

Leaf area estimation in triticale by leaf dimensions

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ABSTRACT: The objective of this study was to estimate the leaf area of triticale in function of linear dimensions from flags and other (non-flag) leaves. An experiment was conducted with the IPR111 cultivar in the 2016 agricultural year. At 93 days after sowing, 400 leaves were collected in order to generate the mathematical models of leaf area estimation in function of linear leaf dimensions. A total of 200 leaves were collected at 106 days after sowing in order to validate the models. In each of the 600 leaves, the length (L) and the width (W) were measured, and the product of length times width (L×W) and the ratio between length and width (L/W) were estimated. Afterwards, the leaves were digitized and the real leaf area determined by means of digital images. Linear, quadratic and power models were generated and validated for the estimation of the real leaf area (Y). The morphology of flag and other (non-flag) leaves is distinct and, thus, leaf area estimation models should be generated for each leaf type. In triticale, the most precise models of leaf area estimation are those that use L×W as the explanatory variable.

Key words: image processing; mathematical models; × *Triticosecale Wittmack*

Estimação da área foliar de triticale por dimensões foliares

RESUMO: O objetivo deste trabalho foi estimar a área foliar de triticale em função de dimensões lineares de folhas bandeiras e das demais folhas. Foi conduzido um experimento com a cultivar IPR111 no ano agrícola de 2016. Aos 93 dias após a semeadura foram coletadas 400 folhas para a geração de modelos matemáticos de estimação de área foliar em função das dimensões lineares das folhas. Aos 106 dias após a semeadura foram coletadas 200 folhas destinadas à validação dos modelos. Em cada uma das 600 folhas, foram mensurados o comprimento (L) e a largura (W) e estimados o produto comprimento vezes largura (L×W) e a razão comprimento largura (L/W). A seguir, as folhas foram digitalizadas e a área foliar real determinada por meio de imagens digitais. Foram gerados e validados modelos do tipo linear, quadrático e potência para a estimação da área foliar real (Y). A morfologia das folhas bandeiras e das demais folhas é distinta e, portanto, modelos de estimação de área foliar devem ser gerados para cada tipo de folha. Em triticale, os modelos mais precisos de estimação da área foliar são aqueles que utilizam L×W como variável explicativa.

Palavras-chave: processamento de imagem; modelos matemáticos; × *Triticosecale Wittmack*

Introduction

The triticale (\times *Triticosecale* Wittmack) is a hybrid cereal originated from the crossing between wheat (*Triticum* sp.) and rye (*Secale* sp.), aiming to combine the growth ability under unfavorable conditions from rye with the food use versatility from wheat (Zhu, 2018). According to the author, triticale was initially used mainly as animal feed and currently exhibits potential in human diets as a complementary cereal in several foods and beverages. According to data from the Conab (2019), the Brazilian production of triticale was 53.9 thousand tons in the 2018 agricultural year in an area of 19.9 thousand hectares and productivity of 2709 kg ha⁻¹. The Brazilian southern region produced 40.7 thousand tons of the grain in 2018.

Leaf area modeling is important in biological and agronomic studies because it is a yield indicative of a given crop, as coffee, being directly related to physiological processes, especially photosynthesis (Favarin et al., 2002). In general, the productivity tends to be greater when the plant quickly reaches the leaf area peak and remain at that level for the longest possible period (Alvim et al., 2010). According to Liu et al. (2018), the leaf size is an important factor that contributes to the photosynthetic capability, affecting various agronomic traits while the flag leaves contribute significantly to the grain yield in wheat. In this sense, Tang et al. (2018) pointed out that leaf size is the major determinant of the architecture and productive potential of many crops, including cereals. The authors identified 14 and 9 quantitative trait loci (QTLs), respectively, for flag leaf length and flag leaf width in rice and concluded that knowledge of this information is important for increasing yield in breeding programs. QTLs were also identified by controlling flag leaf length, width, area and angle of wheat (Liu et al., 2018; Zhao et al., 2018). Liu et al. (2018) observed positive and significant correlations between flag leaf length, width and area with kernel number per spike and kernel weight per spike. Positive and significant correlations between flag leaf-related traits and yield-related traits were also observed by Zhao et al. (2018), who affirmed that an appropriate flag leaf size could benefit the formation of a high yield potential.

Evaluating five cultivars of oats, wheat and rice in three seasons, Al-Tahir (2014) verified a positive and significant relationship between grain yield and leaf area of the flag leaf. The removal of the flag leaf at the beginning of wheat ear formation contributes to the reduction of 9%, 10.7% and 11.1% of grain weight, grain yield and number of grains per ear, respectively (Souza et al., 2013). Furthermore, in wheat cultivars, Hashim et al. (2017) verified the reduction of 7.04% to 35.29% of the productive traits, such as spike length, spikelet number per spike, number of grains per spike, grain weight and spike yield due to the early removal of the flag leaf. Wu et al. (2017) indicate that the flag leaf is the main photosynthetic organ with a key role in grain yield of rice and according to the authors, 106 loci associated with the morphology (length and width) of the flag leaf were identified.

According to Kara (2016), some physiological traits of flag leaf from triticale have a positive correlation with each other, such as transpiration rate with the leaf temperature, stomatal conductance and transpiration rate, stomatal conductance with net photosynthesis rate and mesophyll conductance with net photosynthesis rate during booting, anthesis and grain filling stages. However, data presented by the author indicate that the direction and magnitude of the correlations between physiological traits of flag leaf and grain yield oscillate between the different stages of evaluation, not being possible to indicate a single physiological trait that presents significant correlations with grain yield in all growth stages.

