institute of Education, Science and Technology of Minas Gerais (IFMG) in Governador Valadares-MG municipality, from June to September 2017. The trees selected for the collection had exuberant crown, with no apparent signs of pest and insect attack. The collection region has a humid tropical savanna climate, classified as Aw by Köppen International System (rainy summer and dry winter).

Fruits collected were stored in Kraft paper bags and hand-fed at seedling nursery of IFMG São João Evangelista-MG *campus*, located at 18°33'11" south latitude and 42°45'10" west longitude (Datum WGS84). The climate is classified as Cwa (dry winter and rainy summer), with average minimum temperature of 22°C and average maximum of 27°C per year, annual average rainfall of 1.180 mm and altitude of 730 m. The beneficiation consisted of seeds isolation in relation to fruits and elimination of materials that had some atrophy or injury.

The nursery produced 131 seedlings of *H. Heptaphyllus* in two types of containers; 81 units in 280 cm^3 tubes (6.5 cm outside diameter, 5.2 cm inner diameter, 19 cm length, 8 internal ribs) and the rest in plastic bags (height 30 cm, width 25 cm and perforated). The seedlings were cultivated in a shade house, covered with sombrite (50% mesh), until 30 days after sowing and were irrigated three times a day for 15 min (nozzle flow rate of 103 L h^{-1}). Afterwards, the seedlings were conditioned in the open air (full sun) for 92 days, irrigated four times a day for 10 min (nozzle flow rate of

Table 1. Probability Density Functions (PDF) tested for adjustment of the height distribution of *Handroanthus heptaphyllus* seedlings at 122-day old.

Name	Probability Density Functions
Weibull 2P	$f(x) = \left(\frac{\gamma}{\beta}\right)\left(\frac{x}{\beta}\right)^{\gamma-1} e^{-\left(\frac{x}{\beta}\right)^{\gamma}}$
Logistic 2P	$f(x) = \frac{e^{\left(\frac{x-\alpha}{\beta}\right)}}{\beta\left[1+e^{\left(\frac{x-\alpha}{\beta}\right)}\right]^2}$
Log-logistic 2P	$f(x) = \frac{\gamma\left(\frac{x}{\beta}\right)^{\gamma}}{x\left[1+\left(\frac{x}{\beta}\right)^{\gamma}\right]^2}$
Cauchy	$f(x) = \frac{1}{\pi\beta\left[1+\left(\frac{x-\alpha}{\beta}\right)^2\right]}$
Gamma 2P	$\begin{cases} f(x) = \frac{x^{\gamma-1}e^{-x/\beta}}{\beta^{\gamma}\Gamma(\gamma)}, 0 < x < \infty \\ 0, for\ other\ values\ of\ x \end{cases}$
Normal 2P	$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$
Log-normal	$f(x) = \frac{1}{x\sigma\sqrt{2\pi}} e^{-\frac{(ln(x)-\mu)^2}{2\sigma^2}}$

x = biometric class center, $x \geq 0$; α= location parameter, $\alpha > 0$; β= scale parameter, $\beta > 0$; γ= shape parameter, $\gamma > 0$; $\Gamma(\gamma)$ = parameter Gamma function γ, $\gamma > 0$; μ= mean (location parameter); σ= standard deviation (scale parameter); e = neperian constant; and Π= 3,14159265359.

118 L h^{-1}). The seedlings did not present a tender or brittle caulinar system.

The census was carried out measuring the total height (H, cm) of all seedlings produced with the aid of a millimeter ruler. The total height was characterized by linear distance from collection to last leaf. Data were submitted to descriptive statistical analysis (minimum, mean, median, mode, maximum, coefficient of variation, and by the moment's method, asymmetry and kurtosis). The unpaired *t* test was applied to compare the height averages of the seedlings produced in the different container types.

Data were grouped into biometric classes with regular intervals of 2.5 cm in height. The functions tested were: two-parameter Weibull (Weibull 2P); two-parameter Logistic (Logistic 2P); two-parameter Log-logistic (Log-logistic 2P); Cauchy; two-parameter Gamma (Gamma 2P); Normal; and Log-normal. All the functions were adjusted by the maximum likelihood method, using the optimization methodology of Nelder and Mead. The PDF are listed in Table 1.

Adjustments quality was evaluated according to the Mean Absolute Error (MAE), Pearson correlation coefficient ($r_{Y\hat{Y}}$), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Lower MAE values and both criteria of statistical information imply higher predictive quality.

Adherence of the functions to the data was evaluated by the Kolmogorov-Smirnov test (Gibbons and Subhabrata, 1992). It is a test that compares the estimated cumulative frequency with the observed frequency. The point of greatest divergence between distributions is the test statistic value (dn). In addition, graphical analysis was performed between values observed and estimated by obtained equations.

From predictive best efficiency function, identity test model (Graybill, 1976) was applied to identify the statistical equality among estimated distributions for seedling size produced in each container. This hypothesis was tested by F statistic, whose non-rejection ($F_{H0} < F_{\alpha}$) allows to admit that distributions' frequency are not different from each other.

In order to diagnose statistical effect, the significance level of 1% was adopted in all analyses. These were carried out using software R 3.3 (R Core Team, 2017).

RESULTS

Height distribution of the *H. heptaphyllus* seedlings produced in tubes was more asymmetrical than those from plastic bags; both distributions presented negative asymmetry, with the left tail (Table 2). The leptokurtic (positive kurtosis) and platicurtic (negative kurtosis) behaviors were observed in height distributions related to the use of plastic tubes and bags, respectively. The plastic bags exhibited seedlings with a greater amplitude of variation (absolute difference between the maximum and minimum values) and coefficient of variation of height. The mean, median and mode values were close to each other, indicating a trend towards normality.

By the t test, the average height of the seedlings produced in plastic bags was higher than those from tubes. Regarding the use of tubes, all adjustments presented adherence by the Kolmogorov-Smirnov test (mean of test statistics equivalent to 0.47 ± 0.11). When plastic bags were used as containers for seedlings production, adherence was verified only in the adjustments of the Weibull 2P (dn = 0.64) and Cauchy (dn = 0.64); the mean of test statistics of adjustment of the other functions was 0.80 ± 0.09. Functions adjustments showed few deviations, with low MAE values (Table 3). In general, the correlation coefficients were high (above 0.90) for seedlings produced in tubes. The significance ($p \leq 0.01$) of the correlation coefficients was found in all the adjustments.

The efficiency of adjusted functions majority was distinct between containers. According to AIC and BIC criteria, the increasing order of predictive height efficiency of seedlings produced in tubes was: Cauchy, Log-normal 2P, Gamma 2P, Log-logistic 2P, Normal, Logistic 2P and Weibull 2P. For the use of plastic bags as containers, the order was modified to: Cauchy, Log-logistic 2P, Log-normal, Gamma 2P, Logistic 2P, Normal, and Weibul 2P. The predictive superiority of the Weibull 2P function was also confirmed by the lower MAE value, high correlation coefficient and adherence. From the parameters obtained using the PDF (Table 2), followed by the estimates of relative frequency of seedlings per height class. The negative asymmetry of height data (Table 1) was evidenced in the frequency distributions observed (Figure 1). The observed frequencies of height did not show discontinuity (absence of seedlings in one or more biometric classes), considering the respective intervals formed by the minimum and maximum limits (Table 2).

Facing the best predictive efficiency, the Weibull 2P function was selected for graphical analysis (Figure 2) and subsequent comparison. The F Graybill test result showed a significant statistical difference ($F_{H0} \geq F_{\alpha}$) between the estimated height distributions of the seedlings for the two types of containers. The curve concerning the plastic bags was more displaced to the right, showing greater dispersion (standard deviation of 7.03 cm) and of central tendency (average of 43.81 cm).

DISCUSSION

Providing accurate estimates requires good function adherence to structure data. The adherence of the PDF demonstrated potential to describe the hypsometric structure of seedlings produced in tubes. However, not all of them were efficient to model the height distribution of seedlings from plastic bags. The values of dn statistic (Kolmogorov-Smirnov) were smaller with use of tubes; variation amplitude of this statistic was from 0.29 to 0.57 for the tubes and from 0.64 to 0.82 for the plastic bags. The smaller number of seedlings produced in plastic bags, together with the greater variation amplitude of height (25.60 cm), may have negatively affected the predictive performance of PDF Logistic 2P, Log-logistic 2P, Gamma 2P, Normal and Log-normal. It is important to emphasize that other statistical approaches, such as the use of artificial intelligence and regularized regressions (Binoti et al., 2013; Castro et al., 2013; Binoti et al., 2014; Kadyrova and Pavlova, 2014; Diamantopoulou et al., 2015; Chai et al., 2016), can be applied in order to improve predictive quality in complex database modeling. The most common regularization methods for machine learning focused on regression problems are the Ridge and Least Absolute Shrinkage and Selection Operator (LASSO) (Chai et al., 2016). Biological network-regularized logistic models are examples that have been extensively used in the genomic area (Zhang et al., 2013; Huang et al., 2015; Chai et al., 2016; Huang et al., 2016; Kang et al., 2017), but its application has not yet been found in Brazilian silviculture.

It is emphasized that hypsometric distribution can be obtained by measuring height of all seedlings produced (census) or a representative subset of them (sampling). The census for a specific species is justified for a reduced number of seedlings, which is common in nurseries that propagate native species and prioritize the diversification of production. On the other hand, sampling techniques may be indicated to characterize the biometric distribution of large-scale production of a given species.

The graphical analysis showed a greater clarity in the judgment of the height distributions estimated by PDF. When analyzed in Figure 1, Weibull 2P function was the one that best represented data series. All functions underestimated the number of seedlings produced in

Table 2. Height characterization of *Handroanthus heptaphyllus* seedlings at 122-day old, produced in two types of containers.

Statistics	Tubes	Plastic bags
Minimum (cm)	11.20	29.20
Mean (cm)	22.43	43.81
Median	22.60	44.65
Mode	22.20	43.20
Maximum (cm)	28.30	54.80
Coefficient of variation (%)	13.01	16.04
Asymmetry	-0.76	-0.51
Kurtosis	1.85	-0.66

Asymmetry and kurtosis calculated by the moment's method.

Table 3. Coefficients and adjustment quality of the Probability Density Functions (P.D.F.) used to estimate the relative frequency of the *Handroanthus heptaphyllus* seedlings height at 122-day old, produced in two types of containers.

Function	Coefficients		MAE	$r_{Y\hat{Y}}$	AIC	BIC
Tubes						
Weibull 2P	β = 23.7074	γ = 8.5222	0.0866	0.9733	412	417
Logistic 2P	α = 22.5770	β = 1.6853	0.0868	0.9750	413	418
Log-logistic 2P	β = 22.4868	γ =12.8553	0.0873	0.9483	419	424
Cauchy	α =23.1246	β =1.6426	0.0931	0.9705	434	434
Gamma 2P	β = 2.1465	γ = 48.1318	0.0867	0.9200	423	428
Normal	μ = 22.4228	σ = 3.0703	0.0864	0.9533	416	420
Log-normal	μ = 3.0997	σ = 0.1491	0.0870	0.8977	428	433
Plastic bags						
Weibull 2P	β = 46.5998	γ = 7.9708	0.0577	0.6933	334	338
Logistic 2P	α = 44.3161	β = 4.0281	0.0600	0.6079	341	345
Log-logistic 2P	β = 43.9983	γ =10.3913	0.0610	0.5244	346	350
Cauchy	α = 45.6190	β = 4.2709	0.0621	0.6608	359	359
Gamma 2P	β = 0.8363	γ = 36.5878	0.0599	0.4740	343	347
Normal	μ = 43.7500	σ = 6.9101	0.0593	0.5644	339	343
Log-normal	μ = 3.7648	σ = 0.1700	0.0602	0.4229	345	349

α= location parameter; β= scale parameter; γ= shape parameter; μ= mean (location parameter); e σ= standard deviation (scale parameter). MAE: Mean absolute error; $r_{Y\hat{Y}}$: correlation coefficient; AIC: Akaike information criterion; e BIC: Bayesian information criterion.

plastic bags in the extreme height classes. It is easy to notice the non-adherence of those functions whose null hypothesis was rejected by the Kolmogorov-Smirnov test.

Although Weibull 2P and Cauchy PDF were the only ones whose adherence was verified to describe the height of seedlings in both tubes and plastic bags, the first showed a better predictive efficiency of hypsometric distribution of *H. heptaphyllus* seedlings (Table 3 and Figure 1). Adjustment statistics showed that the scale and shape parameters of the Weibull 2P function were not biased. Scale parameter represents the amplitude of the distribution and it was smaller when using tubes for seedlings production, consistent with the limits shown in Table 2. The use of plastic bags resulted in a distribution relatively closer to the axis of symmetry. The values of shape parameter denoted negative asymmetry, revealing

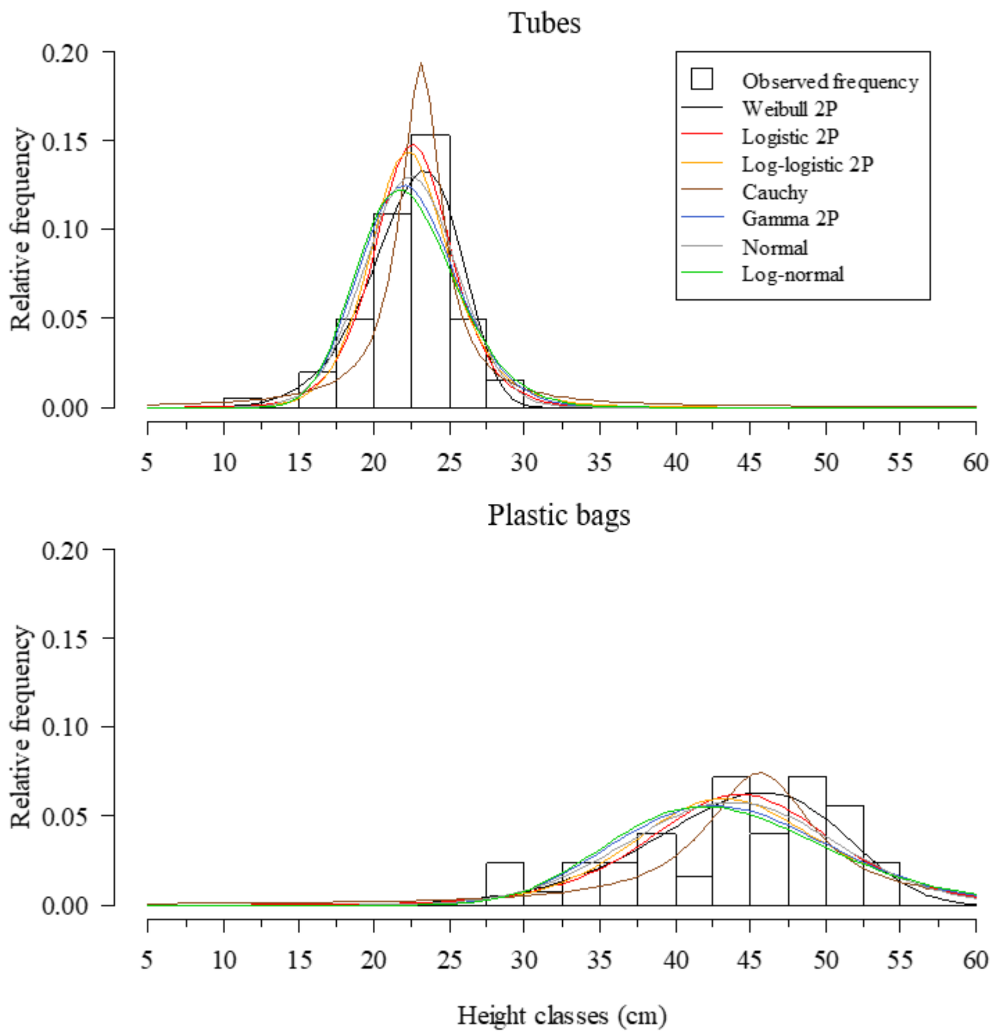

Figure 1. Relative frequency observed and estimated by PDF for *Handroanthus heptaphyllus* seedlings height at 122-day old, produced in two types of containers.

an accumulation of larger size seedlings. This assumption was based on the premise that shape parameter (values above 3.6) increases as the asymmetry becomes progressively more negative (Bailey and Dell, 1973).

