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Chicory (*Cichorium intybus* L.) yield under water stress and estimation of leaf area using allometric relations

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Abstract

Leafy vegetables are mainly grown in small properties using empirical irrigation control and frequently applying excess water. Considering these aspects, this study aimed at (i) evaluating the chicory (*Cichorium intybus* L. cv. “Folha Larga”) production, grown in a greenhouse under different irrigation levels, proportional to the reference evapotranspiration (ET_0) and (ii) adjusting models to estimate chicory leaf area from leaf linear measurements. For this, the chicory was grown in a 120 m^2 greenhouse. A completely randomized experimental design was used, applying five irrigation treatments and nine repetitions per treatment. The irrigation treatments consisted of percentages of ET_0, as follows: 45.6; 60.8; 76; 91.2 and 106.4% of ET_0. Measurements of plant height, number of leaves, shoot fresh and dry mass and leaf area were taken. Measurements of 605 leaves were used to adjust ten initial models, from which four models were selected based on statistical coefficients. These models were then submitted to statistical analysis to obtain models which residuals present tendency to normality and homoscedasticity. Measures of 250 new leaves were used to apply the Bland-Altman graphical method. The results show that the increase in one percent of ET_0 applied by irrigation increased the plants height in 0.148 cm, and 0.263 and 0.0158 g in the shoots’ fresh and dry mass (respectively) and decrease in 0.306 cm^2 of the leaf area. Bland-Altman method revealed that the models estimated correlated but non-concordant data, pointing out to the need of adding the biases’ value of each model to the estimated values.

Keywords: Bland-Altman method; *Cichorium intybus*; drip irrigation; evapotranspiration; leaf linear measures; linear regression.

Abbreviations: c _ Camargo and Sentelhas’s performance index; d _ Wilmott’s index of agreement; ET_0 _ reference evapotranspiration; H _ plants’ height (cm); KW _ Kruskal-Wallis non-parametric test; LA _ leaf area (cm^2 $leaf^{-1}$); L _ leaf length; NL _ number of leaves per plant; GQ _ Goldfeld-Quandt homoscedasticity test; R^2_{adj} _ adjusted determination coefficient; r _ Pearson’s correlation coefficient; SDM _ shoots’ dry mass; SFM _ shoots’ fresh mass; SW _ Shapiro-Wilk tendency to normality test; W _ leaf width.

Introduction

Chicory (*Cichorium intybus* L.) is a vegetable from the Asteraceae family, the same family of lettuce, escarole and dandelion. The economic interest of chicory crop is related to the use of leaves in salads or braised, substituting cabbage, spinach and endive in the preparation of hot dishes (Filgueira, 2008). Chicory is grown for other reasons, such as root production for blending with coffee (Patel et al., 2000). It is also used as forage, being widely used as high feed quality perennial herbage (Cranston et al., 2016).

Besides the different cultivars used for each purpose, chicory presents a taproot that enables it to reach deep into the soil to get nutrients and water. The taproots also serve as carbohydrate storage influencing the persistence of plants and enabling plants survival under drought conditions (Nie et al., 2008). The raising world population and the increasing need to produce more food with less water is increasing the search for plants capable of maintaining its growth under drought periods and produce similar yields to those obtained with full irrigation. Chicory’s ability to recover from water deficit periods (Nie et al., 2008) is an important factor when considering crops to grow in semi-arid or arid regions.

Although the purpose of growing chicory was different than in the present study, the studies performed by Cranston et al. (2016) and Patel et al. (2000) present an analysis of chicory yield during periods of water deficit, analyzing shoots fresh mass, number of leaves per plant, leaf mass and root dry mass. Apart from the studies mentioned above and some other research in literature about irrigation and chicory yield, there are some studies regarding the deficit irrigation influence on plants of the same family, such as lettuce (Bandeira et al., 2011; Santos and Pereira, 2004).