There are direct and indirect methods for leaf area determination of a crop. The leaf area estimation in function of linear leaf dimensions can be performed through indirect methods, using mathematical models. In this case, leaves are collected, linear dimensions are measured and real leaf area is determined. The leaf area modeling is based on one or more linear dimensions from the leaves, as well as the validation of the generated models. These models can be used to determine the crop leaf area in the field, maintaining the plant integrity and allowing the monitoring of the crop growth and development. Mathematical models for leaf area estimation in function of length, width or product of length \times width have already been developed for coffee (Favarin et al., 2002), hazelnut (Cristofori et al., 2007), eggplant (Hinnah et al., 2014), rose (Rouphael et al., 2010), crambe (Toebe et al., 2010), Aruana grass (Galzerano et al., 2012), forage turnip (Cargnelutti Filho et al., 2012), snap bean (Toebe et al., 2012a), jack bean (Toebe et al., 2012b), gladiolus (Schawb et al., 2014), pigeon pea (Cargnelutti Filho et al., 2015a) and canola (Cargnelutti Filho et al., 2015b), among other crops.

Due to the importance of the leaf area and mainly the contribution of the flag leaf on grain yield in cereals, numerous studies like those mentioned above seek to identify QTLs associated with leaf morphology. For that matter, precise modeling of the leaf area assumes a relevant support role in this area of science. In general, the models of leaf area estimation available in the literature for crops from the Poaceae family do not distinguish between flag and other (non-flag) leaves. However, the relations between leaf dimensions (length and width) and morphology between flag and other (non-flag) leaves are likely to be distinct and therefore require specific models for the correct prediction of the real leaf area. Thus, the objective of this study was to estimate the leaf area of triticale in function of linear dimensions of flags and non-flag (all other plant leaves) leaves.

Material and Methods

An experiment was carried out with triticale IPR111 cultivar in the experimental area from the Federal University of Pampa (Fundação Universidade Federal do Pampa - UNIPAMPA) campus Itaqui, located in the geographical coordinates of 29°09'S latitude, 56°33'W longitude and 74 m of altitude. According to Köppen climate classification, the

climate of the region is Cfa, humid subtropical without dry season defined (Wrege et al., 2012) and the soil is classified as "Haplic Plinthosol" (Santos et al., 2013).

Seeding procedure was performed on June 3, 2016, with spacing of 0.17 m between rows and final plant population in the harvest of 1294117 plants ha⁻¹. The experimental area consisted of 800 m² (84 rows of 56 m with spacing of 0.17 m between rows). Basic fertilization was carried out with 20 kg ha⁻¹ of N, 80 kg ha⁻¹ of P₂O₅ and 80 kg ha⁻¹ of K₂O. Moreover, two top dressing fertilizations were performed according to cropping recommendations presented by the CQFS (2004), totaling 80 kg ha⁻¹ of N.

At 93 days after sowing, a total of 200 flag leaves and 200 non-flag (all other plant leaves) leaves were collected for generating mathematical models of leaf area estimation in function of the linear dimensions from the triticale leaves. At 106 days after sowing, 100 flag leaves and 100 other (non-flag) leaves were collected for the validation of these models. Sampling at 93 and 106 days after sowing were performed at random within the experimental area, with destruction of the collected plant in the time of evaluation. Length (L) and width (W) of the leaf blade were measured with a millimeter ruler in each of the 600 leaves. Afterwards, the product of length times width (L×W), the ratio between length and width (L/W) were estimated and the real leaf area from each leaf was determined by digital image processing. For this, the leaves were put in sequence in an EPSON brand scanner, Perfection V33/V330 model and scanned in 200 dpi resolution. Then, the digital images were processed with Digimizer v.4.5.2[®] Software (Medcalc Software, 2015) for real leaf area determination.

Measures of central tendency, dispersion and distribution for L, W, L×W, L/W and real leaf area (Y) of the flag and non-flag (others) leaves used for model generation and validation were calculated. Frequency histograms and scatter plots between L, W, L×W, L/W and Y were constructed for flag and other leaves used in the generation of the mathematical models. The mean values of L, W, L×W, L/W and Y were compared between flag and other leaves by means of the t-test for independent samples. Based on the 200 flag leaves and 200 other (non-flag) leaves, models of linear ($Y = a + bx$), quadratic ($Y = a + bx + cx^2$) and power ($Y = ax^b$) types were generated for the estimation of the real leaf area (Y). In these models, x represented the leaf linear dimension (L, W or L×W). In addition to that, general models based on the 400 leaves [flags and other (non-flag) leaves] were generated. Linear, quadratic and power models have already been generated and validated in various agricultural crops (Rouphael et al., 2010; Toebe et al., 2010; Cargnelutti Filho et al., 2012; Galzerano et al., 2012; Toebe et al., 2012a; Toebe et al., 2012b; Hinnah et al., 2014; Schawb et al., 2014; Cargnelutti Filho et al., 2015a; Cargnelutti Filho et al., 2015b).

In the generated models using the product of length times width of the leaf, the diagnosis of collinearity was performed, based on the Variance Inflation Factor: $VIF = 1 / (1 - r^2)$ (Cristofori et al., 2007) and in the Tolerance $T = 1 / VIF$ (Rouphael et al., 2010; Toebe & Cargnelutti Filho, 2013),

where r^2 is the coefficient of determination from the linear regression between L and W. Moreover, the case of $VIF > 10$ and $T < 0.10$ is considered as severe collinearity and the use of the two variables (length and width) is not recommended in the model generation. Therefore, one of the variables should be eliminated, as described by Cristofori et al. (2007), Rouphael et al. (2010) and Toebe & Cargnelutti Filho (2013).

