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Leaf area estimation in muskmelon by allometry

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Abstract

This study developed a method for estimating the leaf area (LA) of muskmelon by using allometry. The best linear measure was evaluated first, testing both a leaf length and width (W). Leaf samples were collected from plants grown in containers of different sizes, leaves of four cultivars, at different develpoment stages, and of different leaf sizes. Two constants of a power equation were determined for relating allometrically a linear leaf measure and LA, in a greenhouse crop. W proved to be a better fit than the leaf length. The maximum attainable W and LA were estimated at W_x = 15.4 cm and LA_x = 174.1 cm^2. The indicators of fit quality showed that the function was properly related to LA and W as: $LA/LA_x = A_o \times (W/W_{Lx})^b$; the allometric exponent was b = 1.89, where $R^2 = 0.9809$ ($n = 484$), the absolute sum of squares, 0.4584, and the standard deviation of residues, 0.03084, based on relative values calculations (LA/LA_x and W/W_{Lx}). The relationship was not affected by the cultivar, crop age, leaf size or stress treatment in the seedling stage. The empirical value of allometric constant (A_0) was estimated as 0.963.

Additional key words: allometric model; *Cucumis melo* L.; growth analysis; nondestructive methods.

Introduction

LA in crops is related mainly to transpiration, light interception, CO_2 exchange, and hence, to photosynthesis and growth. In practice, it is prominent to measure LA at both individual and community levels. The measurement of LA is often required for a growth analysis, studies on plant nutrition, plant competition, soil-water relationship, light reflectance, and energy transfer (Schwartz and Kläring 2001, Blanco and Folegatti 2005, Cemek *et al.* 2011). Moreover, many models for horticultural crops deal with photosynthesis in reference to LA development and light interception and their relationship with growth and maintenance of respiration (Bader and Abdel-Basset 2002). In this view, LA has been found to strongly influence evapotranspiration, crop growth, and productivity (Lizaso *et al.* 2003).

For LA measurement, leaves must be mostly harvested by destructive sampling for direct measurement. Such methods do not allow sequential measurements on the same leaves during crop growth. Generally, this problem can be overcome by increasing the number of the samples and by assuming an increase in variability. However, there are various situations where leaf removal is not desirable, such as in the case of measurements carried out on rare plants (Misle *et al.* 2006) or crops under commercial conditions. This also applies to experiments, which have large numbers of treatments, where a big number of leaves must be collected and the analysis is very laborious and time-consuming, or to those, which demand high-cost equipment for nondestructive determination (Ruiz 1996, Tsialtas and Maslaris 2005). As a consequence, several authors have pointed out the need for an inexpensive, rapid, reliable, and nondestructive method for LA measurement (Peksen 2007, Nabi Ilkaee *et al.* 2011, Olfati *et al.* 2010, Rouphael *et al.* 2010b).

As an alternative to destructive methods, indirect methods can be employed. Montgomery (1911) has been frequently acknowledged as the first one to propose that LA can be calculated using linear measurements, such as a leaf length (L) and W (Bhatt and Chanda 2003, Leroy *et al.* 2007, Karimi *et al.* 2009). A mathematical equation can be obtained by relating L, W or both to the actual LA of leaves using regression analysis. The method has

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Abbreviations: A – area; A_0 – allometric constant; LA – leaf area; LA_x – maximum attainable leaf area; b – allometric exponent of leaf width; L – leaf length; L_x – maximum attainable leaf length; *M* – leaf dry mass; Sy.x – standard deviation of residuals; W – leaf width; W_x – maximum attainable leaf width; α – allometric exponent of leaf dry mass.

