

Evaluation of mathematical equations for estimating leaf area in rapeseed¹

Avaliação de equações matemáticas para estimar a área foliar de canola

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ABSTRACT - The aim of this study was to evaluate estimations of leaf area in rapeseed (*Brassica napus* L.) and the potential for application to different genotypes, environmental conditions and types of crop management. In experiments conducted during 2013, 2014, 2016 and 2017, three genotypes of rapeseed, five doses of nitrogen and three sowing dates were used. Leaves were randomly collected from different plants and positions on the plants. The leaf area (LA), and maximum width (W) and length (L) were determined for each leaf, and the product of L and W (LxW) was calculated. Fifty-six equations for estimating LA in rapeseed, where the independent variables were W, L or LxW, were compiled from the bibliography and evaluated in this work. The evaluation was made using the following statistics: significance of the linear (*a*) and angular (*b*) coefficients of the regression around the 1:1 line, concordance index (*d*), bias index (BIAS), mean absolute error (MAE), mean relative error (MRE), random mean squared error (MSE_r) and systematic mean squared error (MSE_s). Only 11 equations showed the *a* and *b* coefficients as not being different from 0 and 1 respectively. However, only 10 were suitable, as they displayed the lowest values for *d*, BIAS, MAE and RME, and the MSE_s was smaller than the MSE_r. The MAE ranged from 5.4 cm² to 16.2 cm², well within the error range for generating the equations. LA in rapeseed can be estimated by general biometric equations without considering the specificity of the genotype or the morphological type of the leaf.

Key words: *Brassica napus*. Colza. Modelling. Leaf area index.

RESUMO - Objetivou-se avaliar a estimativa da área foliar de canola (*Brassica napus* L.) com potencial de aplicação para distintos genótipos, condições ambientais e de manejo da cultura. Em experimentos conduzidos em 2013; 2014; 2016 e 2017, utilizou-se três genótipos de canola, cinco doses de nitrogênio e três datas de semeadura. Foram coletadas, aleatoriamente, folhas em diferentes plantas e posições na planta. Em cada folha foi determinada a área foliar (AF), a largura (L) e o comprimento (C) máximos e foi calculado o produto de C e L (CxL). Na bibliografia foram compiladas 56 equações de estimativa da AF para canola, cujas variáveis independentes eram L, C ou CxL, as quais foram avaliadas neste trabalho. A avaliação foi feita pelas estatísticas: significância dos coeficientes linear (*a*) e angular (*b*) da regressão em torno da linha 1:1, índice de concordância (*d*), índice BIAS (BIAS), erro médio absoluto (MAE), erro médio relativo (EMR), erro quadrático médio aleatório (MSE_a) e erro quadrático médio sistemático (MSE_s). Apenas 11 equações apresentaram os coeficientes *a* e *b* não diferentes de 0 e 1, respectivamente. Entretanto, apenas 10 foram adequadas, por apresentarem os menores valores de *d*, BIAS, MAE, EMR e o MSE_s foi menor do que o MSE_a. O MAE variou de 5,4 cm² a 16,2 cm², dentro da faixa dos erros da geração das equações. A AF de canola pode ser estimada por equações biométricos gerais sem considerar especificidade de genótipo e/ou tipo morfológico de folha.

Palavras-chave: *Brassica napus*. Colza. Modelagem. Índice de área foliar.

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INTRODUCTION

Leaf area (LA) is the principal structure involved in the interception of solar radiation, photosynthesis, evapotranspiration and other plant processes, and has an effect on grain yield (KIRKEGAARD *et al.*, 2012). A precise estimation of LA is therefore fundamental for understanding responses to the environment and to crop management, particularly in rapeseed, since the plant transfers part of the functions of the leaf to the siliquae at the start of the reproductive period (FOCHESATTO *et al.*, 2016).