The validation of the leaf area estimation models was performed based on the 100 estimated values of leaf area by the model (\hat{Y}_i) and the 100 observed values (Y_i) of real leaf area for the models of flag and other (non-flag) leaves, separately. In the general models, the 200 observed values and the 200 estimated values by the models were compared. In each case, a simple linear regression ($\hat{Y}_i = a + bY_i$) of the leaf area estimated by the model (dependent variable) was fitted as a function of the observed leaf area (independent variable). The hypotheses $H_0: a = 0$ versus $H_1: a \neq 0$ and $H_0: b = 1$ versus $H_1: b \neq 1$ were tested using the Student t-test at 5% probability. Then, the Pearson linear correlation coefficient (r) and the coefficient of determination (R^2) between \hat{Y}_i and Y_i were calculated. For each model, the mean absolute error (MAE), the root means square error (RMSE) and the Willmott index of agreement (d) (Willmott, 1981) were also calculated as indicated by Cargnelutti Filho et al. (2012; 2015a, b).

In order to select the best models of triticale leaf area estimation in function of L, W or L×W, the following criteria were used in the validation of the models: linear coefficient not different from zero, angular coefficient not different from one, Pearson linear correlation coefficient and coefficient of determination closer to one, mean absolute error and root means square error closer to zero and Willmott (1981) index (d) closer to one, according to recommendations of Cargnelutti Filho et al. (2012; 2015a, b). Statistical analyzes were performed using Microsoft Office Excel[®] software and Statistica 12.0[®] software (Statsoft, 2015).

Results and Discussion

The high difference of the minimum and maximum values of flag and other (non-flag) leaves in different evaluated variables ($7.80 \text{ cm} \leq \text{length} \leq 37.00 \text{ cm}$, $0.90 \text{ cm} \leq \text{width} \leq 2.00 \text{ cm}$, $7.80 \text{ cm}^2 \leq \text{length} \times \text{width} \leq 67.20 \text{ cm}^2$, $7.80 \leq \text{length/width ratio} \leq 26.00$, and $5.47 \text{ cm}^2 \leq \text{real leaf area} \leq 50.86 \text{ cm}^2$) indicates wide variability of the size and shape from collected leaves (Table 1). In both flag and other (non-flag) leaves used for generation and validation of the estimation models of leaf area, the mean and median values were similar, indicating proper fitting to the normal distribution.

The coefficient of variation was higher for L×W and Y (Table 1) when compared to the values of the other variables (L, W and L/W), similar to the results observed by Cargnelutti Filho et al. (2015a) in pigeon pea and by Toebe et al. (2012a) in jack bean. The high values from coefficients of variation occurred because of the great data variability, which contributes to obtaining models with a wide use spectrum. Thus, we can affirm that the collected data are adequate for the construction of a leaf area model representative of the crop.

Table 1. Number of leaves (n), minimum (Min.), maximum (Max.), mean, median (Med.), variance (Var.), coefficient of variation (CV, in %), asymmetry (Asym.), kurtosis (Kurt.) and p-value of the Kolmogorov-Smirnov normality test for variables evaluated on flag and other (non-flag) leaves collected respectively in the first and second periods for the generation and validation of leaf area models of Triticale (*× Triticosecale* Wittmack) IPR111 cultivar.