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shown validity under varying environmental conditions (Meza and Bautista 1999, Schwartz and Kläring 2001, Blanco and Folegatti 2005, Zhang and Liu 2010) or when comparing different cultivars (Schwartz and Kläring 2001, Kumar and Sharma 2010; Wu *et al.* 2010; Nabi Ilkaee *et al.* 2011). Various equations relating L and W to LA have been developed for several vegetable and field crops, such as bean (Bhatt and Chanda 2003), crucifers (Olfati *et al.* 2010), cucumber (Robbins and Pharr 1987; Blanco and Folegatti 2005, Cho *et al.* 2007), eggplant (Rivera *et al.* 2007), faba bean (Peksen 2007), muskmelon (Chirinos *et al.* 1997, Lopes *et al.* 2007, Nascimento *et al.* 2002, Wu *et al.* 2010), pea (Galindo and Clavijo 2007), soybean (Nabi Ilkaee *et al.* 2011), sugar beet (Tsialtas and Maslaris 2005), sunflower (Rouphael *et al.* 2007), tomato (Schwarz and Kläring 2001), watermelon (Rouphael *et al.* 2010b), and zucchini (Rouphael *et al.* 2006), in addition to fruit trees, such as pistachio (Karimi *et al.* 2009) and species and medicinal plants, such as *Bergenia purpurascens* (Zhang and Liu 2010), sage (Kumar and Sharma 2010), and even ornamental plants such as roses (Rouphael *et al.* 2010a).

Nondestructive methods based on linear measurements are easy to be carried out and they offer a good precision and high accuracy. Although most scholars in the field have faced the problem of searching for a practical tool through linear or polynomial equations (Ruiz 1996; Chirinos *et al.* 1997, Meza and Bautista 1999, Karimi *et al.* 2009), there are some others, who have employed a power equation (da Silva 2000, Schwartz and Kärling 2001, Nascimento *et al.* 2002, Galindo and Clavijo 2007, Leroy *et al.* 2007, Antunes *et al.* 2008, Zhang and Liu 2010). However, only Schwartz and Kärling (2001) briefly refer to their background as being supported by general allometry.

Allometric relationships can be frequently found as unnoticed ratios in growth analysis (*i.e.*, leaf mass ratio, harvest index or yield components); but these are just morphological relationships. Although the allometric approach seemed to be disregarded by scientists (France and Thornley 1984), recent research has revaluated it to serve not only for morphological but also functional analysis. The theoretical understanding behind this lies in its condition for optimizing the exchange of resources of an organism, or a part of it, with the environment (root area, LA, lungs or gut surface); at the same time, scaling across a wide species and body size range has been verified (West *et al.* 1997, Enquist 2002). Moreover, Niklas (1994) did not consider this analysis an intellectual exercise but he assigned to it a legitimate and meaningful place in the technological approaches of the biological as well as the physical sciences, facilitating the quantitative description of complex systems. The same author offered some particular examples of scaling on morphological factors as stem diameter, plant height, or proportions on reproductive structures. However, a practical tool as a linear measure to estimate LA through a power function has been scarcely employed.

Theory

A first approach to the problem is a simple and practical linear relationship between LA and a linear measure (L or W) on the leaf. Frequently, a good fit is found to be enough for practical purposes, but this fortuitous situation is a geometric impossibility: one of the simplest shapes to consider is the circle, where it is easy to note that the increase in a diameter is not linearly related to an increase in the area; instead, a power function is evident ($A \propto \text{radius}^2$). When simple geometric shapes are considered, allometric relationship occurs and the allometric exponent $b = 2$ can be observed. For leaves, it must be assumed that leaf blades are symmetrical and have an invariant shape, regardless of their size and position in the plant; this means that varying leaf areas result from proportional enlargement or reduction of this fixed shape (Sezer *et al.* 2009). According to Niklas *et al.* (2009), this situation ($A \propto L^2$) is valid if no variation in the density of the tissue is assumed when considering the mass allometry; but they warn that the scaling exponent governing the relationship between LA and L can significantly differ, depending on the species under study. However, they analysed the data from 25 species in Niklas and Cobb (2008), which showed that the scaling exponent for LA *vs*. L was 2.07 ($R^2 = 0.973$).

The practical use of leaf allometry for estimating the LA in muskmelon was used by Misle *et al.* (2004), who determined the same exponent, identifying the power function with allometry similarly as Nascimento *et al.* (2002).