Mathematical equations are currently important tools for estimating LA. In general, they relate LA to the dimensional variables of the leaves, such as length (L) and width (W) (LIMA *et al.*, 2012; RICHTER, *et al.*, 2014). There are various equations for estimating LA from the L and W of the leaf, and from the product of these dimensions (CARGNELUTTI FILHO *et al.*, 2015, CHAVARRIA *et al.*, 2011; TARTAGLIA *et al.*, 2016); however, these relationships are not always consistent, neither do they maintain the same response pattern, due to changes that occur in the leaf morphology of rapeseed as a result of such environmental factors as air temperature (STELANOWSKA *et al.*, 1999). This points to the need to evaluate estimating equations for LA in rapeseed, considering the variability of different environments and strategies of crop management.

The representativeness of LA estimates from mathematical equations is another important aspect. According to Chavarria *et al.* (2011), LA in rapeseed was best estimated from leaf W, but the proposed equation has a relatively low coefficient of determination (R^2), which may lead to imprecise estimates (TARTAGLIA *et al.*, 2016). On the other hand, for Cargnelutti Filho *et al.* (2015), the best estimate of LA in rapeseed was from leaf L; this was also verified by Tartaglia *et al.* (2016). However, these authors did not explore the interannual variability of rapeseed growth and development and/or different crop management strategies. In addition, the work of Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016) were carried out in the southern half of the State of Rio Grande do Sul (RS), a region not traditionally used for cultivating rapeseed. As such, there is a need for broader assessments, which would consider the variability of different environmental conditions and types of crop management, applied to the region of greater cultivation, i.e. the north of Rio Grande do Sul.

Furthermore, Cargnelutti Filho *et al.* (2015) state that specific equations should be sought for each genotype. This contradicts the biological nature of rapeseed: high plasticity with a great ability to compensate for area, and adaptation to different environments (JULLIEN *et al.*, 2011;

KRÜGER *et al.*, 2016). In this context, the hypothesis is that it is possible to find general equations for estimating LA in rapeseed from linear dimensions, including the biological nature and new genotypes that are launched for cultivation in the market (TARTAGLIA *et al.*, 2016).

The aim of this study, therefore, was to evaluate equations for estimating LA in rapeseed with the potential for application to different genotypes, environmental conditions and types of crop management.

MATERIAL AND METHODS

The study was carried out with data collected in the field during 2013, 2014, 2016 and 2017, in the experimental area of Embrapa Trigo, Passo Fundo, RS (28°11'40" S, 52°19'20" W, at an altitude of 713 m). According to the Köppen classification, the region has a type Cfa climate (ALVARES *et al.*, 2013), with soil classified as a humic Dystrophic Red Latosol (STRECK *et al.*, 2008).

The experiments were set up in a randomised block design with four replications, except for 2013, when there were five replications. In 2013 and 2014, the treatments consisted of nitrogen doses (N) of 10, 20, 40, 80 and 160 kg ha⁻¹ as cover fertiliser, using the Hyola 61 genotype, with sowing on 22 April 2013 and 29 April 2014 respectively. For the other years, the experiments were factorial: sowing dates and rapeseed genotypes. There were three sowing dates each year: 18 May 2016, 3 June 2016 and 15 June 2016, and 8 April 2017, 22 May 2017 and 16 June 2017, using the Hyola 61, Diamond and ALHTM6 genotypes.

In 2013 and 2014, sowing was by seeder-fertiliser coupled to a tractor; in 2016 and 2017 sowing was manual with only the base fertiliser added to the soil. The spacing between rows was 34 cm, at a plant density of 40 plants per m². The base fertilisation and N treatments in the form of urea, are described in Fochesatto *et al.* (2016) and Pinto *et al.* (2017) for 2013 and 2014. In 2016 and 2017, base fertilisation followed the same suggestions of Fochesatto *et al.* (2016) and Pinto *et al.* (2017), with 80 kg N ha⁻¹ as cover fertiliser. The other cropping treatments were carried out according to the need of the crop and following suggestions for each specific situation.