Variable (unit.)	n	Min.	Max.	Mean ⁽¹⁾	Med.	Var.	CV (%)	Asym. ⁽²⁾	Kurt. ⁽³⁾	P-value
Collection at 93 days after sowing – Flag leaves used in the generation of the model										
Length (cm)	200	9.70	34.40	19.59b	19.00	25.68	25.87	0.45*	-0.04 ^{ns}	0.21
Width (cm)	200	0.90	2.00	1.48a	1.50	0.06	17.24	-0.05 ^{ns}	-0.59 ^{ns}	0.11
Length × Width (cm ²)	200	8.73	67.20	29.98b	28.20	146.07	40.31	0.63*	0.00 ^{ns}	0.10
Length / Width ratio	200	8.44	20.25	13.17b	12.93	4.31	15.75	0.53*	0.38 ^{ns}	0.22
Real leaf area (cm ²)	200	5.89	50.49	21.88b	20.46	81.61	41.28	0.73*	0.22 ^{ns}	0.10
Collection at 93 days after sowing – Others (non-flag) leaves used in the generation of the model										
Length (cm)	200	15.30	37.00	26.52a	26.15	18.28	16.12	0.02 ^{ns}	-0.29 ^{ns}	0.66
Width (cm)	200	0.90	1.90	1.38b	1.40	0.04	15.15	-0.01 ^{ns}	-0.28 ^{ns}	0.01
Length × Width (cm ²)	200	15.30	65.34	37.13a	36.13	106.30	27.77	0.30 ^{ns}	-0.17 ^{ns}	0.66
Length / Width ratio	200	11.44	26.00	19.38a	19.33	6.55	13.20	0.02 ^{ns}	0.09 ^{ns}	1.00
Real leaf area (cm ²)	200	11.38	50.86	29.44a	28.34	63.57	27.08	0.26 ^{ns}	-0.23 ^{ns}	0.35
Collection at 93 days after sowing - Flag and others (non-flag) leaves used in the generation of the model										
Length (cm)	400	9.70	37.00	23.06	23.35	33.97	25.28	-0.04 ^{ns}	-0.61*	0.61
Width (cm)	400	0.90	2.00	1.43	1.40	0.06	16.66	0.08 ^{ns}	-0.40 ^{ns}	0.00
Length × Width (cm ²)	400	8.73	67.20	33.55	32.36	138.65	35.09	0.31*	-0.31 ^{ns}	0.40
Length / Width ratio	400	8.44	26.00	16.28	16.11	15.07	23.85	0.22 ^{ns}	-0.90*	0.01
Real leaf area (cm ²)	400	5.89	50.86	25.66	25.08	86.72	36.29	0.28*	-0.40 ^{ns}	0.49
Collection at 106 days after sowing - Flag leaves used in the validation of the model										
Length (cm)	100	7.80	28.60	16.45b	15.20	20.37	27.44	0.59*	-0.13 ^{ns}	0.07
Width (cm)	100	0.90	2.00	1.32a	1.30	0.06	19.12	0.71*	-0.02 ^{ns}	0.02
Length × Width (cm ²)	100	7.80	56.20	22.65b	20.65	105.50	45.34	1.10*	0.97*	0.21
Length / Width ratio	100	7.80	17.73	12.38b	12.50	4.42	16.99	0.15 ^{ns}	-0.41 ^{ns}	0.96