Allometry was used first in plants by Pearsall (1927) but the current terminology and the use of the power function were established by Huxley (1924). In this relationship, the proportional rates of increase in LA and W (or L) are related by the constant factor *b* (France and Thornley 1984):

$$\frac{1}{\mathrm{LA}}\frac{d\mathrm{LA}}{dt} = b\frac{1}{\mathrm{W}}\frac{d\mathrm{W}}{dt} \quad (1)$$

The integration of this relationship results in the power function. In addition, maximum LA ($\mathrm{LA_x}$), and maximum leaf width ($\mathrm{W_x}$) or length ($\mathrm{L_x}$) must be known for a practical use. Thus, the integrated expression becomes:

$$\frac{\mathrm{LA}}{\mathrm{LA_x}} = \mathrm{A_0}\left(\frac{\mathrm{W}}{\mathrm{W_x}}\right)^{b} \quad (2)$$

A_0 is a normalization constant, also called allometric constant, theoretically $A_0 = 1$.

The objectives of this study were (*1*) to search for a linear measure of either leaf width or leaf length, which may be allometrically related to the LA of muskmelon, (*2*) to determine the equation parameters, and (*3*) to assess the ability of this relationship in estimating LA under different conditions.

Materials and methods

Whole plants of muskmelon (*Cucumis melo* cv. Durango) were sampled at different growth stages during the season of 2004 with the aim to measure leaf L and W (at different growth stages) as well as LA. Such measurements were a part of an experiment carried out to determine growth components of the muskmelon under greenhouse cultivation, located close to the city of Curicó (34°58'60"S, 71°13'60"W), in the irrigated valley of central Chile. Plants were grown vertically due to a plant-support netting set up for guiding plant branches. Light pruning was done by early terminating tertiary ramifications after the first flower. Seedlings at the first true leaf stage were transplanted from multi-container trays in rows covered with plastic mulch and they were drip-irrigated and fertigated following Misle (2013). Later, leaf samples were cropped and carried in bags to the laboratory. Maximum W of the first third of the leaf from the proximal border (towards the petiole) and L measured from the base of the leaf to the distal end (tip) with a metric scale in cm were taken into account. Analyses were performed to determine whether W or L fitted better Eq. 2, supported by R^2, the absolute sum of squares, and the standard deviation of residues, calculated on basis of relative values. The first data set (75 leaves) from the sampling 22 d after transplanting was used for this purpose. Leaves were taken from plants grown in 72 ml containers (no restriction for roots).

At the same time, the effect of stressful conditions was tested. Plants were grown from seedlings produced in the greenhouse in containers of different sizes. This condition was proved to restrict a yield and quality of muskmelon grown in containers smaller than the size of 72 ml, especially during the first harvest (Alvarado 2005). Thus, the samples from plants grown in containers of 25, 32, 43, and 72 ml were evaluated after 22, 29, and 93 d following transplantion with n = 75, 36, and 22 for each date, respectively. Only the leaves of the main stem were measured. Since LA sampling was done at the same stand as in the experiment of Alvarado (2005), randomized blocks were used as the main experiment design. In addition to this comparison, most of the samples, including treatments and replicates, were employed in a single regression with n = 484 for fitting the parameters of Eq. 2.

During 2006, another set of samples was taken in the same location, but this time the evaluation involved four cultivars: Colima, Durango, Early Brew, and Zeus. Cultivars from different companies were selected in order to have varieties, which were not so closely related to each other. In each cultivar, 30 leaves were simultaneously sampled on the same day (λ = 0.67) in order to measure LA and W with the aim of verifying the equation previously determined. All four cultivars were drip-irrigated and fertilized in the same way with soluble fertilizers through the irrigation system according to the method described in Misle (2013). LA measured in four cultivars were compared with the estimates from the equation fitted previously.

In all the cases, leaves were placed on a flat surface of white paper for taking photos. LA was later determined by using the software *Compu Eye*, *Leaf & Symptom Area* (Bakr 2005). This method for estimating LA from digital photos has been proven to be highly precise (Tavares-Júnior *et al.* 2002; Lopes *et al.* 2007). Regressions were made for the power equation, evaluating separately W and L. The discriminants for evaluating fits were R^2, the absolute sum of squares, and the standard deviation of residuals, the same indicators, which were used for the final determination of the parameters of Eq. 2. Analysis of variance was performed for evaluating the effect of growing plants from different container sizes at the stage of transplant production and the *Tukey*'s test was used for mean comparisons ($P<0.05$) as needed. When comparing cultivars, the correlation coefficient and the standard deviation of residuals were used to evaluate LA measured and estimated by Eq. 3.