The leaf area (LA) is a very important factor for the majority of the agricultural and physiological studies that involve vegetative growth, light interception, photosynthetic efficiency, evapotranspiration and answer to fertilizers and to irrigation. An indirect and non-destructive method mostly used to estimate LA involves the use of mathematical equations. These equations estimate LA from linear measurements of leaf length (L) and width (W) which does not require the destruction of leaves. These equations usually present high precision and accuracy (Blanco and Folegatti, 2005). José et al. (2014), studying the basil crop (*Ocimum basilicum* L.), adjusted equations for LA estimative from leaf linear measurements, using the adjusted coefficient of determination (R^2_{adj}), Pearson's correlation coefficient (r), Wilmott's index of agreement (d), Camargo and Sentelhas's performance index (c), and Bland-Altman graphical method for the verification of the equations with data from different leaves. Studying the relations between sunflower LA and leaf linear measures Firouzabadi et al. (2015) adjusted regression models with high r. In this study the authors have used only the determination coefficient (R^2) and r. To validate the models the authors used the measurements of 50 sunflower leaves. Considering the lack of information in literature about the response of the chicory crop to the irrigation, this study aimed at: (i) studying the response of chicory to different irrigation water heads calculated according to the percentages of the reference evapotranspiration, analyzing variables associated to the vegetative growth of chicory, and; (ii) adjusting equations for the chicory LA estimative from linear measures of leaf length and width.

Results

Chicory yield in response to the deficit irrigation

The Shapiro-Wilk and Barlett tests revealed that the original data of the following variables: plants height (H), number of leaves (NL) and shoots' dry mass (SDM) presented tendency to normality and homogeneity of variances. The data of shoots' fresh mass (SFM) did not present tendency to normality nor homogeneity of variances, requiring appropriate outliers' removal through Boxplot method, obtaining data with tendency to normality and homoscedasticity. For the leaf area (LA) variable, results of p-value lower than 0.01 were obtained for the Shapiro-Wilk and Bartlett tests, even after data transformation, demanding the use of non-parametric statistical techniques to the original data. The Shapiro-Wilk and Bartlett tests' results for the original data of all the variables except SFM are presented at Table 1. For the SFM the results shown are regarding the data after outlier removal and data transformation. Table 1 also presents the results of mean square obtained with the analysis of variance of the variables H, NL, SFM and SDM, as well as the results of the Kruskal-Wallis test obtained for the LA data. From these statistical results, it is possible to affirm that there was significant difference between the treatments of ET_0 percentages for the H (p-value < 0.01), NL, SFM and SDM (both with p-value < 0.05) and LA (p-value for the Kruskal-Wallis test < 0.01). It is also possible to state that there was significant difference between repetitions for H and SFM. The average values of NL for each treatment are presented in Table 2, with the indicators obtained through the Tukey test. It is noted that there is no significant difference between the treatments of ET_0 percentages for NL according to the Tukey test. This may have occurred because the plants were exposed to a lower water deficit at the beginning of the experiment, a moment in which there is larger growth in terms of number of leaves and when, possibly, the final number of leaves per plant is determined. Figure 1A shows data regarding H and SFM according to the irrigation treatment and also the representation of the equations adjusted through linear regression. The linear models adjusted for H and for SFM according to the ET_0 percentages applied by irrigation were defined, respectively, by the equations y=17.831+0.148x (in which y is H and x is the $\%ET_0$) and y=6.995+0.263x (in which y is the SFM and x is the $\%ET_0$). According to the equations presented in the last paragraph, and in Figure 1A, it can be emphasized that an increase of one percent of ET_0 applied by irrigation resulted in an increase of 0.148 cm in H and of 0.263 g in SFM. Consequently the treatment of 106.4% of ET_0 was the treatment that produced higher plants and with higher shoot's fresh mass. The plants submitted to this ET_0 percentage were, in average, 9.18 cm higher and 15.81 g heavier (SFM) than the plants submitted to the treatment of 45.6% of ET_0.

The best linear regression models for SDM and LA according to the ET_0 percentages applied by irrigation are represented in Figure 1B, being defined by the equations y=1,335 +0.0158x (in which y is SFM and x is % ET_0 applied by irrigation) and y=108.04-0.306x (in which y is LA and x is $\%ET_0$ applied by irrigation). It is noticeable that an increment of one percent of ET_0 produced an increase of 0.0158 g in SDM and a decrease of 0.306 cm² in LA. The plants that were submitted to the treatment of 106.4% of ET_0 were, in average, 0.97 g heavier (SDM). On the other hand, plants submitted to the treatment of 45.6% of ET_0 presented leaves, in average, 18.61 cm² larger than the leaves of the plants submitted to the treatment of 106.4% of ET_0.