LA, W and L were determined during the vegetative phase of the rapeseed, between the end of rosette formation and the end of main stem elongation, from a random sampling of leaves from different plants in the plot and different positions on the plants. In 2013, two leaves were sampled per plot, giving a total of 50 leaves; in 2014 nine leaves were sampled per plot, totalling 180 leaves, and in 2016 and 2017 four and five leaves were sampled per plot,

for a total of 144 and 180 leaves respectively. Nine leaves were discarded due to damage during the transportation and handling of the plant material. This generated a series of 546 independent observations representative of the main causes of variability in the rapeseed canopy.

A LICOR model 3100-L optical planimeter was used to determine the individual LA of each leaf. The maximum linear dimensions for leaf L and W were determined by ruler. The L was measured along the central vein, starting from the insertion of the petiole in the plant to the apex of the leaf, and the maximum W was measured across the central part of the leaf blade. The product LxW was then calculated.

The data were evaluated using descriptive statistics: minimum, mean, median, maximum, standard deviation, coefficient of variation (CV), kurtosis and asymmetry, with data normality verified by means of the Kolmogorov-Smirnov test (KS). In addition, LA dispersion profiles were generated as a function of L, W and LxW to verify the distribution pattern of the data.

The equations for estimating LA in the rapeseed were compiled from Chavarria *et al.* (2011), Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016), for the Hyola 61, Hyola 433, Hyola 76, Hyola 411 and Hyola 420 genotypes. In all, 56 equations were studied, being at present the only equations available for the growing conditions in the south of Brazil. The equations under study were generated based on linear, quadratic and power models.

To meet the objectives of the study, the data for LA, W, L and LxW were grouped into six distinct sets of data, representing the six distinct conditions for variability. The first set involved all the data from the experiments in 2013, 2014, 2016 and 2017, irrespective of genotype and N treatment (TD). The second was composed of all the TD, but minus the extreme N treatments (10, 20 and 160 kg ha⁻¹) of 2013 and 2014 (TD_{MN}), i.e. keeping only treatments within the range normally used for N as cover fertiliser in rapeseed (between 40 and 80 kg N ha⁻¹). The third, fourth, fifth and sixth sets of data comprised the following: H61 - all the data for the Hyola 61 genotype from the four experiments; H61_{MN} - the same data as H61, but minus the extreme N treatments (10, 20 and 160 kg ha⁻¹); Diam - data for the Diamond genotype from the 2016 and 2017 experiments; and M6 - data for the ALHTM6 genotype from 2016 and 2017.

The performance of the equations for accuracy and precision of the estimates was evaluated by different statistics (WILLMOTT *et al.*, 1985), assuming that to be considered suitable, the equations should meet the criteria of all the statistics used. First, data dispersion around the 1:1 line was evaluated, considering the observed

(measured) values along the ordinate and the simulated (estimated) values along the abscissa (PIÑEIRO *et al.*, 2008), as per equation 1.

$$O_i = a + bS_i \quad (1)$$

where: O_i is the observed (measured) value, S_i is the value simulated by the model as a function of the leaf dimensions (W, L and LxW), and a and b are the angular and linear coefficients of the regression line respectively. The coefficients, a and b , were tested by t-test at 5% probability of error as to the significance of their variation relative to 0 and 1 respectively. The ideal model is one in which coefficient a does not differ from 0 and coefficient b does not differ from 1 (SMITH; ROSE, 1995).

Data dispersion around the 1:1 line was complemented (PIÑEIRO *et al.*, 2008) by the following statistics: concordance index (d), BIAS index, mean absolute error (MAE), mean relative error (MRE), random mean squared error (MSE_r) and systematic mean squared error (MSE_s).

For the bias index, which is the mean value of the deviations of the simulated values in relation to the observed values, the calculation was adapted from Leite and Andrade (2002) as per equation 2:

$$\text{BIAS} = (\Sigma S_i - \Sigma O_i) / \Sigma O_i \quad (2)$$

where: O_i is the observed (measured) value and S_i is the value simulated by the model as a function of the leaf dimensions (W, L and LxW). The bias index shows the trend of the model, where the closer the value is to zero, the lower the trend and the better the model.