Results

Defining the best linear measure: The analysis of the first data set (75 leaves) indicated that both, W and L, fitted LA estimation. Although both measures exhibited high R^2, the absolute sum of squares as well as the standard deviation of residuals revealed a better fit when using W than L (Fig. 1).

As a consequence, the next steps of the present study employed only W as the linear measure for estimating LA.

Evidence of constancy in a shape: We selected the data presented in Fig. 2 to illustrate the constancy in a shape since it was not possible to include all data collected.

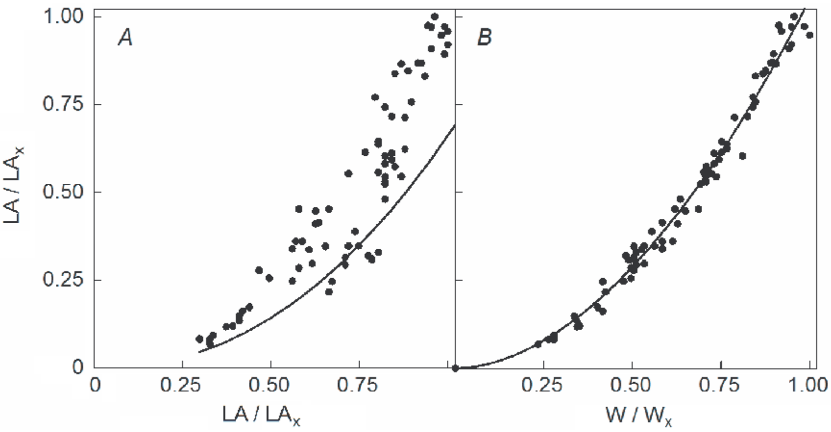

Fig. 1. Allometric curves for (*A*) relative leaf length (L/L_x) and (*B*) relative leaf width (W/W_x) of muskmelon related to relative leaf area (LA/LA_x), for 75 samples obtained 22 d from transplanting. L_x – maximum leaf length; W_x – maximum leaf width; LA_x – maximum leaf area. The fit indicators R^2, absolute sum of squares, and Sy.x, standard deviation of residuals, were: (*A*) 0.9981, 34.89, and 0.6913; (*B*) 0.9854, 0.00876, and 0.03465, respectively.

Each point in Fig. 2 is the mean of 3 leaves sampled. Despite obtaining plants with significantly different sizes, regarding the 72 ml containers (Alvarado 2005), this factor did not affect the relationship between LA and W as all four curves fully overlapped. With respect to the magnitude of LA of the leaves sampled, there were no significant differences when using *ANOVA* ($P \leq 0.05$), which could be visually noted in Fig. 2*E*.

Determining the curve parameters: The invariant shape of curves in Fig. 2 persisted at different stages of the crop, which was evidenced through the accumulation of all data in a single fit of $n = 484$ from the whole crop development cycle. The resulting allometric exponent was $b = 1.893$ with $R^2 = 0.9809$; the absolute sum of squares equaled 0.4584, and the standard deviation of residuals was equal to 0.03084, on relative value basis. Thus, the allometric function properly described the relationship between LA and W, determining the following expression to estimate LA:

$$LA = 0.963\ LA_x \left(\frac{W}{W_x} \right)^{1.89} \tag{3}$$

The maximum attainable LA and W were estimated from the mean value of the highest measures, thus the magnitudes for these constants became $W_x = 15.4$ cm and $LA_x = 174.1$ cm^2. The resulting curve is shown in Fig. 3*A*.

Finally, the correlation between estimated and measured values is shown in Fig. 3*B*.

Model verification in different cultivars: The parameters previously determined with the dataset of cv. Durango in 2004 were employed directly in Eq. 3 to estimate LA of different cultivars, which were compared with measured LA (Table 1). The Eq. 3 produced LA estimates, which highly correlated with the measured values (Niklas *et al.* 2009, Sezer *et al.* 2009).