The choice of the function for modeling distributions determines the accuracy of the estimates. The evaluation of biometric distribution models should consider interpretations of qualitative nature (biological realism) and quantitative (statistical). The Weibull 2P function proved to be an efficient probabilistic model capable of accurately representing reality, even on occasion with reduced data and large variation amplitude. Currently, this function is intensely adjusted in forest area due to its flexibility to assume different asymmetries and shapes, modeling several distribution tendencies, from normal to exponential (Bailey and Dell, 1973; Leite et al., 2010; Tsogt and Lin, 2014; Diamantopoulou et al., 2015). The ability to model distributions with different asymmetries and shapes was confirmed in the present work (Table 3 and Figure 1). There is a consensus of the superiority of the Weibull function over the other PDF for the description of biometric attributes, above all, the diametrical structure of trees (Campos and Leite, 2017). However, no studies have been found using the function to describe the hypsometric structure at nursery level.

Comparing the hypsometric distributions estimated by the Weibull 2P function in each container (Figure 2), rejection of the similarity hypothesis was recorded ($F_{H0} \geq F_{\alpha}$). This difference between distributions hinders the predictive performance of a possible fit for the entire data set, and it is indicative that the seedlings in some cases require different management per container. In terms of mean, the difference in height was also observed by the t test. The definition of stratification criteria for PDF adjustment, as silvicultural treatments, provides detailed information about the effect of the management in

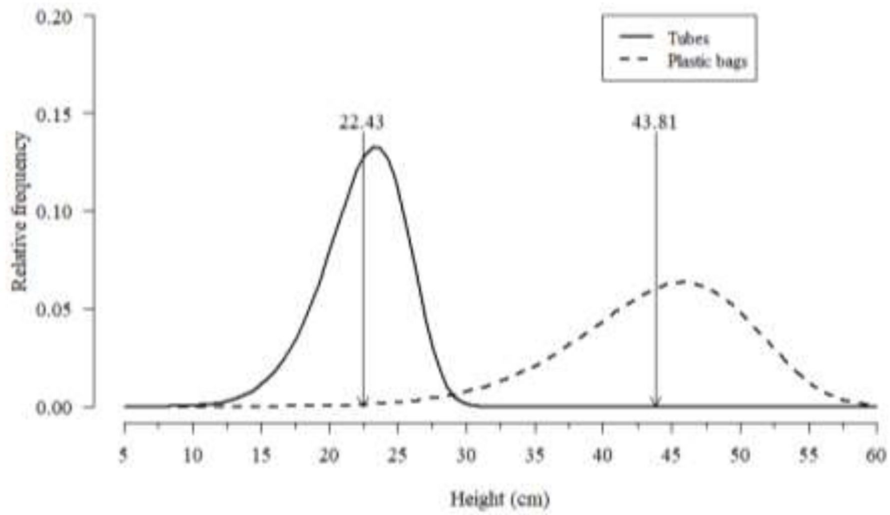

Figure 2. Estimated relative frequency with the Weibull 2P function for *Handroanthus heptaphyllus* seedlings height at 122-day old produced in two types of containers. The arrows indicate average of observed distribution of the database.

seedlings production in commercial and technological routine.

Size is crucial for the establishment of seedlings in field or urbanized areas, small differences may influence the establishment and/or dominance of the species (Dominguez-Lerena et al., 2006). The classification of nursery seedling lots in terms of height is a quality control methodology (Gomes et al., 2002). The adjustment of the hypsometric distribution for each container proved that it is possible to sketch the quality of seedlings using PDF, because the larger the value of the scale parameter, the greater the growth rate. In this perspective, the seedlings produced in plastic bags (distribution more asymmetric and with lower value of the shape parameter) presented higher growth potential in height than those originating from tubes. It is emphasized that the standardization of an index age, or reference, is essential for making inferences about the classification of productive potential (Campos and Leite, 2017). In the presence of seedlings with different ages or periodic measurements, the adjustment of regression models for height estimates in the index age is possible.

Knowledge of productive potential stimulates the search for silvicultural strategies that minimize reducing factors and limiting plant development. The containers used in seedlings production influenced the growth rate of seedlings, corroborating Dominguez-Lerena et al. (2006) for *Pinus pinea* L. The *H. heptaphyllus* seedlings produced in plastic bags presented a higher growth rate, reducing their time in nursery and making possible the anticipated expedition in relation to those of tubes. This fact was probably due to the greater volume of plastic bags, which provide more nutrients, space for the development of the root system and water utilization, whose losses in tubes can reach 78% of the volume applied (Bomfim et al., 2009; Barroso et al., 2000). In addition, the depth of the plastic bags was 1.58 times greater than that of the tubes. According to Dominguez-Lerena et al. (2006), the depth of the container is one of the most important attributes that act on the morphology of seedlings.

A greater disuniformity of seedling height produced in plastic bags was observed, with a flattened distribution (platicurtic) and greater data dispersion. The seedlings from tubes had lower hypsometric variation, resulting in greater production uniformity. The use of tubes proved to be a promising technique to homogenize seedlings propagation. With regard to the choice of growing container to meet a rapid demand for seedlings, plastic bags can be a viable alternative to be considered, provided all the costs involved in the production chain are analyzed and waste is avoided.

The information of hypsometric structure of seedlings allows a better targeting of the inputs. The accelerated growth rate favors the production of high-quality seedlings, which are more valued for commercialization and frequently used in urban afforestation. Seedlings that grow faster require more attention to the application of silvicultural treatments, such as the additional application of N based on nutritional balance. As for the height, the plastic bags were the most suitable containers for the production of high-standard seedlings of *H. heptaphyllus*.

The most frequent intervals, formed by three consecutive size classes (Figure 2), concentrated 30.99% (20.00 to 27.50 cm) of the seedlings produced in tubes and 17.94% (42.50 to 50.00 cm) of those coming from plastic bags. Assuming a hypothetical scenario in which seedlings with ≥90% of the height average of those 10 higher are ideal to be conducted in high-standard, 40% (height ≥24.30 cm) and 28% (height ≥ 46.58 cm) of the seedlings from plastic bags and tubes met this condition, respectively. Therefore, investment in fertilizers and larger containers, such as buckets, should be considered for maximize yields.

Seedlings with slow growth rates require more time to reach specific quality standards. Decisions to invest in the growth of these seedlings or their immediate expedition should be evaluated with caution, if possible, considering the market requirements and available inputs. Smaller seedlings, which meet a certain level of quality, can be targeted to medium and small-scale farmers, as they are traditionally a public with greater financial limitations for forest recovery (Ferraz and Engel, 2011; Pinto et al., 2011). Another target audience are companies with capital restrictions to start reforestation projects. Thus, larger seedlings are indicated for reforestation in places where there is clear possibility of survival and establishment of the species (Pinto et al., 2011).

The detailed statistical analysis of biometric surveys performed in nursery for better control of seedling production is recommended, evaluating size distributions and general measures of position and dispersion. The hypsometric distribution is an indicator of growing stock (Amaral et al., 2009), with potential use in the planning and management of seedlings production, helping to define silvicultural strategies and logistic strategies in nurseries. It is important to point out that difficulties in adjusting biometric distributions have been solved due to advances in computer science and statistical techniques, making adjustments ever simpler and facilitating the choice of the best function in relation to the dataset (Lana et al., 2013).

Conclusion

The two-parameter Weibull function is efficient to model the height distribution of *H. heptaphyllus* seedlings at 122-day old, produced in tubes and plastic bags. This function is flexible and promising for adjusting seedling height.

The hypsometric distribution is an efficient tool to classify seedlings and to support strategic decisions about logistic and silvicultural treatments in nurseries.

The hypsometric distribution of *H. heptaphyllus* seedlings at 122-day old may be different between plastic bags and tubes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Identification and estimation of the sugarcane production potential of Mozambique

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Identifying the potential areas that can produce sugarcane is extremely important, especially for developing countries. Most of the territories in Mozambique have soils with high and medium agricultural potential but are hampered by water conditions. Therefore, irrigation is the only solution to ensure certainty in agricultural production. In addition, to set up new business a minimum of infrastructure is required, like roads. The objective of this work is to estimate sugarcane productivity and production potential of Mozambique from agrometeorological data and soil maps using the intersection buffers of 25 km of rivers and 50 km of land transport routes. The analysis allowed the identification of potential areas and it is concluded that irrigation is necessary for high yield of sugarcane in Mozambique, but there are areas where it is possible for the cultivation just by rain fed. The estimated available area was 11,943,071 ha for irrigated areas and 11.640.221 ha for rainfall areas (15% of the country area). From the yield on these areas, it was possible to estimate the potential production of 1,030 Mt year^{-1}, with full irrigation and 611 Mt year^{-1} by rainfall. The productivity values generated by the model showed satisfactory results compared to the data observed in a production unit located in Marromeu, both for rainfed and irrigated.

Key words: Area, geotechnology, modeling, yield.

INTRODUCTION

Mozambique has a large agricultural potential for the production of bioenergy, mainly due to availability of potential areas (Watson, 2011). Furthermore, the energy shortages in the country underscore the need for identifying the possibility of production of either ethanol as electric energy from the use of biomass. There is an opportunity for investment in bioenergy because the country is largely rural and imports all fossil fuels

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(Econergy International Corporation, 2008).

To identify potential areas, the characterization of the climatic conditions is important since it is the main cause of variability of agricultural production. The knowledge of these variables during the growing season associated with agrometeorological yield models allows analysis of reduced productivity. Thus, the use of simple models with essential climatic elements (temperature and precipitation) facilitates the practical application and its integration into the process of crop management.

For the application of agrometeorological yield models, there is a need for meteorological data, which are generally derived from conventional or automatic weather stations. However, these data may have errors or are difficult to access. Agro-meteorological data from global models like the European Center for Medium-Range Weather Forecast (ECMWF) are an alternative. These are available free of charge by the meteorological database Joint Research Centre (JRC), a research center of the European Commission which collects weather information from stations scattered throughout the world, radar, satellites and other sources (Person and Grazziani, 2007).

As a developing country, investments in Mozambique need to be near a minimum of infrastructure such as roads and connection to ports (Schut et al., 2010). On the other hand, in places where the irregularity of rainfall and the influence of El Niño are frequent, the irrigation of sugarcane is a mandatory aspect (Ellis and Merry, 2004).

So in this context, the aim of this study is to estimate the potential yield and total production of sugarcane for Mozambique from agro-meteorological data and soil map, using the intersection of 25 km of river buffers and 50 km of land transportation routes buffers.

MATERIALS AND METHODS

This study considered the total area of Mozambique, considering the years 2008 to 2013 (due to data availability), the annual period from July to June (12 months), and totaling five periods. The analysis was divided into two stages, the first being the estimated average degree days, water deficit and productivity and total production (Figure 1); the second, determining potential areas (Figure 2).

For the first stage, estimated data of average air temperature (°C), precipitation (mm) and reference evapotranspiration (mm) of the ECMWF global model, with spatial resolution of 0.25° and temporal resolution of ten days, available in raster format were used (JRC, 2015). It was also used data, format shape suitable for the production of sugarcane in the type of soil in Mozambique. The potential was classified according to the presentation in CGEE (2009), which was applied in Brazil, as high (81.4 t ha^{-1} $year^{-1}$), medium (73.1 t ha^{-1} $year^{-1}$) and low (64.8 t ha^{-1} $year^{-1}$); areas with unfit classification, restricted and others were considered equal to zero. This productivity was transformed into raster format with a spatial resolution of 0.25°, where each pixel was classified according to the predominance of area classification.

The degree days (DD) were calculated according to the number of hours favorable for growth in basal temperature (Tb) of 18°C (Equation 1).

$$DD = \frac{(Tmax + Tmin)}{2} - Tb \quad (1)$$

The hydric deficit (HD) was calculated as the difference between precipitation (P) and the reference evapotranspiration (ETo) provided by the ECMWF global model (Equation 2).

$$HD = P - ETo \quad (2)$$

The base model for the estimation of productivity (Y) is given by Equation 3, where the factor considered is productivity given by the presented potential CGEE (2009) and the DD and HD values were obtained based on the average of 5 times. B and c factors were considered with the values, 0.01 and 0.1 respectively. These factors were obtained from multiple regression data with historical series of 5 years for rain fed sugarcane areas in Piracicaba, São Paulo, Brazil. In this manner, they are standard values. Due to the absence of historical sugarcane productivity data in Mozambique, it is not possible to calculate calibrated factors for the country.

Therefore, the model is based on simplicity, expressing the importance of the water supply from the water deficit and the efficiency of interception of solar radiation from the degree days during the harvest period. In practice, with lower HD, DD will be lower due to the few hours of sunshine (clouds). Two outputs were considered for the model and productivity considering the water deficit and others, excluding it in the case of irrigation.

$$Y = a + b\,DD - c\,HD \quad (3)$$

In step 2 (Figure 2), potential areas were identified as well as the main features of water availability, that is, the presence of water courses with constant flow and transport infrastructure by land routes, such as main and secondary roads or railway line. Thus, from these data, a 25 km buffer for water availability (perennial rivers) was first made; this distance is related to canals built in Marromeu. A buffer of 50 km of land routes was also made for the displacement of production. According to CGEE (2009), usually sugarcane growing areas are located at an average distance of 25 km of the plants (on average) due to cost of shipping. Potential areas were determined from the intersection of the buffer areas, thus having the two major characteristics considered.

In order to exclude restricted areas of the previous result after processing, the restricted were excluded as follows: bad soil, slope greater than 13%, high carbon, cropland and mixed, high DUAT (right to use and benefit from the land known as Direito do Uso e Aproveitamento da Terra) and reserves. Thus, the productivity from the map obtained in step 1 was determined in the final area of the intersections for the total production, considering only the performance without water deficit, in case of use of irrigation. All phases of the study were performed using spreadsheets in Microsoft Excel and the ESRI ArcGIS 10.2.2 for Desktop to process and generate the maps.

RESULTS AND DISCUSSION

Figure 3 shows the potential yield by soil type and average periods of precipitation, hidric deficit and total degree days.

It appears that there is great potential in the northern region of the country, both in soil characterized as having high potential, required amount of degree days and minimal rainfall, but requiring supplemental irrigation. The total degree days showed results consistent with the

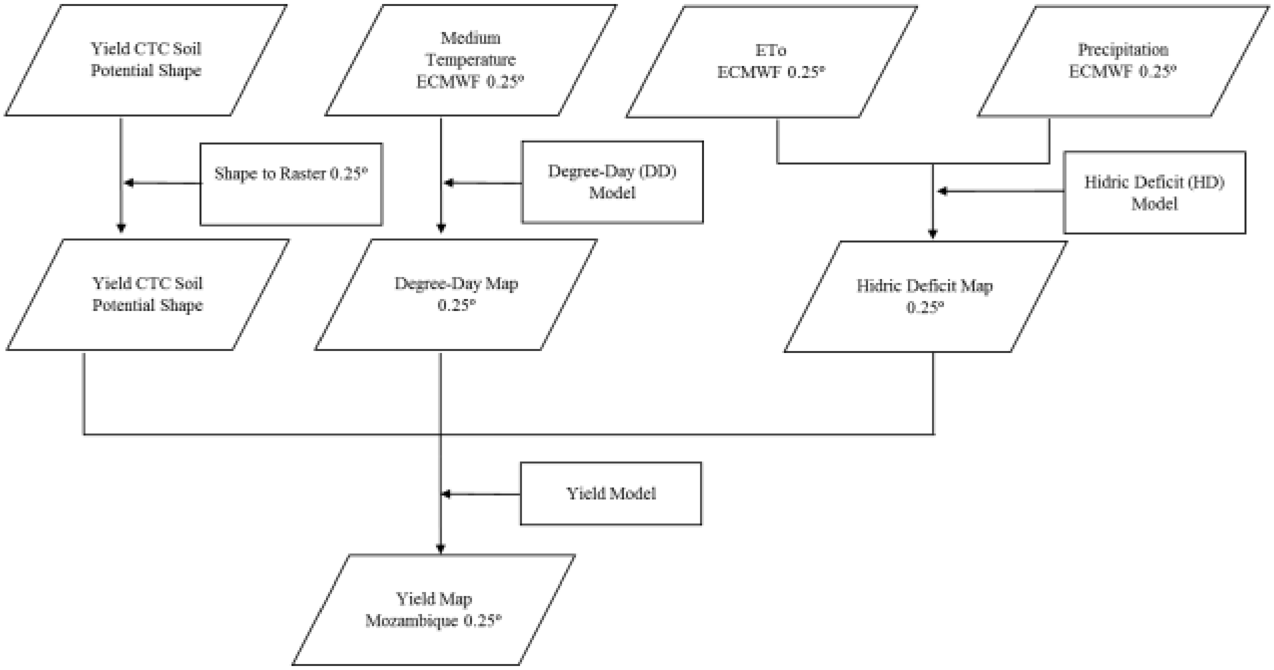

Figure 1. Flow chart of the steps for obtaining the sugarcane productivity in Mozambique.