The concordance index d is the statistic that evaluates the degree to which the simulations are free of error, and represents the accuracy of the model. This varies from 0, where there is no agreement, to 1, where there is perfect agreement between the observed and simulated values; d was calculated as per Willmott *et al.* (1985), from equation 3:

$$d = 1 - \left\{ \frac{\left[\Sigma (S_i - O_i)^2 \right]}{\left[\Sigma \left[(S_i - \bar{o})^2 + (O_i - \bar{o})^2 \right] \right]} \right\} \quad (3)$$

where: S_i is the simulated value, O_i is the observed value and \bar{o} is the mean of the observed values.

The MAE, which is the mean of the absolute values of the errors was calculated as per Willmott and Matsuura (2005), with equation 4:

$$\text{MAE} = [\Sigma (|O_i - S_i|)] / n \quad (4)$$

where: O_i is the observed value, S_i is the simulated value and n is the number of observations. The MAE considers

the magnitude of the errors, assigning the same weight to all the residuals, and is considered a robust indicator (FOX, 1981), as it is less affected by outliers and is recommended for evaluating equations (WILLMOTT; MATSUURA, 2005).

For the MRE, which is used to verify the margin of error in estimating the model, Equation 5 was used:

$$\text{MRE} = 100\{\Sigma[|O_i - S_i|]/O_i\}/n \quad (5)$$

where: O_i is the observed value, S_i is the simulated value and n is the number of observations. The coefficient 100 transforms the values into a percentage.

The random (MSE_r) and systematic (MSE_s) parts of the mean squared error were calculated from equations 6 and 7 respectively, as per Willmott (1982):

$$\text{MSE}_r = [\Sigma(\hat{S}_i - S_i)^2]/n \quad (6)$$

$$\text{MSE}_s = [\Sigma(\hat{S}_i - O_i)^2]/n \quad (7)$$

where:

$$\hat{S}_i = a + bO_i \quad (8)$$

with a and b the linear and angular coefficients of the regression line equation between the simulated and observed values. MSE_r represents the random variations of the measurements and is due to uncontrolled factors affecting each measurement differently, whereas MSE_s represents the difference between the measured value and the true value, and is the same for the total measurement.

The calculations were carried out on spreadsheets using the Excel® software. For the statistical analysis, the R software (R CORE TEAM, 2018) was used.

RESULTS AND DISCUSSION

In general, the values for the leaf dimensions L and W varied between 3.6 and 46.5 cm and between 2.0 and 20.5 cm respectively, while the product $L \times W$ and LA varied between 7.2 and 672.4 and between 3.7 and 461.1 respectively (Table 1). These limits are within or fairly close to those presented by Chavarria *et al.* (2011), Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016) for obtaining the equations. Variations in the limits of the independent variables (L , W , $L \times W$) and in LA were found between the sets of data, and were due to variability between genotypes and the effect of nitrogen fertilisation. On average, the H61 and H61_{MN} genotype had longer and wider leaves than Diam and M6. A rise in N cover fertilisation increased this difference (Table 1). It is therefore evident that variations in sowing

times (NANDA; BHARGAVA; RAWSON, 1995), environmental conditions (STELANOWSKA *et al.*, 1999) and N fertilisation (BOUCHET *et al.*, 2016) affect the leaf morphology in rapeseed.

The experimental strategy adopted, involving some of the main factors of rapeseed management, different environmental conditions (different years of cultivation) and genotypes of a different genetic base, guaranteed the desired variability, necessary for evaluating the equations (Table 1). The CV ranged from 21.1% to 114.6%, being approximately twice as much for $L \times W$ and LA as for leaf L and W , as found by Cargnelutti Filho *et al.* (2015). Among the sets of data, the CV was higher in M6, not only for all the independent variables, but also for LA , whereas for the H61, H61_{MN} and Diam data sets, the values for CV were close (Table 1). This is an indicator that justifies the search for more robust equations which consider variability in the LA of the crop (Table 1).