Real leaf area (cm ²)	100	5.47	39.66	16.31b	14.50	55.81	45.80	1.07*	0.76 ^{ns}	0.08
Collection at 106 days after sowing - Others (non-flag) leaves used in the validation of the model										
Length (cm)	100	13.60	34.60	23.98a	24.10	19.60	18.46	-0.18 ^{ns}	-0.33 ^{ns}	0.92
Width (cm)	100	0.90	1.80	1.29a	1.30	0.04	15.98	0.30 ^{ns}	-0.31 ^{ns}	0.06
Length × Width (cm ²)	100	13.60	58.82	31.37a	31.29	92.47	30.65	0.45 ^{ns}	-0.17 ^{ns}	0.47
Length / Width ratio	100	9.93	24.55	18.79a	18.76	8.73	15.72	-0.32 ^{ns}	-0.06 ^{ns}	0.97
Real leaf area (cm ²)	100	10.22	45.06	24.64a	24.00	53.46	29.68	0.36 ^{ns}	-0.26 ^{ns}	0.81
Collection at 106 days after sowing – Flag and others (non-flag) leaves used in the validation of the model										
Length (cm)	200	7.80	34.60	20.22	20.10	34.14	28.90	0.07 ^{ns}	-0.88*	0.08
Width (cm)	200	0.90	2.00	1.30	1.30	0.05	17.68	0.62*	0.10 ^{ns}	0.00
Length × Width (cm ²)	200	7.80	58.82	27.01	26.12	117.60	40.14	0.55*	-0.23 ^{ns}	0.32
Length / Width ratio	200	7.80	24.55	15.58	15.00	16.89	26.37	0.25 ^{ns}	-0.94*	0.15
Real leaf area (cm ²)	200	5.47	45.06	20.47	19.45	71.78	41.38	0.45*	-0.47 ^{ns}	0.46

⁽¹⁾ Means of flag and other (non-flag) leaves for a given variable followed by the same letter do not differ statistically by the t test for two independent samples via bootstrap resampling with 2000 simulations. ⁽²⁾*Asymmetry differs from zero by the t-test at 5% probability level. ⁽³⁾*Kurtosis differs from zero by t-test at 5% probability level. ^{ns}=Non-significant.

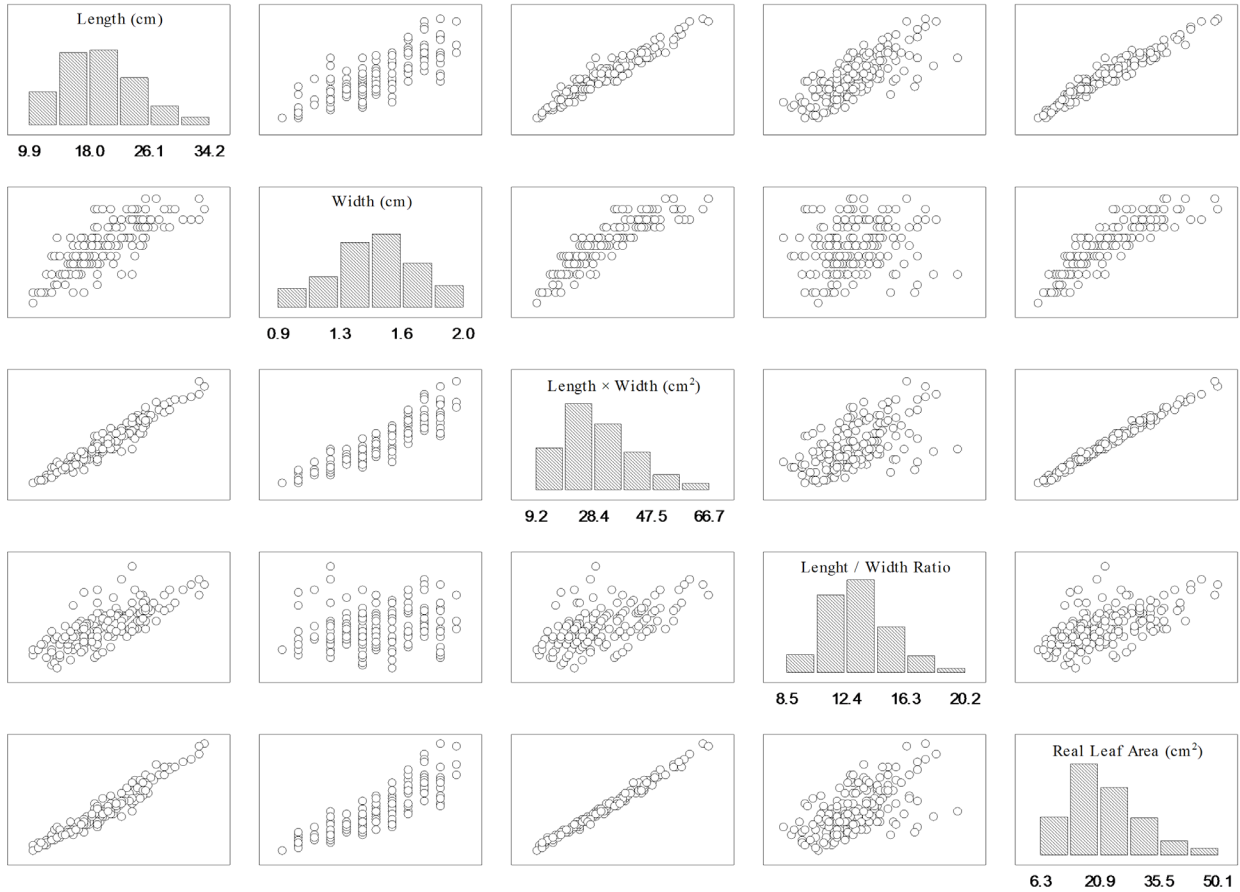
The flag leaves collected for the model generation and validation exhibited some significant deviations, especially regarding the positive asymmetry, as some collected leaves were considerably superior to the other leaves (Table 1 and Figure 1a). In the assessment of other (non-flag) leaves collected for model generation and validation, symmetric data, mesokurtic kurtosis and proper fitting to the normal distribution were observed (Table 1 and Figure 1b).