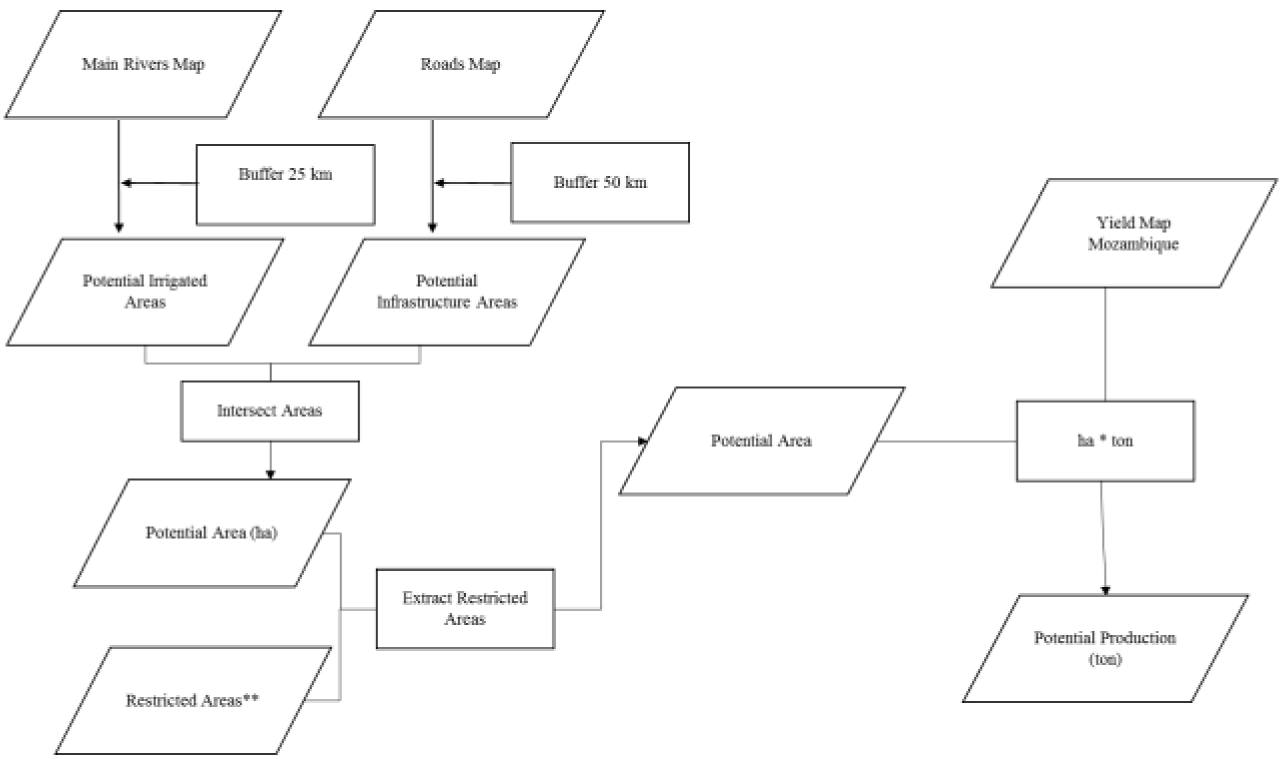

Figure 2. Flowchart of stages for obtaining potential and production areas.

reality of growth. According to Moraes et al. (2014) who quantified the degree days in the cycle of sugarcane in São Paulo, Brazil, the main areas had values between 1500 and 2500 DD, with a maximum of 3398.

The average precipitation available for the period does not exceed 1500 mm in some points, being that most parts of the country have less than 1000 mm. In the case of hydric deficit, there are few areas without problems,

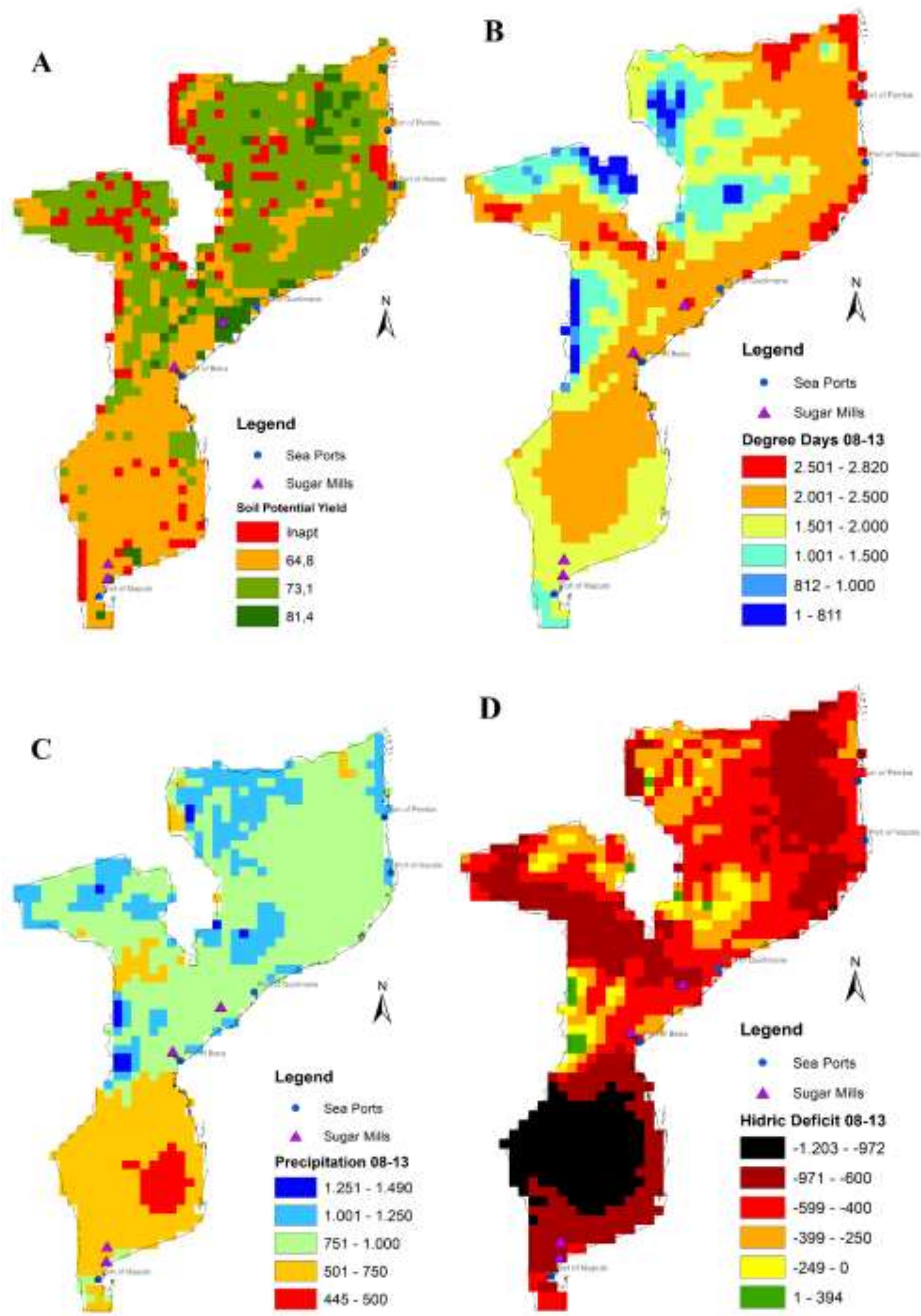

Figure 3. Yield potential for soil type (A); total degree days (B); average rainfall (C); and water deficit (D) in the period.

with insufficient rainfall in almost all. According to Doorenbos and Kassan (1979), a minimum of 1500 mm of rainfall is required during harvest. But Brunini (2010) recommended a minimum of 1000 mm, establishing some criteria where hydric deficit values with smaller deficits than 250 mm are acceptable by sugarcane; between 250 and 400 mm supplementary water is required (CAD 125 mm); between 400 and 600 mm requires irrigation, and for larger deficits, production is impossible but possible only with full irrigation.

Besides the relatively low annual precipitation (Figure 4), there is irregularity of rainfall in Marromeu, located in

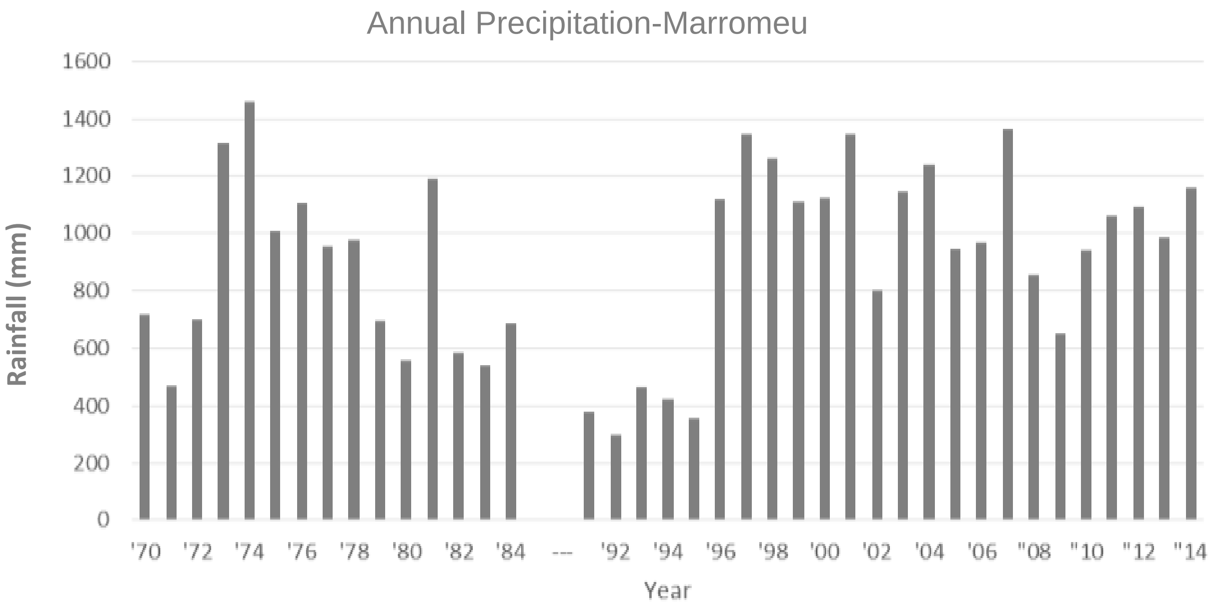

Figure 4. Annual rainfall in Marromeu/Sofala/Mozambique between 1970 and 2014 (except between 1985 and 1990).

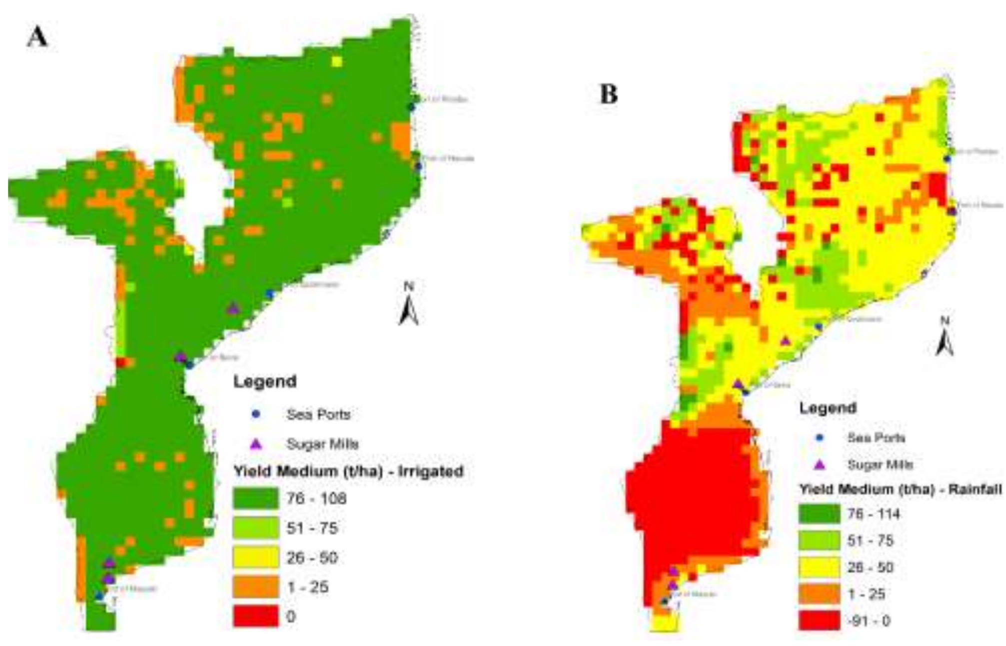

Figure 5. Average productivity estimated from the proposed model, being with no water deficit (A) and with water deficit (B).

Central Mozambique. The annual accumulated is from 1970 to 2014 (except 1985 and 1990). According to Rojas et al. (2014), the influence of El Niño events between 1991 and 1994 favored the water deficit in the region, damaging agriculture. According to Aragón et al. (1998), the drought of 1991 to 1992 strongly affected both Mozambique and the entire southern African region. Matyas et al. (2013) also show that Mozambique is vulnerable to extreme events such as cyclones, causing problems with floods and losses in agriculture.

In Figure 5, the average yield was estimated from the proposed model, being with no water deficit (Figure 5A) and with water deficit (Figure 5B). It was found that without hydric deficit, the productivity of sugarcane is

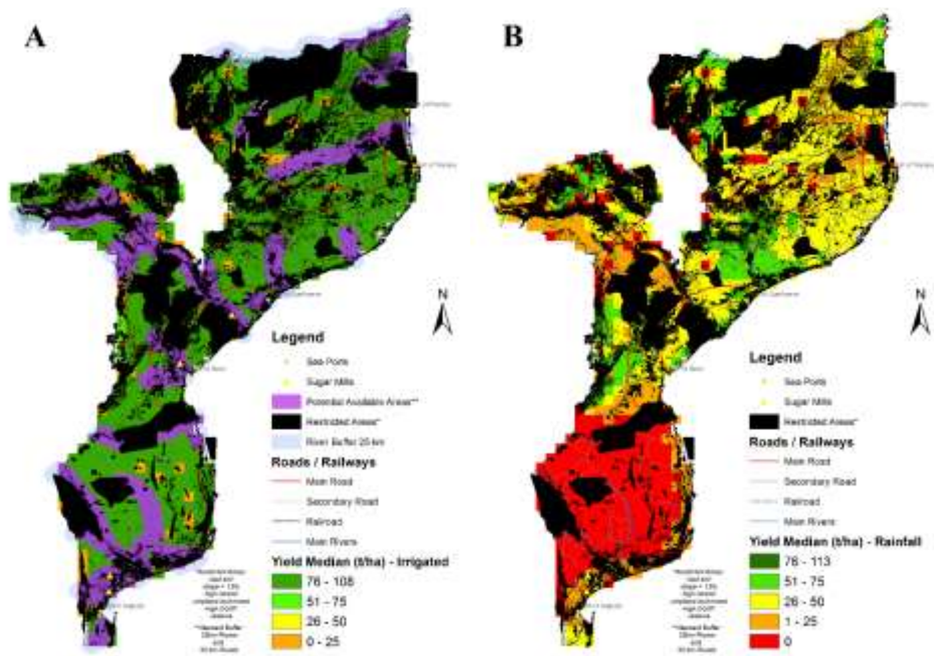

Figure 6. Maps with the potential area in the buffer junction of rivers and roads (A) and potential areas considering just rainfall (B); exclusion of the restricted areas.