Estimation of leaf area using allometric relations

Table 3 presents the best four initial equations for the estimative of wild chicory leaf area through linear measures of leaves (length and width) with its coefficients of determination (R^2_{adj}) and correlation (r); the Willmott's index of agreement (d); the performance index (c) of Camargo and Sentelhas (1997), and the results for the tests of Shapiro-Wilk and of Goldfeld-Quandt regarding the residuals of the equations. It is noticeable that the four initial equations did not presented residuals with tendency to normality and with homogeneity of variances, demanding the use of statistical techniques for outliers' removal (Boxplot) and for data transformation (Box-Cox). The two first equations did not need to have the data transformed ($\lambda = 1$). Using the Boxplot method, with coefficient of 1.3, 25 pairs of data were detected as outliers, and then removed, resulting in equations 1 and 2 (Table 3). However, in the last two equations there was the need to transform data, once lambda (λ) values were equal to zero, with the need to transform data with logarithm. After the transformation, nine data pairs were detected as outliers, using a coefficient of 1.5 in the Boxplot method. After data transformation and outliers' removal, as explained before, new equations were adjusted and the same statistical coefficients and indexes were calculated (Table 3). The residuals of these new equations were submitted to the tests of Shapiro-Wilk and Goldfeld-Quandt (Table 3), obtaining p-values that are higher than 0.05 for these tests. Therefore, the residuals of these equations present tendency to normality and homogeneity of variances. These equations were, then, submitted to the graphic method of Bland-Altman, as described before (Figure 2). Observing Table 3 and the graphics of Figure 2, it is possible to note that there is a high Pearson's correlation coefficient ($r > 0.95$) indicating that the four equations present correlated measures. However, the measures of the equations are non-concordant, as the four equations present statistically significant biases that are distant from zero.

Discussion

Chicory yield in response of deficit irrigation

The results regarding NL, SFM and LA are important as they lead us to consider that the deficit irrigated plots resulted in plants with similar number of leaves, but larger leaves and

Table 1. Shapiro-Wilk and Bartlett tests for the measured variables (after outliers removal and data transformation for the SFM variable) Mean square obtained in the ANOVA and the p-value obtained by the Kruskal-Wallis test.

		H	NL	SFM	SDM	LA
Shapiro-Wilk		0.126	0.414	0.617	0.0266	$7.65\ 10^{-3}$
Bartlett		0.292	0.1405	0.794	0.182	$1.86\ 10^{-8}$
Cause of variation	DF					KW
Treatments	4	130.10**	3.60*	406.4*	1.96*	$2.039\ 10^{-6}$*
Blocks	8	57.08**	1.45^{NS}	56.1^{NS}	1.22^{NS}	0.0538^{NS}
Residuals	32	13.30	1.30	65.6	0.61	-

H – plants' height; NL – number of leaves; SFM – shoots' fresh mass; SDM – shoots' dry mass; LA – leaf area; KW – Kruskal-Wallis test result; DF – degrees of freedom; significance: ** – p-value < 0.01; * – p-value < 0.05; ^{NS} – p-value > 0.05.