In the data set for most of the variables (L , W , $L \times W$ and LA), asymmetry and kurtosis were not significant. Leaf W showed more situations with significant asymmetry. However, as the significant values for asymmetry were low (≤ 0.73) and positive, similar to those found by Cargnelutti Filho *et al.* (2015), this indicates a slight departure from normality. In the vast majority of the situations under study, the p-values of the KS-test were less than $p = 0.05$, proving this slight departure from normality. However, the statistical indices together (kurtosis, asymmetry and p-value) indicate a small departure from normality for L and W , compared to $L \times W$ and LA , as found by Cargnelutti Filho *et al.* (2015), particularly for TD, TD_{MN}, H61 and H61_{MN}, in contrast to a greater departure for M6. This response is also verified by the magnitude of the difference between the mean and the median, which is lower for L and W than for $L \times W$ and LA in each of the experimental situations (Table 1). According to Cargnelutti Filho *et al.* (2015), such statistical characteristics are suitable for validating equations.

The data dispersion (Figure 1) indicated patterns already expected for rapeseed (CARGNELUTTI FILHO *et al.*, 2015; CHAVARRIA *et al.*, 2011; TARTAGLIA *et al.*, 2016), where LA has a non-linear relationship to the leaf L and W (Figures 1a to 1f) and a linear pattern for $L \times W$ (Figures 1g to 1i). This was also found in other species, such as the jack bean (TOEBE *et al.*, 2012) and the forage turnip (CARGNELUTTI FILHO *et al.*, 2012). The dispersion was greater when LA was a function of L , followed by $L \times W$ and W (Figure 1), indicating a probable better response for equations using W as an independent variable.

An analysis of the significance of the a and b coefficients of the regression between the measured and

Table 1 - Number of leaves (n), values: minimum (Mn), mean (M), median (MD), maximum (Mx), standard deviation (SD), coefficient of variation (CV), kurtosis (K), asymmetry (A) and p-value in the Kolmogorov-Smirnov test [KS(p)] for length (L), width (W), the product of length x width (LxW) of the leaf blade, and leaf area (LA) in three rapeseed (*Brassica napus* L.) genotypes and/or for different years of evaluation and for the complete set of data

Data set	Statistic									
	n	Mn	M	MD	Mx	SD	CV(%)	K	A	KS(p)
L – Length of the leaf blade (cm)										
TD	546	3.6	18.2	17.5	46.5	7.7	42.2	-0.41 ^{ns}	0.40 ^{ns}	0.028
TD _{MN}	408	3.6	17.0	15.9	46.5	7.7	45.4	0.05 ^{ns}	0.65 ^{ns}	0.006
H61	336	3.6	20.3	20.2	37.0	7.1	35.1	-0.76 ^{ns}	0.10 ^{ns}	0.273
H61 _{MN}	198	3.6	19.1	18.5	37.0	7.4	38.9	-0.67 ^{ns}	0.27 ^{ns}	0.329
Diam	105	4.0	14.9	14.0	29.0	5.7	37.8	-0.53 ^{ns}	0.41 ^{ns}	0.422
M6	105	4.3	15.0	12.5	46.5	8.9	59.6	1.36 ^{ns}	1.28 ^{ns}	0.040
W – Width of the leaf blade (cm)										
TD	546	2.0	8.4	7.7	20.5	3.6	42.3	-0.11 ^{ns}	0.58	<0.001
TD _{MN}	408	2.0	7.7	7.0	20.5	3.3	43.5	0.71*	0.82*	0.007
H61	336	2.0	9.5	9.2	20.5	3.6	37.8	-0.28 ^{ns}	0.43*	0.024
H61 _{MN}	198	2.0	8.6	8.2	20.5	3.5	41.0	0.54*	0.73*	0.010
Diam	105	2.1	6.9	6.8	14.5	2.5	35.5	0.18 ^{ns}	0.57*	0.669
M6	105	2.1	6.6	6.0	15.3	3.2	48.3	-0.02 ^{ns}	0.83*	0.118
LxW – Product of the length and width of the leaf blade (cm ²)										
TD	546	7.2	178.0	136.2	672.4	137.5	77.3	0.57 ^{ns}	1.06 ^{ns}	<0.001
TD _{MN}	408	7.2	153.0	114.2	672.4	129.2	84.4	0.73 ^{ns}	1.08 ^{ns}	<0.001
H61	336	7.2	214.0	185.0	672.4	140.1	65.5	-0.11 ^{ns}	0.76 ^{ns}	0.003
H61 _{MN}	198	7.2	187.8	148.0	672.4	137.8	73.4	0.73 ^{ns}	1.08 ^{ns}	0.007
Diam	105	8.4	116.2	93.8	420.5	81.2	69.9	1.23 ^{ns}	1.15 ^{ns}	0.010
M6	105	11.4	124.3	71.4	660.3	135.1	108.7	3.58 ^{ns}	1.91 ^{ns}	<0.001
LA – Leaf area (cm ²)										
TD	546	3.7	90.6	66.8	461.1	75.0	82.8	2.05 ^{ns}	1.35 ^{ns}	<0.001
TD _{MN}	408	3.7	76.1	54.6	461.1	69.2	91.0	3.75 ^{ns}	1.67 ^{ns}	<0.001
H61	336	4.2	214.0	185.0	461.1	140.1	65.5	-0.11 ^{ns}	0.76 ^{ns}	0.001
H61 _{MN}	198	4.2	95.9	75.5	461.1	79.8	83.2	3.75 ^{ns}	1.67 ^{ns}	0.003
Diam	105	3.7	58.1	45.3	214.6	42.4	73.0	1.49 ^{ns}	1.21 ^{ns}	0.066
M6	105	4.9	56.8	35.1	260.5	58.7	103.4	2.64 ^{ns}	1.73 ^{ns}	<0.001