Table 1. Area data, productivity and total production for areas considering the water deficit and with full irrigation.

Parameter	Area	Yield (t ha^{-1})	Total production
Irrigated	11.943.071	86	1.030.722.901
Rainfall	11.640.221*	71	611.929.424

*Considering areas where the yield was greater than 40 t/ha.

above 75 t ha^{-1} in almost all the territory of Mozambique. Already considering the water deficit, this is extremely high in some places like the southern region; while applying the model, the values become negative. It is only in the north central region that there are areas with yields above 50 t ha^{-1} for rain fed crop, but with chances of harvest loss due to these tracks of small extent.

In Figure 6A, there is a representation of potential areas considering water availability, given by large flow rivers, and infrastructure displacement given by the main roads, secondary and railway roads. This was added to the map, the position of the four currently existing plants in Mozambique, where they are present in the areas classified as potential. On the other hand, in Figure 6B, there are potential areas considering only the rainfall, including restrictions.

Table 1 shows the existence of large areas available for growing sugarcane countrywide, unrestricted and immediate implementation. The available area is equal to 11.943.071 ha for irrigated areas and 11.640.221 ha for rainfall areas (considering areas where the yield was greater than 40 t/ha), that is some 15% of the territory (considering Mozambique area equal to 799.380 km^2). From the yield in these areas, it was possible to estimate the potential production of 1.030 Mt $year^{-1}$, with full irrigation and 611 Mt $year^{-1}$ by rainfall. In 2013, in Mozambique, only 46.149 ha of sugarcane with a total of 3.166.110 tons of production were collected (Mussuale, 2014), representing a small fraction of its potential.

The data obtained from the model were compared with the observed data of irrigated and rain fed income collected at the plant installed in Marromeu. Also, available data were used, the period considered in the study, the average air temperature for calculating degree days and rainfall for estimating the water deficit. Here, Thornthwaite methodology was used with CAD 100 mm. The methodology differs from the model used in the deficiency of agro-meteorological data to estimate evapotranspiration reference. Table 2 shows the results comparing the model with the observed data.

Table 2. Comparison of model results and observed (Obs) of the total degree days (DD), precipitation (P) and water deficit (HD) during the study period; and model performance with and without HD with rain fed and irrigated areas.

Parameter	Period July-June	
	2009/2010	2010/2011
Total Degree-days (Obs)	4053	4009
DD estimated (ECMWF)	2300	2270
Total Precipitation (Obs)	784	1133
P (ECMWF)	700	960
Total HD (Thornthwaite)	1062,1	723,1
HD estimated (ECMWF)	745	437
Yield Rainfall (Obs)	48 (39*)	76.9 (62.55**)
Model $Y_{rainfall}$	33	61
Yield Irrigation (Obs)	61 (93***)	64.5 (106.1***)
Modelo $Y_{irrigated}$	105	104

*Average larger area. **Average larger area except high yield. ***High yield attained.

According to Table 2, the model is coherent with the results observed in the cultivation area located in Marromeu. As previously shown, data of degree days were high, but it should be considered that the estimated ECMWF data contains average data in pixels of approximately 25 × 25 km. But the observed data are obtained in a point and open area, where the daily averages tend to be higher.

In relation to precipitation, the values were approximated, considering the coverage area of the ECMWF model. The water deficit, despite being higher at the station, showed similar variation, decreasing from 2009/2010 to 2010/2011.

The yield values generated by the model reflected relative similarity with those observed at the sugar mill. For rain-fed areas, we estimated in the first period 33 and 61 t ha^{-1} in the second. In the sugar mill, the values observed for rain fed, for the first period, were an average stands of 48 t ha^{-1}. However, considering only the largest field for the periods, the average was 39 and 62.55 t ha^{-1} respectively, similar to the values obtained by the model. It is worth considering that the value of 62.55 for 2010/2011 was derived from the average of the fields, excluding only a high yield field, which was considered spurious.

For the irrigated areas, considering the average yield of the stands the values were similar in periods, 61 and 64.5 t ha^{-1}. However, considering the areas with the highest yields were 93 and 106.1 t ha^{-1} for each period, respectively, with values similar to those obtained by the model, 105 and 104 t ha^{-1}.

Figure 7 shows the daily average temperature and total precipitation from July 2010 to June 2011 in Marromeu. It can be observed that the temperatures are extremely high in summer, as well as precipitation; but in the winter and early spring, higher average temperature (above 21°C) and practically no rainfall were observed. Scarpari and Beauclair (2004) show that accumulation of sucrose is important for water deficit and accumulation of negative degree days. Thus, interruption of irrigation is necessary, but can have problems due to the absence of negative degree-days.

Conclusion

From a simple model and data free access it was possible to estimate potential production of sugarcane in Mozambique, with a value of 1,030 Mt year^{-1}, being significant for the country. In addition, the analysis identified potential areas and concluded that irrigation is necessary for high yield of sugarcane in Mozambique, but there are areas where the cultivation is possible just by rain fed. It is important to remember that the rain fed crop has a low yield and instability in yield crop predictability. A larger-scale analysis of the locations identified with high agricultural potential is recommended for further studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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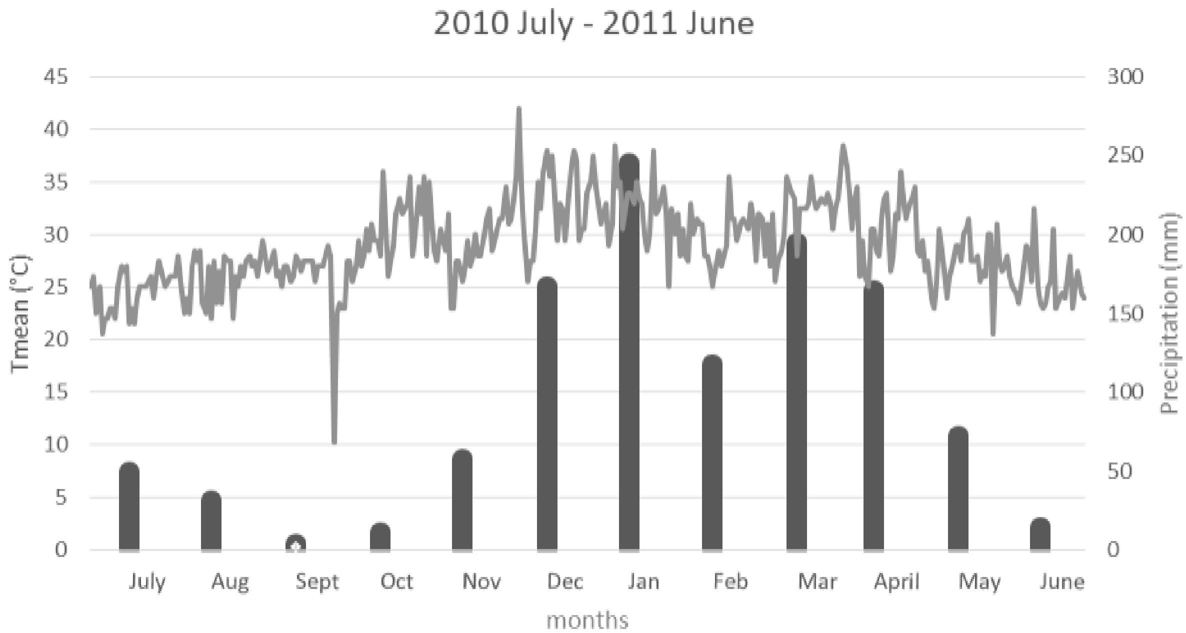

Figure 7. Daily temperature and monthly rainfall in the period considered as harvest from July to June for Marromeu.

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Full Length Research Paper

Evapotranspiration and crop coefficient for Radish under protected cultivation

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The objective of this study was to evaluate the real crop evapotranspiration and radish crop coefficient, "Crimson Gigante" cultivar, for different phenological stages under protected cultivation. The research was performed from March to April 2015 in pots arranged in greenhouse located at the Federal University of Campina Grande (UFCG). Dripping irrigation system was used for plants irrigation and the management was based on reference evapotranspiration (ET_0). The water efficiency application of irrigation system (E_a), gross irrigation depth (LB), real crop evapotranspiration (ET_r) and crop coefficient (k_c) were determined during radish cycle cultivation. The results showed E_a = 89,30%, average daily LB of 4.31 mm, accumulated values of ET_o and ET_r of 175 and 150 mm. The maximum k_c (0.94) was obtained in the intermediate phenological stage (III) and the minimum k_c (0.58) occurred in the final stage (IV) with ET_r of 75.4 and 14.5 mm, respectively. The crop coefficients were similar to those in the literature, except in the last growth plant stage. The increase in leaf area can be used as a parameter to estimate the water consumption of radish plants.

Key words: Crimson Gigante, greenhouse, water consumption.

INTRODUCTION

Radish (*Raphanus sativus* L.), an herbaceous plant belonging to Brassicaceae family, is a small vegetable which root is their comestible part. This plant is characterized as being very sensitive to variations in soil moisture and in a situation of minimal scarcity or excess water, presents physiological disturbances that interfere with its productivity and commercial root diameter, especially due to cracks that results in tubercle (Filgueira, 2008).

Among the irrigation systems recommended for this crop, drip irrigation with high efficiency of water use in agriculture is evidenced, because it is applying the water irrigation depth only in root zone of crops and maintaining soil moisture close to field capacity (Mantovani et al., 2012).

However, it is very important to know the water

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requirements of radish crop so that an efficient management of the irrigation can be carried out and, in this way, the soil moisture conditions can be maintained so that the crop can satisfy its water needs during the different stages of development (Alves et al., 2017).

The parameter that reflects the plants water requirements is the evapotranspiration with substantial relevance for irrigation, because it represents the amount of water necessary to be replaced by irrigation (Camargo and Sentelhas, 1997). The evapotranspiration parameter means the simultaneous occurrence of soil water evaporation and plant transpiration process as function of energy balance, atmospheric demand and soil water supply to plants (Adorian et al., 2015).

One of the procedures used to estimate crop water requirements involves the determination of reference evapotranspiration (ET_0), which, through the use of appropriate crop coefficient (k_c), allows the estimation of crop evapotranspiration (ET_c) in different crop development stages (Sediyama, 1987). In this way, the k_c is directly related to the phenological and physiological crops stages and their respective water requirements, correlating them with the reference evapotranspiration (ET_0). Crop evapotranspiration under real conditions of atmospheric factors and soil moisture characterizes the real crop evapotranspiration (ET_r).

In this context, the objective of this study was to evaluate the real crop evapotranspiration and the crop coefficient of radish, “Crimson Gigante” cultivar, for different phenological stages under protected cultivation.

MATERIALS AND METHODS

This research was made in a greenhouse located in Campus I at Federal University of Campina Grande (UFCG), Campina Grande city (7º 13'50" S, 35º 52'52" W, 551 als), State of Paraíba, Brazil. The region climate was defined as AS (tropical, with winter rains and dry summer), and mean value hottest month above 28°C (Coelho and Soncin, 1982; Köppen and Geiger, 1928).

The radish cultivar used was the “Crimson Gigante” with the seeds produced in expanded polyethylene trays of 128 cells filled with commercial substrate. Transplanting occurred at 8 days after sowing. The crop was cultivated in 60 polyethylene pots with 12 L and with 1.0 m spacing between the rows and 0.50 m between plants. In each pot, a 1 cm layer of gravel number 1 was placed, covered with geotextile blanket and 14 dm^3 of soil. To convert the pots into drainage lysimeters to perform water balance, a hole was made at base of each pot. The soil used was classified as medium texture according to Embrapa (2013) methodology.

A dripping irrigation system with a flow of 2.3 L h^{-1} and irrigation management was utilized based on Hargreaves and Samani (1982) model, according to methodology proposed by Medeiros et al. (2013) for reference evapotranspiration (ET_0) determination (Equation 1):

$$ET_0 = 0{,}0023(T_{méd} + 17{,}8) * (T_{máx} - T_{mín})^{0{,}5}(R_a * 0{,}408) \quad (1)$$

where:

ET_0 – reference evapotranspiration (mm day^{-1})
$T_{méd}$ – average temperature (°C)
$T_{máx}$ – maximum temperature (°C)
$T_{mín}$ – minimum temperature (°C)
R_a – extraterrestrial radiation (MJ m^{-1} day^{-1})

The air temperature and air relative humidity data during the experimental period from sowing until harvesting, equivalent to 37 days, were collected by sensors installed inside the greenhouse. The Christiansen uniformity coefficient (CUC) of irrigation system was 94%, considering a potential efficiency of the irrigation system (E_{ap}) of 95 % according to Equation 2 (Bernardo et al., 2008):

$$E_a = CUC * E_{ap} \quad (2)$$

where:
E_a – water application efficiency of the irrigation system (%)
CUC – Christiansen uniformity coefficient (%)
E_{ap} – potential application efficiency (%)

The gross irrigation depth was determined as a function of the crop evapotranspiration and the efficiency of irrigation system through Equation 3 (Mantovani et al., 2012):

$$LB = \left(\frac{ET_c}{E_a}\right) * 100 \quad (3)$$

where:
LB – gross irrigation depth (mm day^{-1})
ET_c – crop evapotranspiration (mm day^{-1})

The real crop evapotranspiration (ET_r) was quantified through the water balance (Silva et al., 2005), according to Equation 4:

$$\Delta ARM = P + I \pm R + AC - DP - ETr \quad (4)$$

where:
ΔArm – soil water storage variation(mm dia^{-1})
P – precipitation (mm dia^{-1})
I – irrigation (mm dia^{-1})
R – run off (mm dia^{-1})
AC – capillary water ascension (mm dia^{-1})
DP – deep drainage (mm dia^{-1})
ET_r – real crop evapotranspiration (mm dia^{-1})

In the conditions of radish cultivation, the terms ΔArm, P, R, AC and DP were considered null, because it is an experiment in pots, with frequent irrigation in greenhouse. Rearranging the terms of Equation 4, we have Equation 5:

$$ETr = I \quad (5)$$

Thus, it was assumed that the ET_r value between two irrigation events was equal to quantity of water applied to soil in question, considering that moisture was uniform throughout the soil profile. The crop coefficient (k_c) was estimated according to Equation 6:

$$k_c = \frac{ET_r}{ET_0} \quad (6)$$

where,
k_c – crop coefficient, dimensionless.

The results of k_c were compared with the values found by Doorenbos and Pruitt (1977), Marouelli (2007), FAO-56 (Allen et al., 1998) for each phenological phase of the crop studied. The crop cycle according to Doorenbos and Pruitt (1977) comprises the initial stage in trays, phenological phase I; the second phase comprises the growth phase with duration characterized as phenological phase II; the third phase comprises the intermediate phase

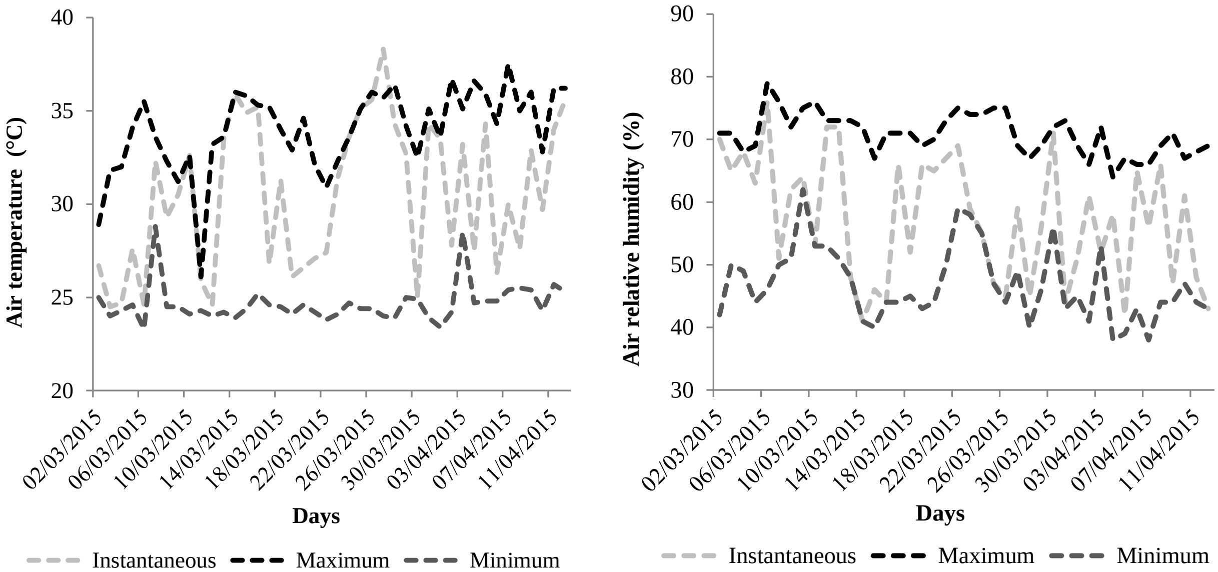

Figure 1. Air temperature over the (A) air relative humidity and (B) over the experimental period.

characterized as phenological phase III; and the final phase characterized as phenological phase IV.