The flag and other (non-flag) leaves differed statistically ($p < 0.05$) when compared to each other. The non-flag leaves displayed greater length and smaller width and greater length/width ratio in relation to the flag leaves (Table 1 and Figure 2 a, b). For flag leaves, the VIF was of 2.79 and the tolerance index was 0.36 between the variables leaf length and width. Meanwhile, for other (non-flag) leaves, the VIF was

of 1.68 and the tolerance was 0.60. For the general data (flag and other leaves), the VIF was of 1.26 and the tolerance was 0.80, indicating no existence of serious collinearity problems (Cristofori et al., 2007; Roupael et al., 2010; Toebe & Cargnelutti Filho, 2013) between length and width. Therefore, the two linear dimensions can be used jointly in triticale leaf area estimation models.

Based on the 400 leaves used to generate the models for both flag and other leaves, greater precision was observed in the linear, quadratic and power models generated using the length × width product due to they having a coefficient of determination greater than 0.97 (Table 2). Thus, the most accurate models for predicting triticale leaf area are those that use two leaf dimensions for the appropriate leaf area prediction. Galzerano et al. (2012), Bianco et al. (2001) and

A. Data of 200 flags leaves used in the generation of leaf area models of Triticale



B. Data of 200 others (non-flags) leaves used in the generation of leaf area models of Triticale

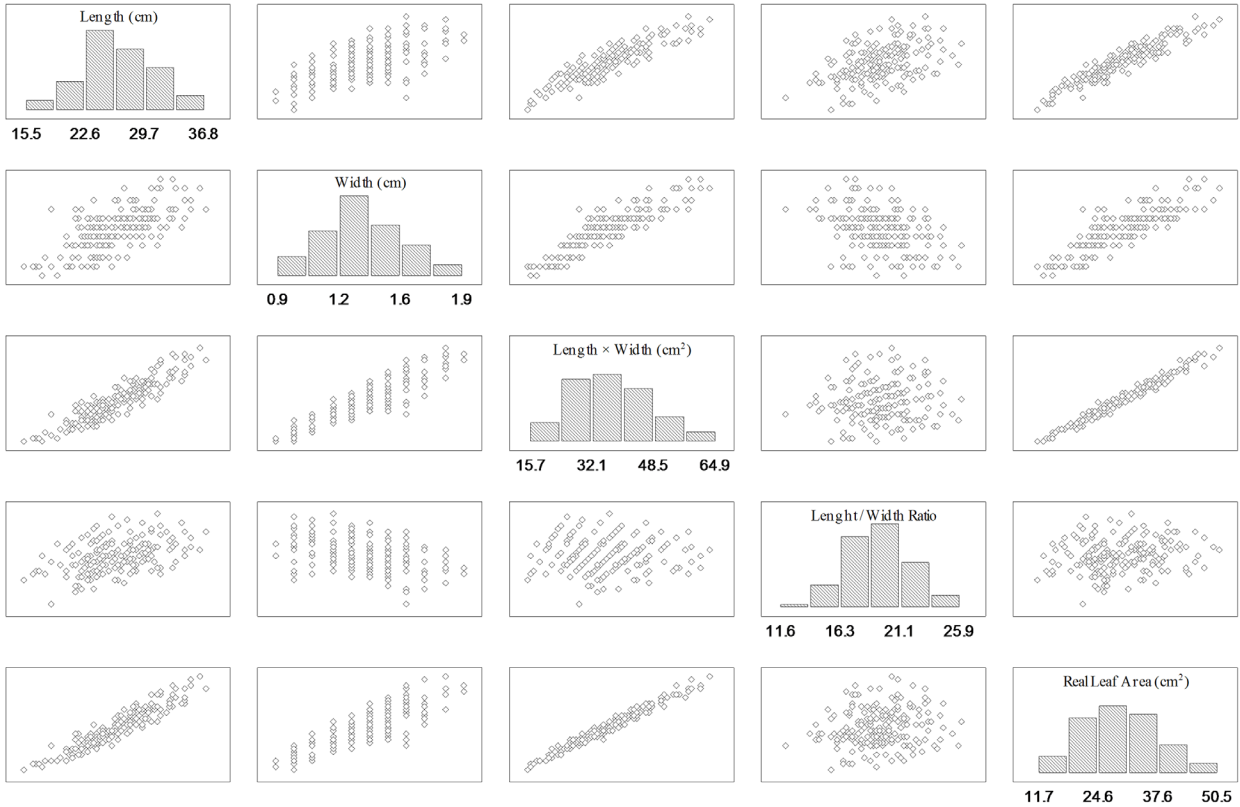


Figure 1. Matrix with frequency histograms (diagonal) and scatter plots of length (cm), width (cm), length versus width (cm²), ratio between length and width (units) and real leaf area (cm²) from 200 flag leaves (A) and 200 other (non-flag) leaves (B) collected at 93 days after sowing and used in the generation of leaf area models of Triticale (*Triticosecale* Wittmack) IPR111 cultivar.

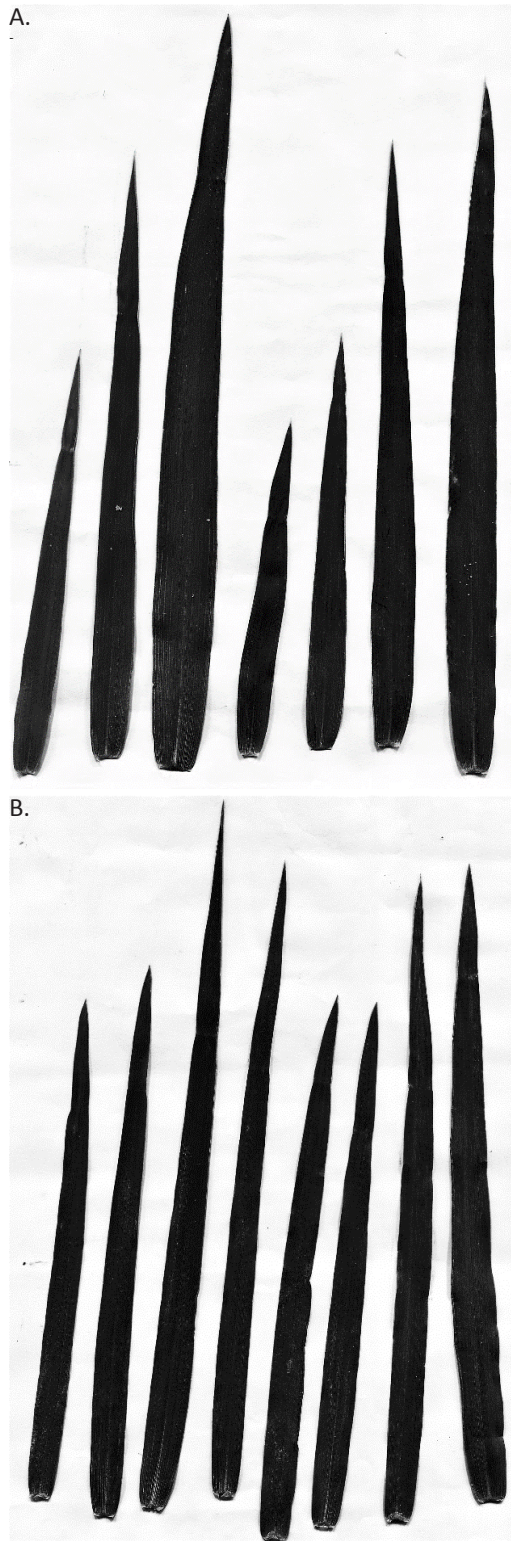


Figure 2. Variability in size and leaf shape in flag (A) and other (non-flag) leaves (B) used in the generation of leaf area models of Triticale (*x Triticosecale* Wittmack) IPR111 cultivar.

Silva et al. (2013) respectively studied the crops Aruana grass, *Panicum maximum* and xaraes and massai grasses and also observed a better equation fitting with the use of two dimensions from the leaf blade.

In order to estimate the leaf area of flag leaves, the linear ($\hat{Y} = -0.4088 + 0.7435x$; $R^2 = 0.98$), quadratic ($\hat{Y} = 0.7992 +$

$0.6602x + 0.0012x^2$; $R^2 = 0.98$) and power ($\hat{Y} = 0.7212x^{1.0029}$; $R^2 = 0.98$) models can be utilized, where x represents the length \times width product. All these models present validation criteria superior to the other models generated from a single leaf dimension (Table 3), i.e., linear coefficient not different from zero, angular coefficient not different from one, Pearson linear correlation coefficient (r) and coefficient of determination (R^2) closer to one, mean absolute error (MAE) and root means square error (RMSE) closer to zero and Willmott index (d) closer to one, among the real values observed and the values predicted by the model. This means that when the real leaf area is zero, the one estimated by the model will also be close to zero. The model will also estimate the increase close to 1:1 for each unit of real area increase. The prediction indicators will be high (r , R^2 , MAE, RMSE and d) and, therefore, these models should be used. Other models, such as Model 6 ($\hat{Y} = 8.8859x^{2.1717}$ – Table 2), are not recommended because when the real leaf area is zero, the estimated leaf area will be greater than zero ($a = 2,541$ – Table 3) and when the leaf area increases by 1 cm, the estimated leaf area will increase by only 0,891cm ($b = 0,891$).