*Kurtosis or asymmetry differ from zero by t-test at 5% probability of error. ^{ns}Kurtosis or asymmetry differ from zero by t-test at 5% probability of error

estimated LA revealed the high accuracy for estimating LA in rapeseed of equations 01, 02, 03, 04, 05, 06, 07, 08, 09, 10 and 11 (Table 2) for at least one of the sets of data. These equations met the desired statistical assumption, which is to show, at the same time, the *a* and *b* coefficients as not different from 0 or 1 respectively (Table 3), demonstrating a broad and more general application potential. The selected equations were obtained by Cargnelutti Filho *et al.* (2015) specifically

for the Hyola 61 (4 equations), Hyola 76 (3 equations) and Hyola 433 (2 equations) genotypes. Only one of the equations obtained by Tartaglia *et al.* (2016) was suitable, being generated for the set of oval-shaped leaves collected from the Hyola 61, Hyola, 433, Hyola 420 and Hyola 411 hybrids and represented by the number 11 (Table 3). The other equations generated by Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016), and those generated by Chavarria *et al.* (2011), showed the *a* and *b* coefficients as

Figure 1 - Leaf area dispersion in rapeseed (*Brassica napus* L.) as a function of length and width, and the product of length x width, in leaves of the Hyola 61 (H61 and H61_{MN}), Diamond (Diam) and ALHTM6 (M6) genotypes and for the complete set of data (TD and TD_{MN}). (NOTE: MN in subscript means no extreme doses of nitrogen as cover fertiliser in the T1, T2 and T5 treatments for 2013 and 2014)