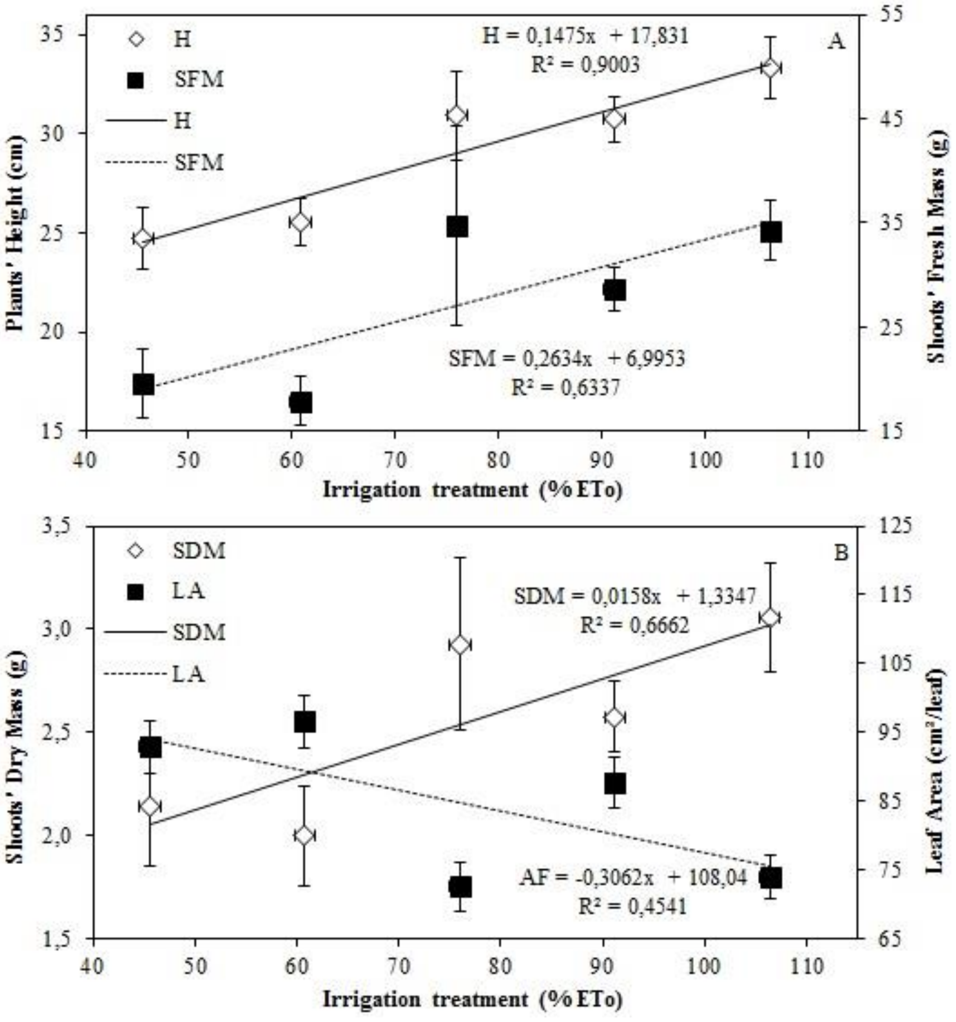

Fig 1. Graphic representation of the equations adjusted through regression analysis for H and SFM (A); and for SDM and LA (B). Vertical lines represent the standard error of the mean. LA – leaf area; SFM – shoots' fresh mass; SDM – shoots' dry mass; H – plants' height.

Table 2. Tukey test of averages for the values of number of leaves per plant.

Treatments	NL
106.4 % ET_0	10.17 a
91.2 % ET_0	9.67 a
76 % ET_0	10.07 a
60.8 % ET_0	8.59 a
45.6 % ET_0	9.41 a

Table 3. Initial and final equations for the estimative of leaf area through leaves linear measures.

		R^2_{adj}	r	d	c	SW	GQ
Initial equations							
AF=0.510 L W		0.988	0.967	0.935	0.904	$1.33\ 10^{-8}$	$4.76\ 10^{-6}$
AF=0.649 π (L W)/4		0.988	0.967	0.935	0.904	$1.33\ 10^{-8}$	$4.76\ 10^{-6}$
AF=0.455 L+3.333 W + 0.4193 L W		0.990	0.935	0.939	0.878	$1.77\ 10^{-8}$	$1.04\ 10^{-5}$
AF=2.441 W + 0.441 L W		0.990	0.968	0.935	0.905	$5.09\ 10^{-4}$	$5.02\ 10^{-5}$
Final equations							
AF=0.571 L W	(1)	0.991	0.975	0.951	0.928	0.0268	0.0153
AF=0.659 π (L W)/4	(2)	0.991	0.975	0.951	0.928	0.0268	0.0153
log(AF)=0.674 log(L) + 1.161 log(W)	(3)	0.999	0.977	0.957	0.935	0.0665	0.0912
log(AF)=0.448 log(W) + 0.691 log(L W)	(4)	0.999	0.979	0.957	0.937	0.0665	0.0909

R^2_{ajust} – adjusted determination coefficient; r – Pearson's correlation coefficient; d – Willmott's index of agreement; c – performance index of Camargo and Sentelhas; L – leaves' length; W – leaves' width; SW – Shapiro-Wilk test; GQ – Goldfeld-Quandt test