To estimate the leaf area of other (non-flag) leaves, the linear ($\hat{Y} = 1.1000 + 0.7633x$; $R^2 = 0.97$), quadratic ($\hat{Y} = -0.1144 + 0.8310x - 0.0009x^2$; $R^2 = 0.97$) and power ($\hat{Y} = 0.8945x^{0.9668}$; $R^2 = 0.97$) models can be used, where x represents the length \times width product. All of these models exhibited superior validation (Table 3), i.e., linear coefficient not different from zero, angular coefficient not different from one, correlation and coefficient of determination between the real and estimated leaf area closer to one, along with the lowest values of MAE and RMSE and greater scores of the Willmott d -index of agreement. In general, the models for flag leaves exhibited superior fitting in relation to the models generated from other leaves.

In order to estimate the leaf area of flag and other leaves altogether, the linear ($\hat{Y} = -0.5138 + 0.7801x$; $R^2 = 0.97$), quadratic ($\hat{Y} = -1.1491 + 0.8206x - 0.0006x^2$; $R^2 = 0.97$) and power ($\hat{Y} = 0.6855x^{1.0302}$; $R^2 = 0.97$) models can be utilized, where x represents the length \times width product. All these models exhibited higher validation (Table 3). However, we should note that these models were generated from flag and other (non-flag) leaves with different leaf morphology (Table 1). Therefore, we recommend the use of specific models for each type of leaf.

In all three cases (flag leaves, other leaves and flag and other leaves altogether), the three types of models (linear, quadratic and power) based on the $L \times W$ product showed higher fitting and all could be used to estimate the triticale leaf area with good prediction (Tables 2 and 3). However, considering the simplicity and good fit of linear models, the $\hat{Y} = -0.4088 + 0.7435x$; $R^2 = 0.9893$ model is recommended to estimate the real leaf area of flag leaves and the $\hat{Y} = 1.1000 + 0.7633x$; $R^2 = 0.9743$ model for estimating the real leaf area of other (non-flag) leaves of triticale, where x represents the $L \times W$ product. Linear models using the $L \times W$ product were also recommended for the estimation of real

Table 2. Linear, quadratic and power models for real leaf area determination (\hat{Y}) of flag and other (non-flag) leaves of Triticale (\times *Triticosecale* Wittmack) IPR111 cultivar in function of linear leaf dimensions and coefficient of determination (R^2) of each model.

Number and type of model	Independent variable (x)	Model	R ²
Generated models based on 200 flag leaves			
1) Linear	Length	$\hat{Y} = - 12.0240 + 1.7308x$	R ² = 0.9428
2) Quadratic	Length	$\hat{Y} = - 4.6197 + 0.9680x + 0.0184x^2$	R ² = 0.9475
3) Power	Length	$\hat{Y} = 0.1942x^{1.5768}$	R ² = 0.9499
4) Linear	Width	$\hat{Y} = - 24.5000 + 31.382x$	R ² = 0.7837
5) Quadratic	Width	$\hat{Y} = 7.8446 - 13.9460x + 15.4070x^2$	R ² = 0.8007
6) Power	Width	$\hat{Y} = 8.8859x^{2.1717}$	R ² = 0.8324
7) Linear	Length \times Width	$\hat{Y} = - 0.4088 + 0.7435x$	R ² = 0.9893
8) Quadratic	Length \times Width	$\hat{Y} = 0.7992 + 0.6602x + 0.0012x^2$	R ² = 0.9899
9) Power	Length \times Width	$\hat{Y} = 0.7212x^{1.0029}$	R ² = 0.9886
Generated models based on 200 others (non-flag) leaves			
10) Linear	Length	$\hat{Y} = - 16.8190 + 1.7441x$	R ² = 0.8746
11) Quadratic	Length	$\hat{Y} = - 7.3883 + 1.0157x + 0.0137x^2$	R ² = 0.8762
12) Power	Length	$\hat{Y} = 0.1442x^{1.6174}$	R ² = 0.8824
13) Linear	Width	$\hat{Y} = - 14.8140 + 32.1030x$	R ² = 0.7069
14) Quadratic	Width	$\hat{Y} = - 14.4070 + 31.4980x + 0.2196x^2$	R ² = 0.7069
15) Power	Width	$\hat{Y} = 17.4540x^{1.5655}$	R ² = 0.7258
16) Linear	Length \times Width	$\hat{Y} = 1.1000 + 0.7633x$	R ² = 0.9743
17) Quadratic	Length \times Width	$\hat{Y} = - 0.1144 + 0.8310x - 0.0009x^2$	R ² = 0.9746
18) Power	Length \times Width	$\hat{Y} = 0.8945x^{0.9668}$	R ² = 0.9757
Generated models based on 400 flag and others (non-flag) leaves			
19) Linear	Length	$\hat{Y} = - 9.0902 + 1.5073x$	R ² = 0.8898
20) Quadratic	Length	$\hat{Y} = - 2.4919 + 0.8920x + 0.0134x^2$	R ² = 0.8932
21) Power	Length	$\hat{Y} = 0.3056x^{1.4041}$	R ² = 0.9130
22) Linear	Width	$\hat{Y} = - 12.8380 + 26.9560x$	R ² = 0.4744
23) Quadratic	Width	$\hat{Y} = - 16.0940 + 31.6110x - 1.6186x^2$	R ² = 0.4745
24) Power	Width	$\hat{Y} = 13.5900x^{1.6426}$	R ² = 0.4884
25) Linear	Length \times Width	$\hat{Y} = - 0.5138 + 0.7801x$	R ² = 0.9729
26) Quadratic	Length \times Width	$\hat{Y} = - 1.1491 + 0.8206x - 0.0006x^2$	R ² = 0.9730
27) Power	Length \times Width	$\hat{Y} = 0.6855x^{1.0302}$	R ² = 0.9761

Table 3. Linear (a), angular (b), Pearson linear correlation (r) and determination (R^2) coefficients obtained in the fitted linear regression between the estimated leaf area (dependent variable) and the real leaf area (independent variable). Mean absolute error (MAE), root mean square error (RMSE) and Willmott d-index of agreement (Willmott, 1981) were calculated based on the estimated and real leaf areas of flag and other (non-flag) leaves of Triticale (\times *Triticosecale* Wittmack) IPR111 cultivar in function of the leaf linear dimensions and different types of models.