Leaf area correlation (AF) and leaf area index (IAF), leaf area index (IAF) versus actual evapotranspiration (ETr) and reference (ET_0) were determined. The leaf area was measured by Equation 7.

$$AF = C * L * f \qquad (7)$$

Where:
AF = leaf area, in cm^2;
C = length, in cm;
L = width, in cm; and
f = correction factor for radish, (0.57) dimensionless, according to the methodology proposed by Matos et al. (2015).

The leaf area index (IAF, cm^2 cm^{-2}) was calculated by means of the leaf area ratio (AF, cm^2) and the area of the pot available to the plants, (AT, cm^2), according to Equation 8.

$$IAF = \frac{AF}{AT} \qquad (8)$$

Where:
IAF = Leaf area index, in cm^2 cm^{-2};
AF = leaf area, in cm^2;
AT = total pot area, in cm^2.

RESULTS AND DISCUSSION

The average air temperature inside the greenhouse was 29.6°C with variation between 23.4 and 37.5°C (Figure 1A). The average of air relative humidity was 59% with variation between 41 and 79% (Figure 1B).

The water application efficiency of the irrigation system was 89.30% being classified as acceptable by Bernardo (2006) for dripping irrigation system. The average daily gross irrigation depth (LB) was 4.31 mm, corresponding to 91.31% of reference evapotranspiration (ET_0). Lacerda et al. (2017) verified that irrigation depth of 100% ET_0 and values close to this one have a better effect on the development and productivity of the crop, so the depth used in the present study was adequate.

The results of reference evapotranspiration (ET_0) and real crop evapotranspiration (ET_r) can be observed in Figure 2. Reference evapotranspiration showed average daily value of 4.72 mm and accumulated value close to 175 mm. While daily average value of real crop evapotranspiration was 4.05 mm with accumulated value next to 150 mm. ET_r accumulated value was 14% less than accumulated of ET_0.

Oliveira Neto et al. (2011) in research with beet, also a tuberous root, cultivated under different dead coverages, observed that the maximum water consumption of the crop was 4.0 mm day^{-1} and was close to the consumption of the radish of the present study.

The total water consumption of the crop, characterized by its evapotranspiration, for each phenological phase was 23.95 mm (phase I), 46.63 mm (phase II), 75.40 mm (phase III) and 14.50 mm phase IV). In daily average values, the ET_r was 3.42, 4.66, 5.02 and 2.90 mm corresponding to the phases I, II, III and IV, respectively. The duration of each phenological phase of the crop, in the order of the phases, was 7, 10, 15 and 5 days. The ETr increased with the development stages of the plant until the phenological stage III, from which it decreased in the final phase of the crop cycle (phenological phase IV) due to the senescence of the plants. This is because with the development of the crop, there is an increase in the water requirement by the plant to supply their physiological necessity, being phase III of filling of the tubercles the phase with the highest consumption.

Alves et al. (2017) in research with radish, “Crimson

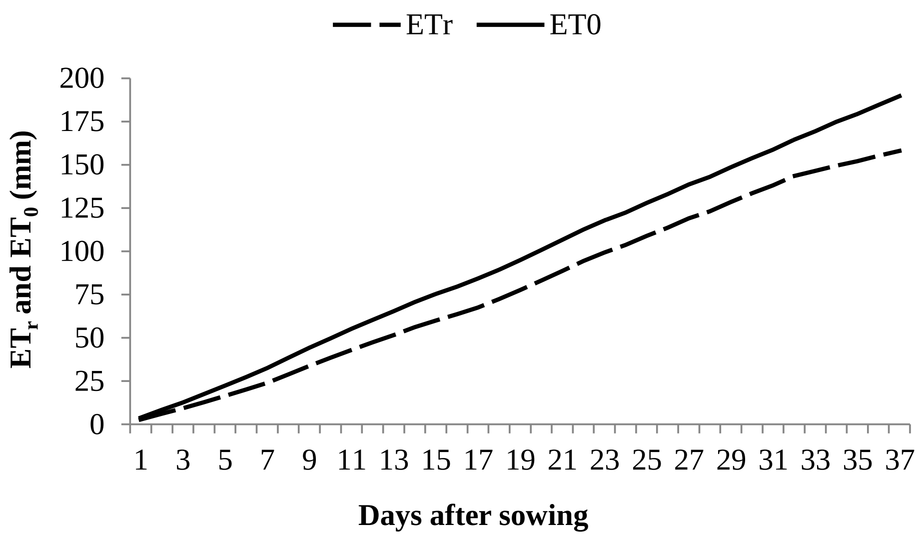

Figure 2. Accumulated values of evapotranspiration (ET_0) and real crop evapotranspiration (ET_r) for "Crimson Gigante" cultivar.

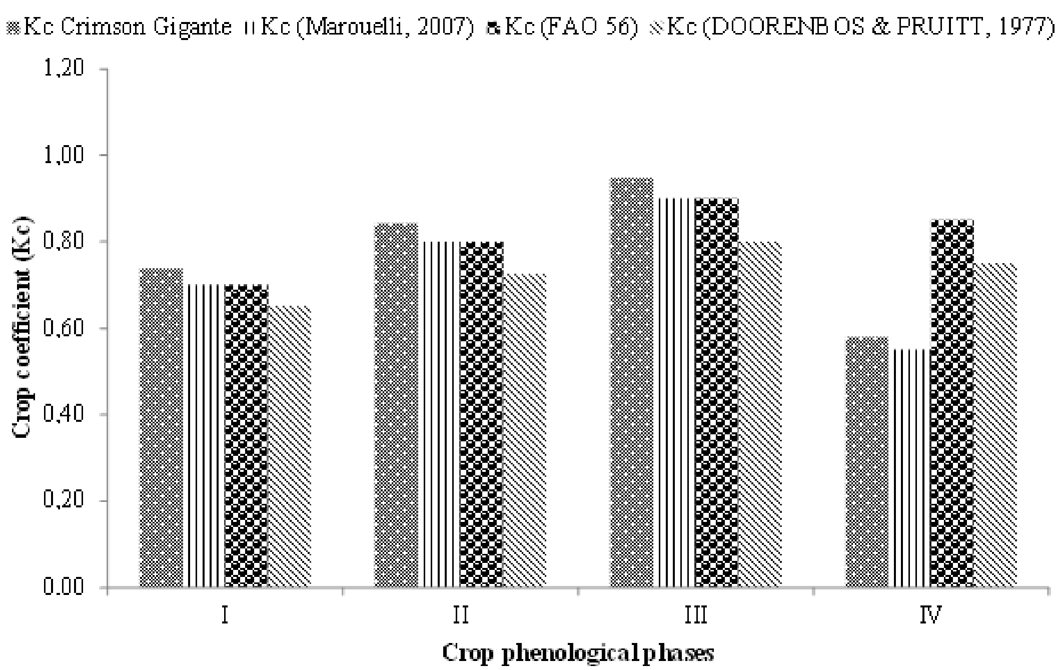

Figure 3. Crop coefficient of "Crimson Gigante" cultivar for the differents phenological phases.

Gigante" cultivar, observed that the highest daily values of crop evapotranspiration were recorded in stage III (1.84 mm day^{-1}). The stage IV presented the lowest water consumption because the culture was in the maturation stage and fruit harvest. These results are consistent with those obtained in the present work. However, ET_r for the same phenological stage was 4.81 mm day^{-1} and it was 2.6 times highest than the crop evapotranspiration found by Alves et al. (2017). This difference can be explained by the fact that climate is one of the main factors in determining the quantity of water evapotranspirated by crops.

Radish crop coefficients (k_c) for the different phenological phases are shown in Figure 3. It was observed that the k_c of radish "Crimson Gigante" cultivar was higher in phenological phase III (0.94), followed by phenological phases II (0.84), I (0.73) and IV (0.58). Considering that the maximum k_c coincided with the

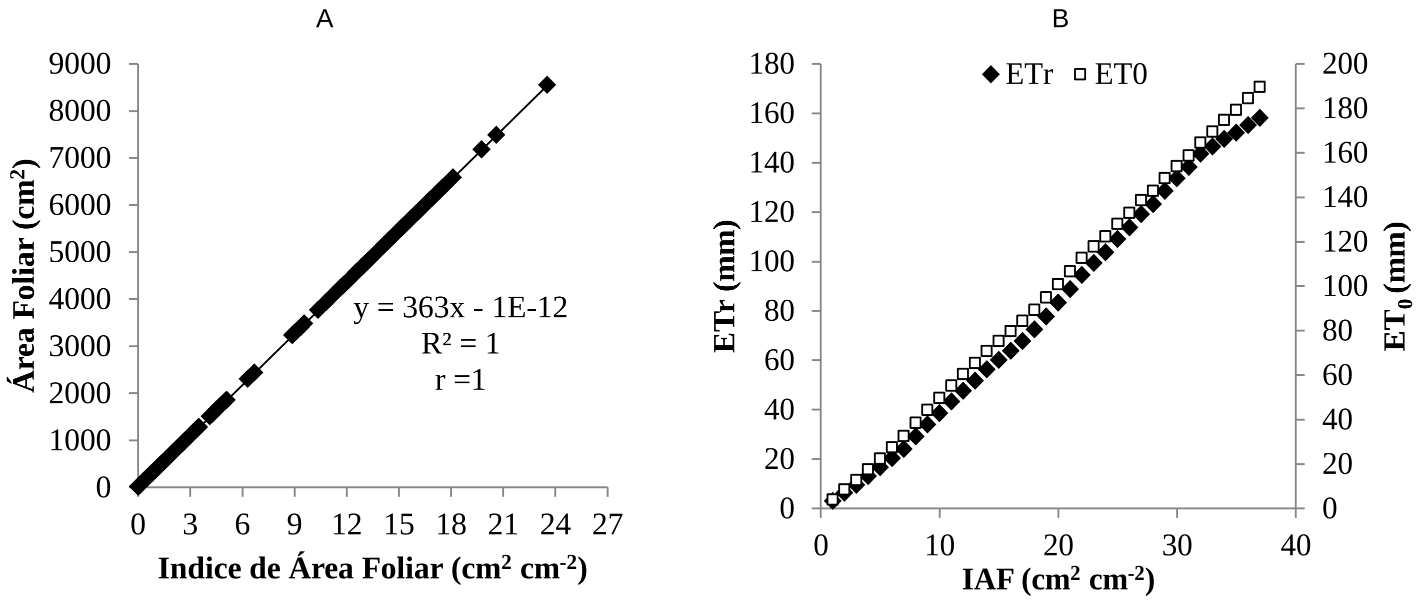

Figure 4. correlação entre área foliar e índice de área foliar(A) e entre índice de área foliar e ETr e ET0 (B).

phase of highest water consumption characterized by the highest ET_r and that the same tendency occurred for other development stages, the consistency of the results obtained for k_c values was verified. When comparing the crop coefficients of the present study throughout the radish cycle with the values obtained by Doorenbos and Pruitt (1977), FAO-56 (Allen et al., 1998) and Marouelli (2007), it was verified that coefficients obtained in this research for all phenological phases (Phase I = 0.70, Phase II = 0.80, Phase III = 0.90 and Phase IV = 0.55) were similar to the coefficients found by Marouelli (2007), followed by FAO-56 values with the exception of phase IV (phase I = 0.70, phase II = 0.80, phase III = 0.90 and phase IV = 0.85) (Figure 3).

This variation of k_c values in relation to the other authors can be explained considering that the k_c was determined as a function of ET_0 that depends on the interaction between the various climatic elements (solar radiation, air temperature and air humidity) and that the ET_r was obtained under greenhouse conditions.

The coefficients obtained in the present study differed from the values found by Alves et al. (2017) for phenological phases I, II and IV (0.45, 0.55 and 0.65, respectively), but presented similarity in phase III (0.95). This fact is justified by the climatic differences of the places where the studies were conducted.

Silva et al. (2014) evaluated beet cultivar Early Wonder and verified maximum ET_r of 2.37 mm day^{-1} in the intermediate stage (phenological phase II) under salt stress conditions. For Itapuã 202's cultivar the highest ET_r was 3.00 mm day^{-1} at the same phase. These results differ from those obtained in the present study and can be justified because of salinity influences that can affect plant physiology and, in this way, reduce crop evapotranspiration. The authors also determined the crop coefficients for the different phenological phases of the beet and observed for phases I, II and III k_c values of approximately 0.3, 1.0 and 0.9 for Early Wonder and 0.20, 0.85 and 0.50 for the cultivar Itapuã 202. These differences can be attributed to local conditions, crop varieties and cultivation conditions.

From the model presented, a strong correlation can be observed between the leaf area and the leaf area index of the radish (Figure 4A). It is noted that as leaf area increases leaf area index increases linearly. These results are consistent with those obtained by Silva et al. (2006) and Matos et al. (2015).

For Lopes et al. (2011), both leaf area and leaf area index are plant growth factors that indicate the capacity of the plant's assimilatory system to synthesize and allocate organic matter in the various organs that depend on the photosynthesis, respiration and translocation of photoassimilates of the sites of carbon fixation to the places of use or storage, where there is growth and organs differentiation.

The radish leaf area index significantly interferes with actual evapotranspiration and reference evapo-transpiration, so that the higher the LAI the ETr and the ET0 increase linearly (Figure 4B). It should be emphasized that the maximum demand coincides with the larger leaf area of the crop, thus evidencing the effect of the photosynthetically active leaf area on the water demand for the plants. These results show a great influence of IAF on the perspiration of radish plants.

According to Reis et al. (2009), the evapotranspiration determined using the Penman-Monteith method, correlates strongly with the leaf area index under protected environment conditions.

Conclusions

i) The water consumption and the crop coefficient of "Crimson Gigante" radish are maximum in the phenological phase III of development and formation of the tubercles and present minimum values in phase IV of maturation.
iii) The crop coefficients are similar to those in the literature, except for the last stage of development of the plant (phenological phase IV).
iv) The inside greenhouse climatic conditions affect the real crop evapotranspiration.
v) The increase in leaf area can be used as a parameter to estimate the water consumption of radish plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of planting dates and spacing on growth and flowering of French marigold Sel. 'FM – 786'

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The present study was carried out in an experimental farm in the Department of Floriculture and Landscaping, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during March to December, 2010. The experiment was laid out in split plot design, comprising of eight different planting dates and two spacing, (viz., 30 × 20 cm and 30 × 30 cm) as sub-plot treatment at monthly intervals from, mid-March to October, 2010. Maximum flower size was recorded for April planting date crop, whereas, June planting date gave the best results for plant height and plant spread. However, maximum number of flowers and maximum yield per plant were recorded in March planted crop. Plant height, plant spread flower size and yield per plant were at maximum in a wider spacing S_2 (30 × 30 cm). Maximum number of flowers per plant as well as maximum yield per plant was recorded in March planting at closer spacing S_2 (30 × 20 cm).

Key words: Horticulture, floriculture, flowering, plant.