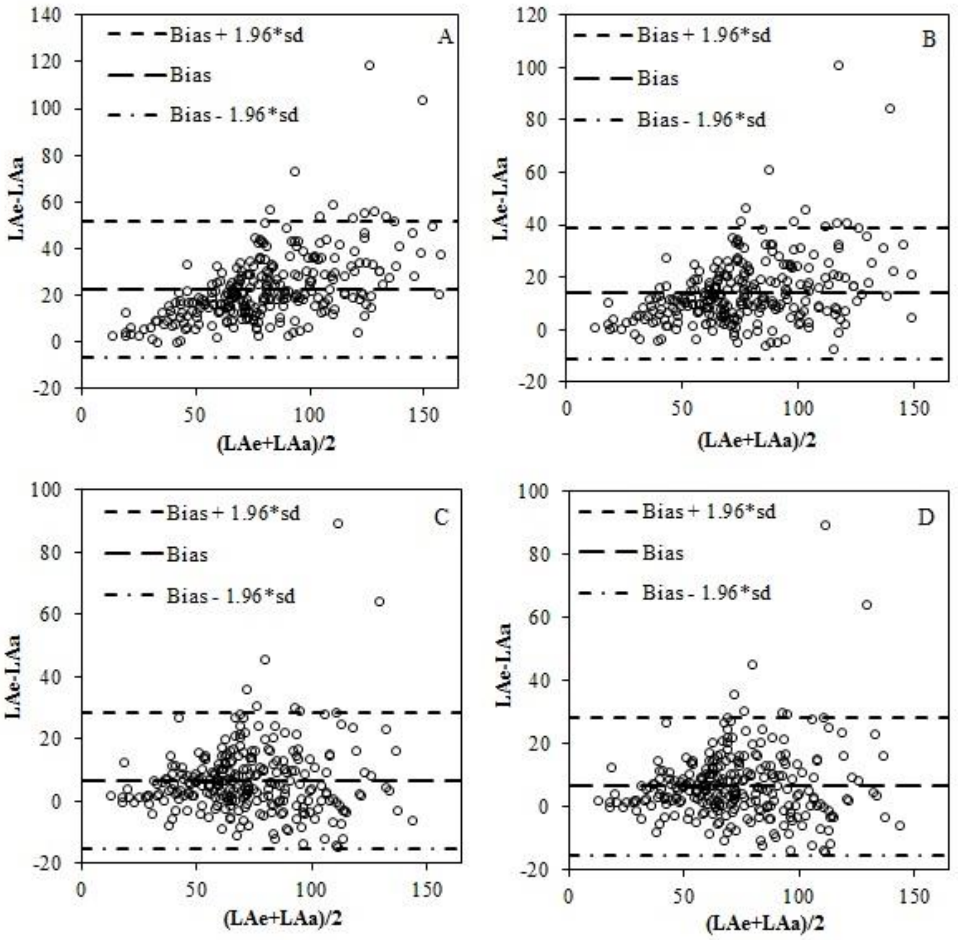

Fig 2. Bland-Altman's graphic method to the evaluation of the adjusted equations 1 (A), 2 (B), 3 (C) and 4 (D) described at Table 3. LAa – actual leaf area; LAe – estimated leaf area.

with less dry matter. This is important to mention because tender and larger leaves are better for consumption as salad.

In a study about the water deficit in lettuce, Santos and Pereira (2004) observed that H and SFM were reduced when the lettuce plants were submitted to higher water deficit levels, similar to the results obtained in the present study (Figure 1A).

Hamada and Testezlaf (1995), studying the response of lettuce to the deficit and excess irrigation, percentages of the evaporation of a class A evaporation pan (ECAP), obtained higher plants when submitted to 120% of ECAP, followed by the treatments of 100, 60 and 80% of ECAP, similar to the results obtained in the present study. These authors obtained a difference of about 50% of the shoot's fresh mass between the treatments of 120 and 60% of ECAP. Regarding the shoot's dry mass, they obtained higher values in plants submitted to the treatment of 100% of ECAP, similar to the present study.

In what refers to SDM, other authors had also obtained results that are similar to those obtained in this study, reporting lower values of SDM in lettuce plants submitted to water deficit in comparison to plants with no water restriction (Bandeira et al., 2011; Cardoso and Klar, 2009). However, Cardoso and Klar (2009) observed that the water excess produced a negative effect in lettuce plants. Bandeira et al. (2011) observed that plants submitted to deficit irrigation presented SDM 31.3% lower than the plants without water restriction. In the study of Cranston et al. (2016) a greater leaf mass was obtained in chicory plants submitted to moderate and severe deficit irrigation in comparison to the fully irrigated plants, suggesting larger leaves, a result that is similar to the ones reported in the present study.

In the present study the equations presented in Figure 1 were adjusted only aiming at observing the answer of the wild chicory to the percentages of reference evapotranspiration applied by irrigation.

Estimation of leaf area using allometric relations

Regarding the obtained models for LA estimation from leaf linear measurements, it is possible to suggest that the equations 3 and 4 are better as they present biases closer to zero and higher determination and correlation coefficients. The four equations can be used; however, the biases of each equation should be added to the values of leaf area predicted by them.

As José et al. (2014), in this study it was possible to adjust equations that take into account linear measures to estimate the leaf area. The use of expensive and destructive methods is not obligatory for the estimative of crop's leaf area. Different from the work of Firouzabadi (2015), the models constructed in the present study were tested with a larger number of statistical coefficients and indexes than only the determination and correlation coefficients. Also, the adjusted models were validated with the data from 250 new leaves with the Bland-Altman's graphic method, many more than in the cited study.