Discussion

Currently, portable area meters can be used for nondestructive single LA measurements. However, the measurement of the surface area of a large number of leaves is often costly, time-consuming, and destructive. A modeling approach involving the relationships between LA and one or more linear measures of leaves is an inexpensive, rapid, reliable, and nondestructive alternative for the accurate measuring of LA (Lu *et al.* 2004, Olfati *et al.* 2010, Rouphael *et al.* 2010b, Nabi Ilkaee *et al.* 2011). In many studies, the adequacy of the model assumptions for estimating LA has not been examined carefully. Various equations relating L and W to its area have been developed for several cucurbit crops such as cucumber (Blanco and Folegatti 2005, Cho *et al.* 2007, Robbins and Pharr 1987), muskmelon (Chirinos *et al.* 1997, Lopes *et al.* 2007, Misle *et al.* 2004, Nascimento *et al.* 2002, Wu *et al.* 2010), watermelon (Rouphael *et al.* 2010b) and zucchini (Rouphael *et al.* 2006). The presence of the allometry in plants is the advantage contrary to using polynomials, linear or other equations. From our point of view, it is because of the allometry present in plants. Young (2010) developed a model for the leaf shape and postulated that leaf shape is allometric. Several authors have used the power function (da Silva 2000, Schwartz and Kärling 2001, Nascimento *et al.* 2002, de Sousa *et al.* 2005, Galindo and Clavijo 2007, Leroy *et al.* 2007, Antunes *et al.* 2008, Zhang and Liu 2010), but only a few of them talk about allometry either with (Schwartz and Kläring 2001) or without a theoretical consideration (Galindo and Clavijo 2007).

In general, this study supported the findings of previous studies (Rouphael *et al.* 2006, 2007, Peksen 2007, Rivera *et al.* 2007, Olfati *et al.* 2010) on the development of a nondestructive equation for predicting LA using simple linear measurements. Olfati *et al.* (2010) demonstrated that equations with a mere measurement of W were more acceptable for estimating LA of cabbage and broccoli. Based on our present study, L did not

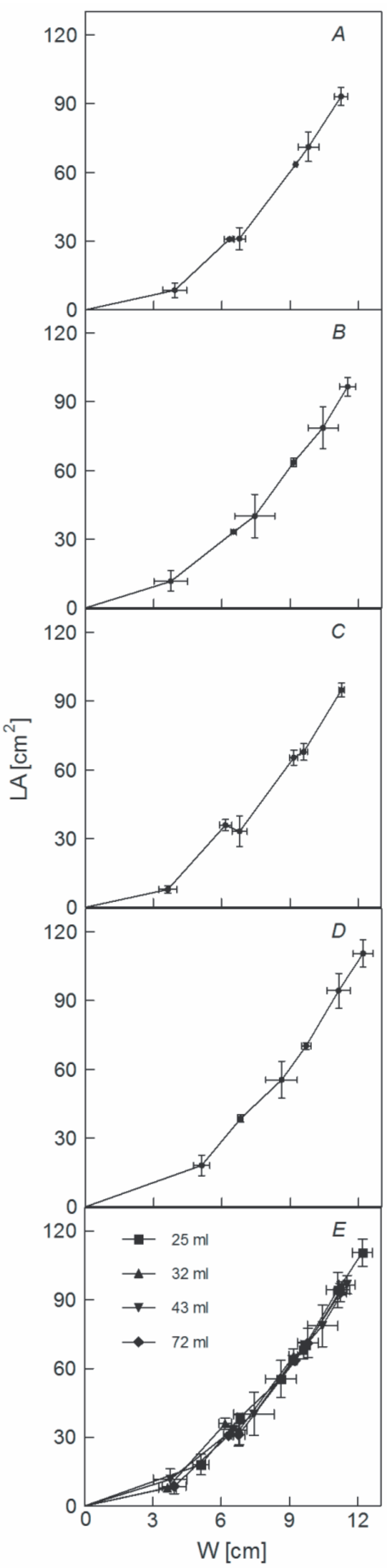

Fig. 2. Relationship between leaf area (LA) and leaf width (W) in plants produced in containers of different size, (*A*) 25 ml, (*B*) 32 ml, (*C*) 43 ml, and (*D*) 72 ml, at 22 d after transplanting. (*E*) All treatments in a single graph overlapping. Standard error bars are indicated.