INTRODUCTION

French marigold (*Tagetes patula*), belonging to family Asteraceae, is a native of Central and South America, especially Mexico. They are compact, dwarf (about 35 to 40 cm in height) and bushy in habit. This is popular among the grower because of it wide spectrum of attractive colours, shape, size and good keeping quality. Flowers are small, either single or double, borne on proportionately long peduncles, its colour varies from deep scarlet, mahogany and rusty red, primrose, yellow, golden yellow, orange and combination of these colours. The recent dwarf types (15 cm in height), which look like cushions and remain fully covered with blooms. The flowers in this group may be self- coloured, striped spotted and blotched. It is highly suitable as bedding plant to provide colours to fill the space. It is used as loose flower, pot plant and also in great demand for making garlands, religious and social functions. French marigold is also ideal for edging, hanging baskets and window boxes. Growth and flowering in marigold is generally governed by the day length and temperature. To meet out the increasing demand of flowers throughout the year, its staggered planting is required. Therefore, this experiment was undertaken to evaluate the effect of planting dates and spacing on growth and flowering parameters of French marigold Sel. 'FM-786' is use in order to regulate the continuous flowering with the help of

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planting dates and to identify the most suitable planting time and spacing of marigold in the mid hill conditions of H.P.

MATERIALS AND METHODS

The present study was undertaken at the experimental farm in the Department of Floriculture and Landscaping, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2010. The experiment was laid out in split plot design with eight main plot treatments as planting dates and two sub-plot treatments as spacing, with three replications. The main plot treatments includes eight planting dates, viz., 16th March, 16th April, 16th May, 16th June, 16th July, 16th August, 16th September and 16th October, whereas, sub-plot treatments include two spacing, viz., 30 × 20 cm (S_1) and 30 × 30 cm (S_2). Observations on various growth and flowering parameters viz., plant height, plant spread, days taken to bud formation, days taken to flowering, duration of flowering, number of flowers per plant , yield per plant and yield per square meter were recorded on five randomly selected plants per replication for each treatment.

RESULTS AND DISCUSSION

Planting dates and spacing showed a significant effect on the plant height, plant spread and days taken to bud formation of French marigold Sel. ‘FM – 786’. Maximum plant height (64.50 cm) was recorded in June planting followed by May planting (63.43 cm). The plants of May and June plantings got exposure to considerably longer day lengths and higher temperatures and hence, higher temperature cause elongation of internodes and this led to the growth of the plant. These results are in close conformity with the findings of Raju et al. (2006), where shorter plants were produced when planting was done in the month of October. This may be ascribed to the season that, October planted crop could not get the requisite temperature for its vegetative growth, and has resulted in the stunted growth. In case of spacing, more plant height (42.11 cm) was obtained with the wider spacing S_2 (30 × 30 cm).

Among the interactions between planting dates and spacing, maximum plant height (65.19 cm) was recorded with spacing S_2 (30 × 30 cm) in May planting and the spread (47.90 cm) was also recorded in June planting followed by April planting (45.39 cm). This may be due to congenial growing environment that led to more vegetative growth besides the delay in bud initiation and flowering as well. Therefore, higher temperature promotes vegetative growth and delay flowering in marigold. The results are in conformity with the findings of Raju et al. (2006).

May planting took maximum time for flower bud initiation, whereas, minimum number of days for bud initiation was recorded in October planting. This might be due to the occurrence of long days available for May planted crop and shortening in day length, when planting was commenced in October that results in early bud initiation. These results are in accordance with the findings of Raju et al. (2006). More time for bud formation (52.65 days) was observed at wider spacing S_2 than spacing S_1. Among interactions, May planted crop took maximum number of days for bud formation (104.73 days) with spacing S_1 (30 × 20 cm). October planting (59.87 days) had taken minimum number of days for flowering. Shortening in day length during October has resulted in early flowering (Raju et al., 2006). May planting has recorded maximum number (115.93 days) of days for flowering. This might be due to the occurrence of long days during May to June which delayed flowering.

Maximum duration of flowering (31.41 days) was recorded in April planting. This might be due to the optimal climatic conditions during its vegetative as well as flowering stage, which is available for crop planted in April. Chanda and Roychoudhary (1991) also reported similar findings where, interactions between spacing and planting dates showed none significant effect. Maximum number of flowers per plant (63.57) was recorded in March planting date this might be due wider spacing. Plants grew more luxuriantly by taking up nutrients which could spread largely as compared to the plants grown at closer spacing, where there was less availability of nutrients and space. Therefore, the maximum number of flowers per plant could be attributed to an increase in plant spread and number of branches. It was also observed that with an increase in plant density, the number of flowers per plant decreased significantly. Chanda and Roychoudhary (1991) reported similar results for marigold.

Maximum flower weight per plant was observed in March planting (151.73 g) and minimum weight of flower per plant was recorded in May planting (20.33 g). This could be attributed to the existence of congenial climatic conditions during the crop growth period, which enabled them to produce an increased amount of photosynthates and intern resulted in more dry matter accumulation. It was observed that, unfavorable climatic conditions exist for optimum vegetative growth and better flowering, during the July to September planted crops. Similar variation was also observed by Guruprasad (1999) and Nagaraju et al. (2004).

In French marigold Sel. ‘FM 786’, maximum flower which yield per square meter was observed in March planting (2172.00 g) with closer spacing of 30 × 20 cm and minimum flower yield per sq. meter, which was recorded in May planting (221.97 g) when the crop was planted at a wider spacing S_2 (30 × 30 cm). The reason for maximum yield per sq. meter might be due to more number of plants. These results are in close conformity with the findings of Ravindran et al. (1986), Bhati and Chitkara (1987) and Mohanty et al. (1993).

This may conclude that, March planting date is

Table 1. Effect of planting dates and spacing on growth and flowering of French marigold Sel. 'FM- 786'.

Planting dates	Plant height (cm)	Plant spread (cm)	Days for bud formation (days)	Days for flowering (days)	Duration of flowering (days)	No. of flowers /plant	Yield/plant (g)	Yield/sq. m (g)
March,16	45.92	41.08	29.47	46.00	24.60	63.57	151.73	1999.55
April,16	51.99	45.39	42.80	62.10	31.41	36.67	93.17	1116.37
May,16	63.43	35.31	104.07	115.93	24.85	12.53	20.33	244.07
June,16	64.50	47.90	54.73	81.43	21.80	25.35	59.97	815.57
July,16	36.70	25.43	69.12	86.93	19.10	17.77	30.80	348.30
August,16	28.33	24.73	52.57	65.70	24.17	25.83	43.50	526.90
September,16	22.98	22.28	43.03	59.87	20.57	17.53	31.80	339.20
October,16	17.66	15.65	23.00	41.20	29.20	12.53	23.70	279.68
$CD_{(0.05)}$	1.27	1.91	1.90	1.10	1.54	4.16	15.33	218.47
Spacing								
S_1	40.77	31.99	52.05	69.58	24.54	26.07	55.51	843.25
S_2	42.11	32.46	52.65	70.21	24.38	26.88	58.24	574.16
$CD_{(0.05)}$	0.80	NS	0.39	0.39	NS	NS	NS	80.33
Planting dates × Spacing								
$PD_1 \times S_1$	44.91	38.97	29.80	46.27	24.60	62.73	144.80	2172.00
$PD_2 \times S_1$	53.38	45.81	40.73	61.33	31.02	36.93	94.27	1414.00
$PD_3 \times S_1$	61.66	34.24	104.73	114.67	25.47	12.27	20.80	312.00
$PD_4 \times S_1$	64.73	50.88	53.67	81.13	22.13	24.97	59.73	1076.00
$PD_5 \times S_1$	34.59	23.96	69.93	87.13	18.73	16.40	29.13	404.40
$PD_6 \times S_1$	26.87	24.09	52.07	64.67	24.13	27.80	45.13	677.00
$PD_7 \times S_1$	22.70	21.53	43.00	60.27	20.40	15.93	27.47	353.20
$PD_8 \times S_1$	17.31	16.40	22.47	41.20	29.87	11.53	22.73	337.40
$PD_1 \times S_2$	46.94	43.20	29.13	45.73	24.60	64.40	158.65	1827.10
$PD_2 \times S_2$	50.59	44.97	44.87	62.87	31.80	36.40	92.07	818.73
$PD_3 \times S_2$	65.19	36.37	103.4	117.20	24.23	12.80	19.87	176.13
$PD_4 \times S_2$	64.27	44.91	55.80	81.73	21.47	25.73	60.20	555.13
$PD_5 \times S_2$	38.82	26.89	68.30	86.73	19.47	19.13	32.47	292.20
$PD_6 \times S_2$	29.79	25.37	53.07	66.73	24.20	23.87	41.87	376.80
$PD_7 \times S_2$	23.27	23.03	43.07	59.47	20.73	19.13	36.13	325.20
$PD_8 \times S_2$	18.01	14.90	23.53	41.20	28.53	13.53	24.67	221.97
$CD_{(0.05)}$	2.28	1.69	1.10	0.96	NS	NS	NS	227.20

optimum for maximum flower production, whereas, May and June planting dates are not suitable for continuous flowering. In case of spacing, closer spacing (30 × 20 cm) is found to be more suitable for different flowering characters (Table 1).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Broadcast and in line application of phosphorus in soils with different densities

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The efficiency of broadcast versus in line phosphate fertilization has been studied; however, soil density as a determining factor for soil efficiency has been less studied. Thus, the objective of this study was to evaluate the effect of broadcast and in line phosphate fertilization in soil with different densities on corn development. The experiment was conducted in pots under greenhouse condition. A completely randomized design was used in a 2x3 factorial arrangement with four replications. Treatments were two levels of soil densities (1.2 and 1.6 g cm^{-3}) and three forms of P application (broadcast, in line and without P). Corn stem diameter, plant height, root volume, root and shoot dry matter, and root and shoot phosphorus content and accumulation were evaluated. Application in line resulted in higher P accumulation by corn plant. There was higher plant dry matter accumulation at soil density 1.2 g cm^{-3} than at 1.6 g cm^{-3}. Phosphate fertilization in line at soil density 1.2 g cm^{-3} enhances dry matter accumulation in corn plants.

Key words: Compaction, macronutrient, phosphorus, soil fertility.

INTRODUCTION

Soil compaction is one of the most severe degradation processes that occurs in the soil and have been affecting about 68 million hectares around the world from the vehicular traffic (Nawaz et al., 2013). Its effect on the soil structure results in higher soil density (Souza et al., 2012) and lower soil porosity (Singh and Hadda, 2014). The availability of nutrients for plants is reduced in compacted soils (Barzegar et al., 2016), such as phosphorus (Novais et al., 2007). Besides that, soil compaction leads to lower crop yield due to increased resistance to root growth and reduced efficiency of water and nutrient use (Twum and Nii-Annang, 2015).

The nutrient P participates on plant metabolism as the main component of adenosine triphosphate (Taiz et al., 2017), this nutrient has an essential role in physiological and biochemical processes, acting as an energy transfer agent within the plant; thus enabling photosynthesis and respiration (Hawkesford et al., 2012), having essential

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role on plant development (Amanullah and Almas, 2012).

The soils with a high degree of weathering help to maintain low levels of available phosphate resulting from high P adsorption capacity of iron and aluminum oxides and hydroxides in soils (DeLong et al., 2013; Fink et al., 2014). Slow diffusion contributes to low level of available P in soil which limits plant uptake (Shen et al., 2011). Moreover, in line fertilization (close to root system) should be chosen over other methods to favor plant uptake of P (Prado, 2008).

The most common method of P application in grain production is soil surface with or without incorporation, in row placement and in band placement (Galetto et al., 2014). Among the determining factors for the best P application method are soil physical and chemical proprieties, fertilizer type and crop type (Ceretta et al., 2007). The phosphate fertilizer applied on the surface will keep contact with soil, causing the P fixation and affecting the absorption by the plants (Barbosa et al., 2018).

Reports have shown that application of phosphate fertilizers using broadcast method without incorporation in a no till system leads to different distribution of P within the soil profile compared to in line application, this will enhance P concentration in superficial layer from 0 to 5 cm (Souza et al., 2016). However, soil compaction might affect the broadcast phosphate fertilization management in no till areas (Santos et al., 2005), as root development and crop production especially in areas under water deficiency might be reduced (Valadão et al., 2015).

Therefore, it is possible that in line phosphate fertilization has higher efficiency than broadcast application, especially in compacted soils. The objective of this study is to evaluate the effect of broadcast and in line phosphate fertilization in soil with different densities on corn development.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse and in the Laboratory of Soil, Fertilizer and Foliar Analysis (LASAF) of University of Mato Grosso State – UNEMAT (09° 51' 42'' S and 56º 04' 07'' W), campus Alta Floresta – MT, in 2016. According to Köppen classification, the weather is classified as Aw with two well defined seasons: raining summer and dry winter, with temperatures around 26 °C and average rainfall between 2000 and 3000 mm (Alvares et al., 2014).

A red-yellow dystrophic oxisol (Embrapa, 2013) was collected from 0-20 cm. The chemical and physical characteristics of the soil are: pH ($CaCl_2$) = 5.0; organic matter (OM) = 26.9 g dm^{-3} ; H^++Al^{3+} $_{(calcium\ acetate)}$ = 28.5 $mmol_{(c)}$ dm^{-3}; P $_{Mehlich\ 1}$= 1.3 mg dm^{-3} ; K $_{Mehlich\ 1}$= 1.66 $mmol_{(c)}$ dm^{-3}; Ca = 11.2 $mmol_{(c)}$ dm^{-3}; Mg = 4.5 $mmol_{(c)}$ dm^{-3} ; base saturation = 38%; Cation Exchange Capacity (CEC) of 46.0 $cmol_{(c)}$ dm^{-3}; sand fraction = 578 g kg^{-1}; silt fraction = 57 g kg^{-1}; and clay fraction of 365 g kg^{-1}.

The experiment was conducted in a completely randomized design in a factorial 2x3 with 4 replications. The treatments were two soil densities D1 1.2 g cm^{-3} (normal) and D2 1.6 g cm^{-3} (compacted), without P application (T1), with superficial broadcast P (T2), and with in line P (T3). Each experimental unit was composed using a pot of 4 dm^{-3} capacity.

Soil density was determined using Beutler and Centurion (2004) methodology. The soil was pressed 15 times with a 3.5 kg wood of 50 cm high and a diameter lower than the 4 dm^{-3} plot of PVC (17.5 cm of height and 17.3 cm of diameter). The soil was compacted on each 1.7 cm soil layer until the soil densities was 1.2 and 1.6 g cm^{-3}.

The soil was dried and sieved before dolomite lime application (with 85 relative efficiency of reactivity – RER) so as to enhance soil base saturation to 60%, as suggested by Sousa and Lobato (2004). The soil was incubated in pots with periodic irrigation for the duration of 90 days. After this period, 200 mg dm^{-3} of P (Mono-Ammonium-Phosphate – 50% of P_2O_5) was applied to broadcast and in line pots, according to the methodology of Malavolta (1981). In line application was placed under and next to the seed in order to simulate field situation. Corn sowing was done with six seeds per pot. All pots were applied with 60 kg ha^{-1} of K_2O (Potassium chloride – 60% of K_2O), 20 kg ha-1 of N at sowing and 40 kg ha^{-1} of N later (urea - 45% of N) (Sousa and Lobato, 2004). Boron was applied during sowing at 2 kg ha^{-1} (boric acid 17% of B) and zinc at 6 kg ha^{-1} (zinc sulfate - 20% of Zn) (Sousa and Lobato, 2004).

Irrigation with tap water was done every day, in order to maintain soil water capacity of about 60%. After 35 days of emergency, the stem diameter, shoot height, root volume, root and shoot dry matter, and root and shoot P content and accumulation were evaluated.

The roots were separated from shoots, washed with tap water followed by distillated water, and left submerged inside a graduated cylinder containing low level of water. The difference between initial and final volume was used to determine root volume. Roots and shoots were placed into Kraft papers and dried in a forced-ventilated oven at 65°C for 72 h to determine its dry matter content. Shoots and roots samples were ground in a Wiley grinder and chemically analyzed for P content using Embrapa (2009) methodology.