Number and type of model	Independent variable (x)	Model validation indicators						
		a ⁽¹⁾	b ⁽²⁾	r ⁽³⁾	R ²	MAE	RMSE	d
Generated models based on 200 flag leaves								
1) Linear	Length	0.084 ^{ns}	1.003 ^{ns}	0.959*	0.920	1.821	2.208	0.979
2) Quadratic	Length	1.202*	0.947*	0.965*	0.931	1.610	1.994	0.982
3) Power	Length	1.315*	0.937*	0.966*	0.934	1.562	1.944	0.982
4) Linear	Width	1.446 ^{ns}	0.954 ^{ns}	0.898*	0.807	2.811	3.577	0.944
5) Quadratic	Width	2.476*	0.911*	0.899*	0.809	2.742	3.525	0.943
6) Power	Width	2.541*	0.891*	0.901*	0.812	2.598	3.393	0.946
7) Linear	Length \times Width	-0.133 ^{ns}	1.015 ^{ns}	0.993*	0.987	0.618	0.877	0.997
8) Quadratic	Length \times Width	0.258 ^{ns}	0.996 ^{ns}	0.993*	0.986	0.638	0.898	0.996
9) Power	Length \times Width	0.219 ^{ns}	0.997 ^{ns}	0.993*	0.987	0.609	0.865	0.997
Generated models based on 200 others (non-flag) leaves								
10) Linear	Length	1.355 ^{ns}	0.960 ^{ns}	0.909*	0.826	2.718	3.252	0.951
11) Quadratic	Length	2.439*	0.920 ^{ns}	0.913*	0.834	2.554	3.092	0.954
12) Power	Length	2.613*	0.909*	0.915*	0.838	2.474	3.025	0.955
13) Linear	Width	7.791*	0.758*	0.840*	0.706	3.327	4.395	0.897
14) Quadratic	Width	7.808*	0.757*	0.840*	0.706	3.328	4.394	0.897
15) Power	Width	7.634*	0.752*	0.839*	0.705	3.275	4.280	0.901
16) Linear	Length \times Width	0.679 ^{ns}	0.989 ^{ns}	0.985*	0.971	1.059	1.324	0.992
17) Quadratic	Length \times Width	0.348 ^{ns}	1.001 ^{ns}	0.985*	0.971	1.045	1.315	0.992
18) Power	Length \times Width	0.386 ^{ns}	0.998 ^{ns}	0.985*	0.971	1.049	1.310	0.992

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Continued from Table 3

Number and type of model	Independent variable (x)	Model validation indicators							
		a ⁽¹⁾	b ⁽²⁾	r ⁽³⁾	R ²	MAE	RMSE	d	
Generated models based on 400 flag and others (non-flag) leaves									
19) Linear	Length	-7.341*	1.903*	0.986*	0.973	11.264	13.809	0.746	
20) Quadratic	Length	2.305*	0.936*	0.941*	0.886	2.413	3.066	0.966	
21) Power	Length	2.115*	0.937*	0.941*	0.886	2.365	3.018	0.967	
22) Linear	Width	11.597*	0.524*	0.714*	0.510	5.276	6.217	0.807	
23) Quadratic	Width	11.482*	0.528*	0.714*	0.511	5.268	6.207	0.809	
24) Power	Width	10.544*	0.528*	0.709*	0.504	5.172	6.044	0.816	
25) Linear	Length × Width	0.391 ^{ns}	0.985 ^{ns}	0.986*	0.973	1.061	1.392	0.993	
26) Quadratic	Length × Width	0.198 ^{ns}	0.993 ^{ns}	0.986*	0.974	1.051	1.383	0.993	
27) Power	Length × Width	0.314 ^{ns}	0.986 ^{ns}	0.986*	0.973	1.066	1.402	0.993	

⁽¹⁾* Linear coefficient differs from zero by t-test at 5% probability level. ⁽²⁾* Angular coefficient differs from zero by t-test at 5% probability level. ⁽³⁾* Correlation coefficient differs from zero by t-test at 5% probability level. ^{ns} Non-significant.

leaf area of hazelnut (Cristofori et al., 2007), rose (Rouphael et al., 2010), gladiolus (Schawb et al., 2014) and pigeon pea (Cargnelutti Filho et al., 2015a).

Conclusions

The morphology of flag and other (non-flag) leaves is distinct. The flag leaves generally have smaller length and greater width and, consequently, smaller length/width ratio.

In triticale, the most precise models of leaf area estimation are those that use the leaf length × width product as the explanatory variable. In this regard, the linear models for estimating the leaf area of flag leaves ($\hat{Y} = -0.4088 + 0.7435x$; $R^2 = 0.9893$) and others (non-flag) leaves ($\hat{Y} = 1.1000 + 0.7633x$; $R^2 = 0.9743$) can be utilized, where x represents the L×W product.

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