provide a good measure to estimate LA. It is in contrast with da Silva (2000), Cho *et al.* (2007), and Rivera *et al*. (2007), who established that L is a suitable variable to estimate LA. However, Lopes *et al*. (2007) and Wu *et al*. (2010) studied LA of muskmelon and determined that W was better than L as a linear measure to estimate LA, which was supported also by our evaluation. Similar results were obtained by Rouphael *et al*. (2007) and Maldaner *et al*. (2009) with sunflower, Zhang and Liu (2010) with *Bergenia purpurascens*, and Tsialtas and Maslaris (2005) with sugar beet. Moreover, it should be noted that W was almost 17% higher than L in our leaves, comprising a greater part of LA and suggesting a geometrical advantage for estimating LA. However, measures on leaves of a different shape than those of muskmelon leaves, where L is higher than W, such as cherry leaves, resulted also in a better fit for W (Misle 2007, unpublished data). The reasons lie probably in allometry, since some authors have emphasized the role of the leaf veins in governing the leaf shape when trying to model the shape of the leaf (Young 2010, Abd El-Latif 2011). As a line across the leaf, maximum W covers the main venation of the leaf unlike a line along the midrib (maximum L), a better fit of W is plausible to be found since the allocation of resources is at the base of the allometric theory (West *et al*. 1997). If so, W is a more meaningful measure. At the same time, Niklas *et al*. (2009) confirmed that LA scales with respect to increase in leaf dry mass (M) within and across species as LA $\propto M^{\alpha < 1.0}$ and they advanced an explanation. From their hydraulic argument, it can be observed that the number of hydraulic tissue strands is proportional to lamina breadth, a suggestive support for W as a measure for estimating LA.

The stressful condition evaluated in this work did not affect the relationship between LA and W or LA at all, as all the curves fully overlapped (Fig. 2*E*). However, the stress during transplant production has been reported to affect the whole crop development even until harvest (Alvarado 2005, Hanley and Fegan 2007). In contrast with our results, Meza and Bautista (1999) evaluated linear measures for estimating LA of *Manilkara achras* (Mill.) Fosberg, under transparent plastic film and under a rain shelter. They found that L better fitted for LA in plants growing under transparent plastic, while W better fitted LA in the rain shelter treatment. In this case, the treatment was a permanent growing condition, thus, it was expected to result in plants with differences in their leaf size. It is not obvious why it would cause a shift in the best linear estimator of LA. Similarly, Schwartz and Kläring (2001) tested the possibilities of LA estimation by allometry in tomato stressed by salinity and the model was robust across the treatment factors studied. Blanco and Folegatti (2005) investigated the estimation of LA in cucumber under varying salinity of irrigation water; they concluded that the relationship between L or W to LA was maintained independently of the water salinity, even for grafted plants.