The results, for normality (Shapiro-Wilk) and homoscedasticity, were analyzed. The analysis of variance (ANOVA) was done with F test ($p<0.05$), and the means compared through Tukey test at 5% of significance, using ASSISTAT software version 7.7.

RESULTS AND DISCUSSION

There was a significant interaction effect between P application and soil density on corn root and shoot P content (Table 1). Root P content at density 1 did not show significant difference among P application, whereas at density 2 higher root P content was observed on plants treated with in line P compared to broadcast and without P (Table 2). There was no effect of soil density on root P content when in line P application was used; however, higher root P content was observed at the lowest density in plants under broadcast P application and without P treatment (Table 2). These results support the hypotheses that soil compaction decrease P diffusion flow, thus reduces ion-root contact and plant uptake. According to Barbosa et al. (2018) the lower levels of P provided by broadcast fertilization P are justified by the low initial level of this nutrient in the soil, causing a reduction in the availability of P within the reach of the root. Amanullah and Almas (2012) affirmed the application of localized P enhances root system and provides upper

Table 1. Effect of P application method and soil density on root P content (RPC), shoot P content (SPC), root P accumulation (RPA) and shoot P accumulation (SPA) of corn plants cultivated under greenhouse condition.

Treatment	RPC	SPC	RPA	SPA
	g kg^{-1}		g pot^{-1}	
Without P	0.57	0.69	0.64	0.65
Broadcast	0.71	0.64	5.30	4.18
In line	0.86	0.55	7.26	5.58
LSM	0.143	0.128	1.061	1.087
Densities		g cm^{-3}		
D1 – 1.2 g cm^{-3}	0.81	0.65	4.87	3.91
D2 – 1.6 g cm^{-3}	0.62	0.61	3.93	3.02
LSM	0.096	0.086	0.710	0.731
		F values		
Density (D)	17.20**	1.25	7.82*	6.41*
P application(PA)	13.63**	3.55*	134.84**	71.34**
Interaction DxPA	3.929*	6.62**	1.450	2.459
CV%	15.69	16.02	18.81	24.55

* and ** of F test significant at $p<0.01$ and $p<0.05$, respectively.

Table 2. Effect of interaction between P application method and soil density on root P content and shoot P content of corn plants cultivated under greenhouse condition.

Density	Treatment		
	Without P	Broadcast	In line
Root P (g kg^{-1})			
D1 – 1.20 g cm^{-3}	0.689^{a}	0.863^{a}	0.868^{a}
D2 – 1.60 g cm^{-3}	0.449^{b}	0.548^{b}	0.854^{a}
Shoot P (g kg^{-1})			
D1 – 1.20 g cm^{-3}	0.712^{a}	0.772^{a}	0.590^{b}
D2 – 1.60 g cm^{-3}	0.662^{a}	0.515^{b}	0.512^{b}

^{ab} means in both column and row of the same superscript are not significantly different ($p<0.05$).

contact with nutrients.

At density 1, in line application of P resulted in higher shoot P content compared to broadcast and without P application (Table 2). In contrast, at density 2 higher shoot P content was observed in plants under in line and broadcast application compared to plants without P fertilization (Table 2). Regardless of soil density effect within P application, no significant effect was observed in plants without P fertilization. However, density 1 resulted in higher shoot P content when P was broadcast and in line applied compared to those without P application. This might be explained by the dilution effect (Martuscello et al., 2009), which is elucidated by the higher dry matter content of corn plants showed on Table 3.

Accumulation of P in shoots and roots was higher with in line P application than with broadcast and without P (Table 1). It was observed that in line P increased 37 and 24% of P accumulation in roots and shoots, respectively, compared to broadcast application. Souza et al. (2016) verified in line application of phosphate fertilizers may increase concentration of P in the zone of initial radicular growth compared to broadcast application. Da Ros et al. (2017) verified that for in plants of corn and sunflower, the location of the phosphate fertilizer is very important in

Table 3. Effect of P application method and soil density on stem diameter (SD), plant height (PH), root volume (RV), root dry matter (RDM) and shoot dry matter (SDM) of corn plants cultivated in greenhouse condition.

Treatment	SD	PH	RV	RDM	SDM
	cm		g L^{-1}	g pot^{-1}	
Without P	4.03	9.48 b	9.86	1.12	0.94
Broadcast	9.06	17.51 a	51.12	7.74	6.41
In line	9.93	18.47 a	84.13	8.46	10.11
CV%	7.39	9.59	17.72	12.43	7.39
LSM	0.72	1.87	10.96	0.92	0.55
Densities					
D1 – 1.2 g cm^{-3}	7.43	15.07	58.33	5.73	6.31
D2 – 1.6 g cm^{-3}	7.79	15.55	38.42	5.82	5.32
LSM	0.48	1.26	7.35	0.62	0.37
F values					
Density (D)	1.11	0.62	32.4**	0.10	31.8 **
P application(PA)	252**	96.8**	151.6**	254.2**	920.1**
DxPA	7.4**	0.18	10.51**	16.71**	13.6 **
CV%	7.39	9.59	17.72	12.43	7.39

** of F test significant at p<0.01, respectively.

the accumulation of P in the plants.

Moreover, in no till areas, broadcast application of P enhances P content in the superficial soil layer compared to sub superficial layers (Santos et al., 2005) which increases contact between phosphate fertilizer and soil. Therefore, higher rate is required in broadcast application to achieve similar fertilizer efficiency than in line application (Prado et al., 2001).

There was higher accumulation of P in shoot and root of plants cultivated in soil with density 1 than in soil with density 2 (Table 1). Therefore, it was noticed that soil density of 1.6 g cm^{-3} was harmful to root and shoot growth and P uptake. Also, soil compaction results in higher resistance to mechanical penetration that limits radicular development and reduces root capacity of water uptake, hence it affects plant growth (Ortigara et al., 2014).

Plants with P applied in line and broadcast were 94.83% and 84.70%, respectively, which are higher than plants without P fertilization (Table 3). This is explained by P essentiality on plant metabolism, the component of nucleotides used in energy metabolism of plants (such as ATP) and in DNA and RNA (Taiz et al., 2017); resulting in smaller plants in soils with lower P availability.

A significant interaction effect between density and P application was observed for the other measured variables (Table 3). At density 1, stem diameter was higher with application of P in line than broadcast fertilization and without P (Table 4). In contrast, similar diameter was observed with in line and broadcast application at density 2, which were higher than those without P application. There was no effect of soil density on plant diameter when P was not applied and applied in line; however, higher stem diameter was observed at density 2 when broadcast was applied.

Regardless of soil density, root volume was higher when P was applied in line compared to broadcast application and without P application (Table 4). Moreover, higher root volume was observed at density 1 when P was in line applied. Since P has low mobility in soil, lower root volume affects uptake of P by corn plants (Santos et al., 2005) and also affects root distribution and biomass within soil profile ((Twum and Nii-Annang, 2015); resulting in reduction in crop yield. This fact elucidates the importance of knowing crop threshold for soil compaction, as well as of monitoring soil compaction, especially in tropical regions in which there is low levels of available P.

Regardless of soil density, root and shoot dry matter accumulation were higher in plants under in line P application, expect for root dry matter in plants broadcast applied with P, in which similar results to in line application were observed (Table 4).

Moreover, plants treated with in line P presented lower accumulation of root and shoot dry matter at soil density 2. The effect of soil density in plants applied with broadcast P was only observed for root dry matter, in which lower values was observed at density 2. No effect of soil density on root and shoot dry matter was observed in plants without P application.

Grzesiak et al. (2014) observed the negative effect of soil compaction in maize and triticale, with a decrease of aerial parts and roots dry matter and changes in roots distribution in the soil profile. Sarto et al. (2018)

Table 4. Interaction effect between P application method and soil density on stem diameter (SD), root volume (RV), root dry matter (RDM) and shoot dry matter (SDM) of corn plants cultivated in greenhouse condition.

Density	Treatment		
	Without P	Broadcast	In line
SD (cm)			
D1 – 1.20 g cm^{-3}	4.02^a	8.34^b	10.28^a
D2 – 1.60 g cm^{-3}	4.04^a	9.77^a	9.57^a
RV (g L^{-1})			
D1 – 1.20 g cm^{-3}	12.50^c	57.25^b	105.25^a
D2 – 1.60 g cm^{-3}	7.25^c	45.01^b	63.00^b
RDM (g pot^{-1})			
D1 – 1.20 g cm^{-3}	1.20^b	8.88^a	9.35^a
D2 – 1.60 g cm^{-3}	1.04^b	6.60^a	7.54^a
SDM (g pot^{-1})			
D1 – 1.20 g cm^{-3}	1.05^c	6.65^b	11.26^a
D2 – 1.60 g cm^{-3}	0.83^c	6.18^b	8.98^a

[abc] means in both column and row of the same superscript are not significantly different ($p<0.05$).

elucidated that in compacted soil, the roots under hypoxia produce high quantity of ethylene. This hormone inhibits root elongation and induces root swelling in plants (Geisler-Lee et al. 2010). This might explain higher root values (60%) in plants applied with in line P at soil density 1.2 g cm^{-3} (D1) compared to soil density 1.6 g cm^{-3} (D2). Grzesiak et al. (2013) indicate that soil compaction reduces plant system roots; consequently, affecting the development and productivity of several crops (Gubiani et al., 2014; Valadão et al., 2015)

Shoot dry matter of plants that received in line P application was 60% higher than broadcast application, and 11 times higher than plants without P fertilization (Table 4). According to Santos et al. (2005), maximum accumulation of shoot dry matter in corn plants cultivated in pots with nutritive solution occurred with application of 400 mg dm^{-3} of P ($NH_4H_2PO_4$). These authors observed a maximum of 6.02 g pot^{-1}, which was lower than shoot dry matter observed in the present study (11 g pot^{-1}). Souza et al. (2008) also observed that application of 400 mg dm^{-3} of P in corn plants cultivated at densities of 1.34 a 1.67 g cm^{-3} resulted in 4.5 a 3.5 g of shoot dry matter, respectively; these were also lower than shoot dry matter accumulation observed for plants treated with in line P in the present study. This might be due to the application method used, in which corn dry matter increases with increasing availability of P through localized application of fertilizers, as observed in the present study with in line treatment.

Therefore, higher root and shoot dry matter (Table 4) might be explained through higher accumulation of P in corn root and shoot (Table 1). These results elucidate the importance of accurate management of phosphate fertilization in order to increase plant nutrition.

Conclusions

Phosphorus application in line resulted in higher P accumulation in plant. There was higher plant dry matter accumulation at soil density 1.2 g cm^{-3} compared to 1.6 g cm^{-3}. In line phosphate fertilization at soil density 1.2 g cm^{-3} enhances dry matter accumulation in corn plants.

CONFLICT OF INTERESTS

The author has not declared any conflict of interest.

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Full Length Research Paper

Reaction of watermelon accessions to *Meloidogyne enterolobii*

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Identification of resistant germplasms is crucial for crop breeding. The aim of this study was to determine the reaction of accessions of watermelon plants to *Meloidogine enterolobii* using two experiments. In the first experiment, 20 accessions were evaluated, and in the second, the four most promising accessions were selected in the first experiment and two controls. Both experiments were implemented under a completely randomized design with ten replications. Each parcel consisted of a plastic pot containing autoclaved soil inoculated with 2,200 eggs of *M. enterolobii*. Among the variables studied, the egg number and reproduction factor showed a high positive correlation. The subsamples indicated a wide variation among and within each accession ($p < 0.01$). At the end, variation in the response among and within the accessions was observed regarding the reproduction factor. Of the accessions evaluated, accessions 9 and 10 exhibited potential for the implementation of breeding programs.

Key words: *Citrullus lanatus*, resistance, vegetable genetic capabilities

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is a species of the family Cucurbitaceae, being cultivated in almost the entire national territory. The Brazilian northeastern region has an estimated average production of 54,117 tons (IBGE, 2014), and Bahia, Rio Grande do Norte and Ceará, Brazil are the largest producing states, with respectively 122,320, 121,688 and 82,424 tons of the fruit. Among the largest producers in

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the Northeast region, the State of Rio Grande do Norte, Brazil, is considered an excellent choice for growing this vegetable due to favorable climatic conditions, besides being a potential market for melon exports.

However, nematode problems caused by *M. enterolobii* already represent high instability for cultivation. The damages caused by this root-knot nematode were reported by the first time for guava culture in the sub-middle region of the São Francisco basin in 1988, in the municipalities of Petrolina-PE and Juazeiro-BA, Brazil (Moura and Moura, 1989). It was also observed in the watermelon culture in the municipalities of Mossoró and Assu, State of Rio Grande do Norte, Brazil (Moura et al., 2002).

Effective control of *M. enterolobii* is one of the major issues to be overcome for an economically sustainable cultivation of irrigated watermelon crops. Even in the case of inexistent susceptible crops, nematodes can survive and reproduce in many weed species (Terao et al., 2012). Difficulties for the parasite control is increased by the reduced number of active ingredients available in the market and the high cost they add to production. Thus, an investigation of the genetic variability present in the species' germplasm, aiming to develop resistant varieties, could be the most effective and economic method of control for the implementation of integrated management practices (Pontes, 2009).

Taking into account that family farmers usually manage their seeds and grow crops without chemicals, especially pesticides, the populations they use are probably resistant to some biotic stresses. Thus, this study aimed to evaluate the reaction of watermelon accessions collected in the Rio Grande do Norte State, Brazil, to *M. enterolobii* parasitism, to verify the hypothesis probable resistant genotypes.

MATERIALS AND METHODS

The germplasm was obtained by collecting samples of seeds stored by farmers of traditional agriculture in the Rio Grande do Norte State (12 accessions), Brazil, and from spontaneously-growing plants in the Experimental Stations (seven accessions) and other areas (one accession). The samples were collected in the towns of Apodi (Latitude: 5° 38' 58'' South and Longitude: 37° 47' 45'' West), Cerro Corá (Latitude: 6° 2' 44'' South and Longitude: 36° 20' 52'' West) and Tibau do Sul (Latitude: 6° 11' 23'' South and Longitude: 35° 5' 29'' West).

Two experiments were carried out to determine the response to *M. enterolobii* nematodes. The first experiment was conducted from March to May, 2011 and the second one from April to June, 2012. The second one was conducted for reaffirmation of the results identified in the accessions that showed parcels with resistance reaction.

Both experiments were carried out and evaluated in a greenhouse and in the Embrapa's Nematology Laboratory of the Semiarid Region located in Petrolina, State of Pernambuco, Brazil, respectively. The climate of Petrolina is BSwh' of Koppen classification agreement, therefore, semi-arid with a rainy season of high annual average temperatures of 26.03ºC order and average rainfall of 522.3 mm.

Seedling plants were cultivated in expanded polystyrene seedling trays with 128 cells. The substrate used was a commercial compost based on expanded vermiculite and vegetable organic matter. Fourteen days after sowing, the plantlets were transplanted into the soil contained in plastic pots. The soil used was alfissol dystrophic.

To obtain the inoculum, roots infected with the plant nematode in areas of guava tree production (*Psidium guajava* L. cultivar Paluma) were collected in Petrolina, and by means of α-esterase revelation of phenotype, according to the vertical electrophoresis technique in polyacrylamide gel (Alfenas et al., 2006), the presence of *M. enterelobii* species was determined in the sample.

The eggs for preparation of the inoculum were extracted ten days after sowing, according to the method proposed by Coolen and D'herde (1972). Aliquots of 1 mL were used, in which the count of eggs and the calibration of suspension for inoculation of the plants in Petri dishes were performed using a microscope.

Inoculation was made the fourth day after transplanting by adding 1.5 mL of the suspension containing 2200 eggs per parcel into two holes separated at a distance of five centimeters from the plant's base and approximately two centimeters deep. The "Santa Cruz Kada Gigante" cultivar of tomato (*Lycopersicon esculentum* Mill.) was used as control to confirm the inoculum viability.

Seventeen days after transplanting, the plants were tied to stakes with plastic strings. The branches were kept vertically until completion of the experiment and the tiers were adjusted every week.

The first experiment was conducted by the 52nd day after inoculation with average temperature of 28.5°C. Harvesting was performed by cutting the plants base five centimeters above the ground. Each plant was stored in separate plastic bag and taken to the laboratory for evaluation.