Materials and Methods

Experimental area

The experiment was performed in a greenhouse located at the Biosystems Engineering Department (LEB) of the Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Campus of Piracicaba, SP (22º 41' 58'' S, 47º 38' 42" W), at approximately 511 m above sea level. The climate at the region is of the kind Cwa (Köppen), subtropical humid, with hot and humid summers and dry and cold winters, with annual average temperature equal to 21.4ºC, and precipitation of 1,257 mm. The greenhouse where the experiment was conducted presented a total area of 120 m² with concrete boxes of 1.3 m² with individualized irrigation control for each box.

An automatic meteorological station inside the greenhouse was used to measure air temperature and relative humidity, wind speed and direction and solar radiation. From the data of maximum and minimum air temperature (ºC), maximum and minimum air relative humidity (%) and daily total solar radiation (MJ m^{-2} d^{-1}) the reference evapotranspiration (ET_0) was estimated daily by the Penman-Monteith method, parametrized by FAO (Allen et al., 1998).

Experimental design and irrigation treatments

The experimental design used was the completely randomized design, with five percentages of reference evapotranspiration (ET_0) applied by drip irrigation (treatments) and nine repetitions for each treatment. The ET_0 percentages applied by irrigation were: 45.6; 60.8; 76; 91.2 and 106.4%.

The chicory seedlings were transplanted in October 11th, and harvested at December 12th, 2014, approximately 50 days after transplanting the seedlings (DAT). Before transplanted, the seedlings were in Styrofoam trays of 288 cells, presented 0.03 to 0.05 m height and were previously treated with insecticides and fungicides.

Final yield evaluation

The assessed variables in the chicory plants were: plants' height (H – cm), number of leaves per plant (NL), shoots' fresh and dry mass (SFM and SDM – g) and leaf area (LA – cm^2 $leaf^{-1}$). SDM was obtained after drying the shoots in a drying oven at 60ºC for 48 hours and weighting the dried material shortly after taking it from the drying oven.

LA was obtained through digital pictures of the chicory leaves over a clear background, using the software ImageJ v 1.47 (Wayne Rasband, National Institutes of Health, US). This software uses a known linear or bi-dimensional scale at the picture and estimates the leaf areas contrasting the leaves green color with the picture's clear background.

Statistical analysis – final yield

The statistical tests of Shapiro-Wilk and Bartlett were used to test the tendency to the normality and the homogeneity of variances, respectively. When a p-value for these tests for a specific variable was greater than 0.05, the analysis of variance (ANOVA) was performed. When one of these tests resulted a p-value lower than 0.05 for some variable, the Boxplot method for the outliers removal and the Box-Cox test were performed to normalize data (Box and Cox, 1964). When the tests of Shapiro-Wilk and Bartlett, even after outliers removal and data transformation, resulted in a p-value lower than 0.05, the non-parametric statistical techniques (Kruskal-Wallis test) were applied to the original data.

The regression analysis was applied to the variables plants' height, shoot's fresh and dry mass and LA, since the treatments and the variables are considered quantitative variables. Yet, for the variable number of leaves per plant, the Tukey average test was used, since these data are qualitative.

If the data were non-parametric the Kruskal-Wallis test was used.

Statistical analysis – estimation of leaf area

To build the models to estimate the chicory LA based on linear data of leaf length and width, the data of 605 wild chicory leaves was used. Ten initial models were obtained, calculating the determination (R^2_{adj}) and correlation (r) coefficients, Willmott's index of agreement (d), and the performance index (c) of Camargo and Sentelhas (1997).

The best four models according to these coefficients and indexes were selected, following with the use of the Shapiro-Wilk and Goldfeld-Quandt tests to check the residuals normality and homoscedasticity. After the appropriate outliers' removal through Boxplot and data transformation through Box-Cox, new measures of 250 leaves were used to verify the quality of the adjusted models by Bland-Altman graphic method (Bland and Altman, 1986).

Conclusion

Considering the obtained results, it is possible to conclude that the variables plants' height and shoots' fresh and dry mass responded positively to the increase of ET_0 percentage applied by drip irrigation. However, for leaf area the response was negative. The irrigation treatments did not affected the number of leaves per plant. It was possible to adjust four equations to estimate the wild chicory leaf area from linear measures of leaves' length and width.

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