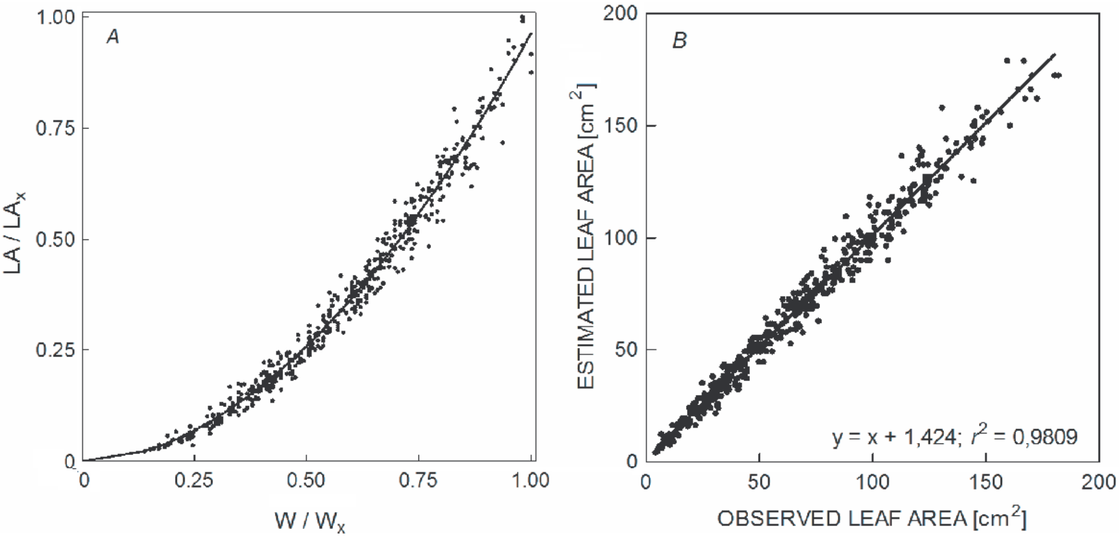

Fig. 3. (*A*) Allometric relationship between relative leaf area (LA/LA$_x$) and relative leaf width (W/W$_x$) in muskmelon. Final fit for determination of parameters A$_0$ and b; *n* = 484 (*see* Eq. 3). (*B*) LA$_x$ – maximum leaf area; W$_x$ – maximum leaf width. Correlation between observed leaf area and estimated leaf area in muskmelon.

Table 1. Correlation parameters between measured and estimated leaf area by Eq. 3 in four cultivars of muskmelon. *r* = correlation coefficient, Sy.x – standard deviation of residuals.

Cultivar	Slope	Intercept	r	Sy.x
Durango	1.043 ± 0.05608	0.8320 ± 4.237	0.9618	2.828
Colima	1.050 ± 0.05286	–0.4424 ± 5.879	0.9663	6.200
Early Brew	1.069 ± 0.06481	2.707 ± 6.111	0.9522	5.557
Zeus	1.006 ± 0.07835	13.33 ± 7.953	0.9246	7.528

With regard to the magnitude of the parameters, our results supported those of Nascimento *et al.* (2002) who determined similar constants and, notably, the same allometric exponent despite having studied a yellow cultivar, belonging to a different group than Durango. Meanwhile, Wu *et al.* (2010) found b = 2 for three different cultivars. It must be noted that the regression in Fig. 3*A* contains data from different treatments, different leaf sizes, and from different stages during the crop development. Durango has been previously employed in LA studies (Chirinos *et al.* 1997, Misle *et al.* 2004). In our test on cultivars, Durango was the hybrid used first for determining the parameters of Eq. 3. Colima was a cultivar genetically close to Durango, thus, it was expected that they both rate slightly higher correlation using Eq. 3 than Early Brew or Zeus, both produced by different companies. Wu *et al.* (2010) investigated an equation to estimate LA in three muskmelon cultivars and W fitted better to all of them. Similarly, Schwartz and Kläring (2001) studied 16 genotypes of *Lycopersicon*, including wild species; they verified the applicability of the allometric relationship, but a general calculation based on L or W for all genotypes was not possible. Similarly, Kumar and Sharma (2010) obtained a linear equation for different genotypes of clary sage and Nabi Ilkaee *et al.* (2011) of soybean.

A considerable body of research supports the use of the power function for estimating LA, whether or not declared as allometry (da Silva 2000, Schwartz and Kärling 2001, Nascimento *et al.* 2002, Galindo and Clavijo 2007, Leroy *et al.* 2007, Antunes *et al.* 2008, Zhang and Liu 2010).

Conclusions: The allometric function allowed to relate properly a linear measure of the muskmelon leaf with LA, an important estimate in experiments, as well as under field conditions for commercial purposes. Leaf W was a more appropriate measure to obtain LA by allometry compared to leaf L. The allometric exponent determined was b = 1.89. The maximum attainable LA and W were estimated as W$_x$ = 15.4 cm and LA$_x$ = 174.1 cm^2. In our study, this relationship was not affected by a treatment concerning the seedling stage (container size), crop age, leaf size or cultivar.

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