To measure the genotypes resistance degree, the gall index (GI) and egg mass index (EMI), egg mass number (EMN), egg number (EN) per plant and the reproduction factor $RF = \frac{EN_f}{EN_i}$, as obtained by the ratio between the final population (EN_f) and the initial population (EN_i) was determined.

The gall index and egg mass were determined by examining the root system of each infected plant under a stereoscopic microscope. To estimate the indices, the score rating of the International *Meloidogyne* Project - IMP, cited by Taylor and Sasser (1978), was used with susceptibility reaction determined when the mean of the gall indices or the egg mass indices was equal to or over three.

For the reproduction factor, plants with *RF* < 1 were considered resistant and the plants with *RF* ≥ 1 as susceptible, as proposed by Oostembrink (1966). Complementarily, the fresh mass of the root system was measured to determine the correlation between the infection severity and the root system mass of each of the accessions evaluated.

The first experiment was performed under a completely randomized design, with 20 treatments corresponding to accessions, and ten replications, each parcel consisting of a plastic pot with three liters of medium textured soil, autoclaved, containing one plant.

For the statistical analysis of data, a variance-stabilizing transformation was applied in $\sqrt[2]{x}$ for the fresh mass of the root system and egg mass number (EMN); in $\log_{10} x$ for the egg number (EN) and $\log(x+1)$, as indicated by Asmus et al. (2005) for the reproduction factor (RF). Analysis of variance was processed for all sources of variation. The post-ANOVA procedures used were the correlation study using Pearson's correlation coefficient method for the RM, EMN and EN quantitative variables, and application of the Scott-Knott's means comparison procedure at a 5% probability level for the RF.

Analysis of the parcels reaction in the treatments was made by comparing the RF mean value of each parcel with a constant value (k), which corresponded to the parcel with the lowest reproduction factor within each accession. Pair comparisons were made usingthe t-test for orthogonal contrasts $t = \frac{(\bar{x} - K)}{\hat{\sigma}}$, with $\bar{x}$: parcel mean; K: constant value relating to the lowest reproduction factor within each accession and $\hat{\sigma}$: standard deviation of the parcels around the treatment mean.

The second experiment comprised accessions 02, 07, 09 and 10 and the Crimson Sweet commercial variety. Accessions 02, 07, 09 and 10 were selected not for presenting the lowest mean RF but because they presented at least one parcel with RF < 1, that is, rated as resistant and with suppressed expression by using the RF arithmetical mean of treatments for rating. The accession 14 was used as a control for the species' susceptibility and the Crimson Sweet variety as a commercial standard genotype.

All planning, installation, conduction and evaluation procedures used in the second experiment were identical to those used in the first experiment.

The only variable determined in the experiment for measurement of the genotypes resistance was the reproduction factor (RF).

For statistical analysis, experiments were subjected to the analysis of variance and the unfolding of the genotypes × environments (G x E) interaction was performed.

RESULTS AND DISCUSSION

With regard to the GI and EMI variables, the 20 accessions examined showed susceptibility to the parasite according to the criteria described by Taylor and Sasser (1978). Severity of the root systems infection, which was found in all accessions assessed, suggests determination of other variables to characterize the germplasm. Taking into account that the infection occurred in a generalized form, it is suggested that for the development of a breeding program for resistance to *M. enterolobii,* variables relating to the reproduction of this parasite be considered, in order to allow identification of genotypes that are potentially capable of suppressing the parasite reproduction after infection. In this context, accessions with reduction character in the pathogen population densities below the economic injury level is configured as a viable alternative for watermelon breeding. Likewise, when Anwar and Mckenry (2010) evaluated seeds from 16 localities in Pakistan, susceptibility reaction of all watermelon genotypes evaluated was found using these criteria. In general, in this study, all accessions were on average susceptible. However, there exists possibility of obtaining resistant genotypes with the completion of new collections in municipalities of Rio Grande do Norte, Brazil.

In this study, the choice of RF was an alternative to quantitative character GI and EMI. The reproduction inhibitor character was suggested as more robust, considering the refinement of the technique of determining the number of eggs per root.

In contrast with the present work, Pontes (2009) observed resistance of four accessions of *C. lanatus* var. *citroides* and one *C. lanatus* var. *lanatus* according to the gall index, using 2,000 and 5,000 eggs as inoculum, room temperature of 32 ± 5°C, and conducted evaluation 45 days after inoculation. Divergence between the results is suggested by the longer period of exposure of the accessions to the parasite in this work, associated with relatively mild temperatures of 27±0.9ºC, which may have favored the *M. enterolobii* reproductive cycle, which, according to Agrios (2005), is optimum at 27ºC.

Thies and Levi (2007), studying the reaction of accessions of *C. lanatus* var. *lanatus* and *Citrullus colocynthis* to *M. incognita* race 3 and *M. arenaria* race 2, observed resistance of the first species as to the gall index, corroborating the results found by Pontes (2009) for the nematode genus and, for this reason, in disagreement with the present work.

Lima (2008), evaluating the reaction of 4 cultivars and 15 accessions of *C. lanatus* var. *lanatus* to *M. javanica*, observed susceptibility to infection in all accessions and, therefore, corroborate the results found in this work. However, the author found a resistance reaction of all genotypes to the nematode oviposition.

The analysis of variance showed significance in the mean squares of RM, EMN, EN and RF, but the intensity of association between the variables was nearly null (Table 1).

High correlation values would allow the selection of genotypes with mitigation potential, or restriction, of *M. enterolobii* reproduction using only one descriptor simple determination (EN;RF), however, not observed in this study. To evaluate the resistance of a host plant, it is necessary to determine the ability of restricting the nematode multiplication in the plant (Mckenry and Anwar, 2006), thus indicating the egg number or the reproduction factor as the most important variables to be recorded.

Regarding the RF variable, it was found that the extreme values presented by some parcels within each accession had a direct influence on the mean RF in each treatment. Thus, the RF mean values seemed to be overestimated (RF > 1), making ineffective the Scott and Knott's test, like any other procedure of multiple means comparisons, to explain the resistance reaction based on relative contrasts to the RF value (Table 2).

The variability observed within each accession, that is, of each representative subsample of a given population was mostly due to the seeds management by the family farmers. The selection of seeds for the next planting, besides the variability that exists in the samples introduced by the African continent (Romão, 2000) by different routes (Correa, 2010), showed to be a very important tool for the maintenance of the variability found in the germplasm existing in traditional farming.

From the analysis of the reaction of each parcel in each treatment assessed, it was possible to identify plants with resistance reaction in the accessions 02, 07, 09 and 10, because they are plants with reproduction factors lower

Table 1. Summary of the analyses of variance and correlation, considering the root mass (RM) variables, egg mass number (EMN), eggs number (EN) and reproduction factor (RF).

Variation source	[a]gl	[b]MS			
		RM	EMN	EN	RF
Accessions	19	1.14**	58.01**	3.46**	2.67**
Error	180	0.36	6.54	0.12	0.09
Total	199				
CV (%)		19.74	27.82	8.12	25.80

Variable	[c]r				
	RM	EMN	EN	EMI	GI
RM	-	- 0.30[ns]	- 0.18[ns]	0.34[ns]	0.00
EMN		-	0.21[ns]	0.25[ns]	0.00
EN			-	0.25[ns]	0.00
EMI				-	0.00
GI					-

[a] Degrees of freedom; [b] mean square; [c] Pearson's correlation coefficient; ** significant by Snedecor's F-test at 1% probability level ($p < 0.01$); CV (%) coefficient of experimental variation; [ns] not significant according to the student t-test for Pearson's correlation coefficient at 5% probability level.

Table 2. Reproduction factors and rating of the accessions regarding the reaction to *M. enterolobii*. Mossoró – RN, UFERSA, 2012.

Genotype	[a]RFm	[b]Scott-Knot's test	[c]Amplitude	Reaction
Accession 01	12.26	C	43.75 - 2.27	Susceptible
Accession 02	7.24	B	19.93 - 0.45	Susceptible
Accession 03	27.39	D	72.73 - 3.41	Susceptible
Accession 04	12.44	C	40.34 - 1.14	Susceptible
Accession 05	22.78	D	56.82 - 1.14	Susceptible
Accession 06	4.84	B	23.30 - 1.14	Susceptible
Accession 07	5.32	B	11.25 - 0.34	Susceptible
Accession 08	7.16	C	11.73 - 4.39	Susceptible
Accession 09	1.94	A	5.68 – 0.57	Susceptible
Accession 10	1.31	A	1.69 – 0.80	Susceptible
Accession 11	12.97	C	28.34 - 3.48	Susceptible
Accession 12	83.02	F	136.36 - 14.20	Susceptible
Accession 13	32.78	E	77.84 – 15.34	Susceptible
Accession 14	113.69	F	147.16 - 84.09	Susceptible
Accession 15	37.33	E	77.84 - 15.34	Susceptible
Accession 16	6.58	B	11.48 - 1.93	Susceptible
Accession 17	67.16	F	151.70 - 26.14	Susceptible
Accession 18	105.27	F	244.32 - 15.80	Susceptible
Accession 19	69.20	F	105.68 - 23.30	Susceptible
Accession 20	13.26	C	22.16 - 1.93	Susceptible

[a]: Mean reproduction factor; [b]: means followed by the same letter are not significantly different according to the Scott-Knott's multiple comparisons test at 5% probability level ($p \leq 0.05$); [c]: difference between the $_{max}$RF and the $_{min}$RF of each genotype.

than one (RF < 1) (Table 3).

The accessions 09 and 10 had the lowest mean reproduction factors in the first experiment, 1.94 and 1.31, respectively, but in the second experiment, all plants indicated higher reproduction factors. The accessions 02 and 07 showed different plant reactions,

Table 3. Orthogonal contrasts between each parcel in accessions 02, 07, 09, 10, 13, 14 and the Crimson Sweet variety in two different trials.

[1]A2	[2]RF		t		A7	RF		t	
	[3](I)	[4](II)	[5]0.45(I)	[6]3.93(II)		[4](I)	(II)	0.34(I)	1.20(II)
01	3.95	10.82	0.60^{ns}	1.30^{ns}	01	1.14	6.20	0.20^{ns}	0.88^{ns}
02	4.27	4.77	0.65^{ns}	0.16^{ns}	02	4.89	14.30	1.15^{ns}	2.31*
03	5.18	13.23	0.81^{ns}	1.75^{ns}	03	4.32	16.25	1.00^{ns}	2.65*
04	0.45	6.41	-	0.47^{ns}	04	11.25	5.32	2.75*	0.73^{ns}
05	1.48	6.82	0.18^{ns}	0.54^{ns}	05	8.86	6.84	2.15*	0.99^{ns}
06	19.93	3.93	3.33**	-	06	4.77	13.36	1.12^{ns}	2.14*
07	4.84	4.64	0.75^{ns}	0.13^{ns}	07	1.48	15.70	0.29^{ns}	2.55*
08	10.34	9.11	1.69^{ns}	0.97^{ns}	08	4.89	1.20	1.15^{ns}	-
09	11.25	21.55	1.85*	3.31**	09	11.25	2.00	2.75*	0.14^{ns}
10	10.73	10.43	1.76^{ns}	1.22^{ns}	10	0.34	5.70	-	0.79^{ns}
A9	**(I)**	**(II)**	**0.57(I)**	**0.82(II)**	**A10**	**(I)**	**(II)**	**0.93(I)**	**1.30(II)**
01	0.68	11.84	0.06^{ns}	1.00^{ns}	01	1.19	1.30	0.72^{ns}	-
02	1.14	LP	0.29^{ns}	LP	02	1.66	5.66	1.99*	0.23^{ns}
03	5.68	35.68	2.65*	3.17**	03	1.69	3.18	2.08*	0.10^{ns}
04	1.36	20.07	0.41^{ns}	1.75^{ns}	04	1.44	66.05	1.40^{ns}	3.34**
05	5.34	8.77	2.48*	0.72^{ns}	05	1.36	13.64	1.18^{ns}	0.64^{ns}
06	0.57	0.82	-	-	06	1.18	20.50	0.69^{ns}	0.99^{ns}
07	0.91	5.27	0.18^{ns}	0.41^{ns}	07	0.93	14.45	-	0.68^{ns}
08	1.94	22.57	0.71^{ns}	1.98*	08	1.91	PP	2.67*	LP
09	0.57	15.80	-	1.36^{ns}	09	0.95	19.45	0.07^{ns}	0.94^{ns}
10	1.25	3.95	0.35^{ns}	0.29^{ns}	10	0.80	17.57	0.37^{ns}	0.84^{ns}

CS	1	2	3	4	5	6	7	8	9	10
(II)	21.23	19.25	50.09	1.39	46.75	45.34	7.32	50.09	35.75	15.20
1.39	1.07^{ns}	0.96^{ns}	2.63*	-	2.45*	2.37*	0.32^{ns}	2.63*	1.85*	0.74^{ns}

LP: Lost parcel; CS: Crimson Sweet. [1]: accession 02; [2]: reproduction factor; [3]: parcels within each accession evaluated in experiment I; [4]: parcels within each accession evaluated in experiment II; [5]: lowest reproduction factor per accession found in experiment I; [6]: lowest reproduction factor per accession observed in experiment II. **: significant contrast according to the Student t-test at 1|% probability level ($p < 0.01$); *: significant contrast according to the Student t-test at 5% probability level ($p < 0.05$); ns: not significant

sometimes lower in the first accession and higher in the second and vice versa. By analyzing the RF magnitude in each accession, it was found that the accession 02 showed one parcel with RF = 0.45 in the first experiment and standard deviation (σ) around the average of 5.85, indicating resistance reaction to *M. enterolobii*. In the second experiment, the accession 02 showed susceptibility reaction to *M. enterolobii* in all parcels. The accession 07 behaved similarly to accession 02.

The accessions 09 and 10 presented a differentiated behavior, with respectively 40 and 30% of their parcels with RF < 1 in the first experiment. The interval of 0.57 ≤ RF ≤ 1.94 comprised 80% of the parcels of the accession 09 with a standard deviation of 1.93. In the second experiment, there was only one plant with RF < 1 (RF = 0.82); however, it reaffirmed its potential as a source of resistance genes for use in breeding programs.

Seventy percent of the parcels showed resistance reaction in the genotype 10 in the first experiment, which synthetized the interval 0.80 ≤ RF ≤ 1.44. In the second trial, no parcel exhibited RF < 1, suggesting that the frequency of individuals with this characteristic, in this seeds subsample, is low, lower than that found in accession 09.

This difference in behavior is likely related to different genus contents in the plants; as even in slightly contrasting conditions, they enabled or mitigated the nematode development. Damasceno (2013), in a study of parents and F_{1s} of watermelon *Citrullus* spp., showed that the general and specific resistance combination ability to the nematode were highly significant, indicating a genetic control of the character. In general, segregation for the RF character can be observed in the plants of all accessions.

Regarding the Crimson Sweet commercial variety evaluated in the second trial, there was variability in the expression of resistance within the cultivar, with a minimum and mean RF of 1.39 and 29.24, respectively.

Commercial lines with high ability of nematode reduction viability can be promising sources of watermelon resistance to the nematode species, either for breeding programs *per se* or crossings.

Similar result was found by Lima (2008), who evaluated six cultivars and 15 accessions of *C. lanatus* var. *lanatus* species, showing resistance reaction of all treatments to *M. javanica* based on the RF.

On the other hand, Pontes (2009) found RF < 1 in two accessions of the *C. lanatus* var. *citroides* species. Also, Damasceno (2013) corroborated the results found by Lima (2008) and Pontes (2009) in accessions of *Citrullus* spp., showing the germplasm potential of traditional farming as a source of tolerance alleles for the characteristics, which was also confirmed by the present study on *C. lanatus* var. *lanatus* accessions.

Conclusions

Variation was found in the response among and within the accessions regarding the ability to inhibit reproduction of *M. enterolobii* species in the root system; as the accessions 9 and 10 showed potential for implementation of breeding programs aiming to develop tolerant genotypes to the reproduction of *M. enterolobii*. However, in future research, the data collection area will be extended to all municipalities in the State of Rio Grande do Norte, Brazil, for characterization and conservation of genetic variability present in germoplasm of traditional agriculture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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