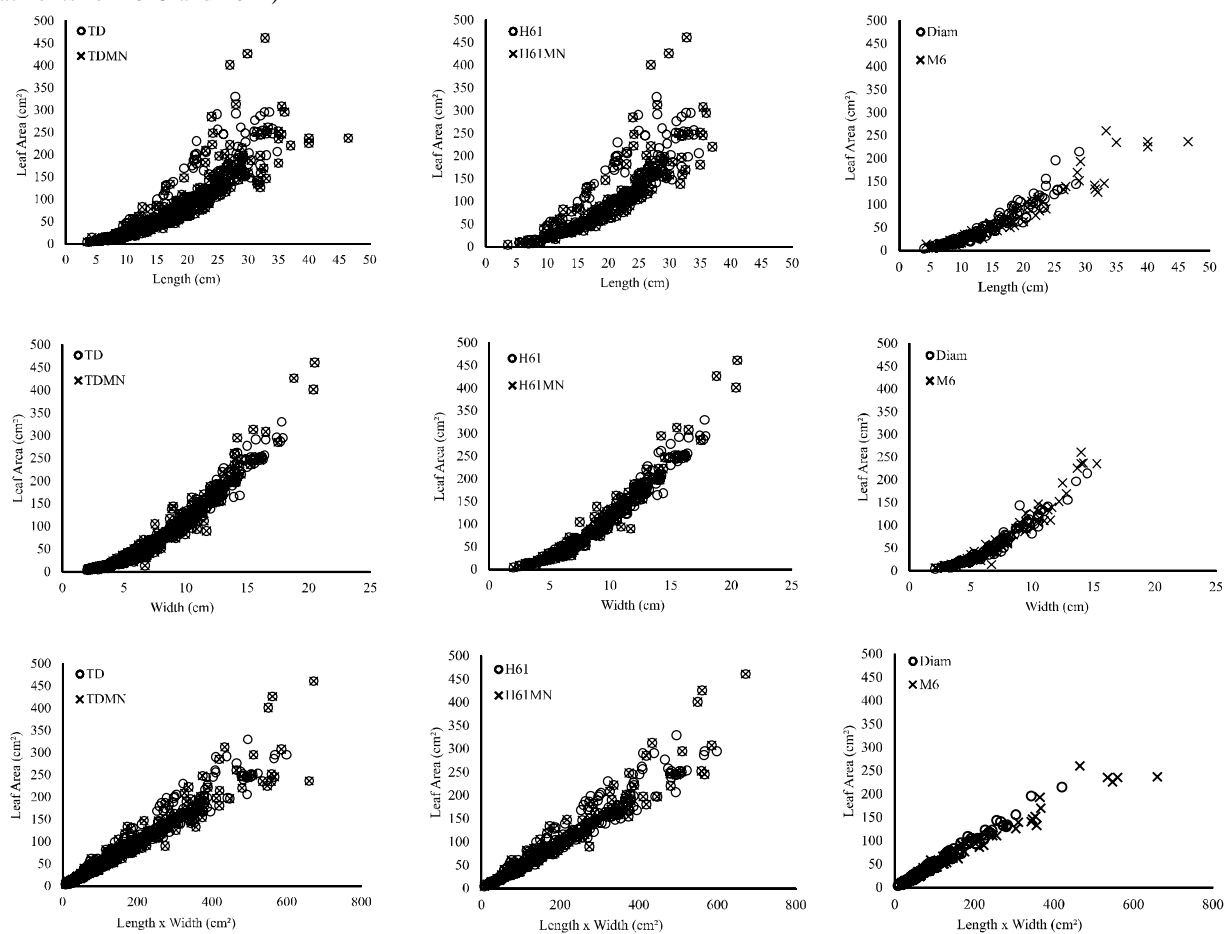


Table 2 - Equations for estimating leaf area (LA) from leaf length (L) and width (W), and the product of length x width (LxW) in rapeseed (*Brassica napus* L.), with the respective coefficients of determination (r^2) and the bibliographic reference to where the equation was obtained

Nº.	Equation	r^2	Bibliographic reference
01	$AF = -1.1711 + 0.7324W + 1.0704W^2$	0.93	Cargnelutti Filho <i>et al.</i> (2015)
02	$AF = 1.3000W^{1.9336}$	0.95	Cargnelutti Filho <i>et al.</i> (2015)
03	$AF = 0.6189LxW^{0.9401}$	0.96	Cargnelutti Filho <i>et al.</i> (2015)
04	$AF = 1.6467 + 0.4525LxW$	0.94	Cargnelutti Filho <i>et al.</i> (2015)
05	$AF = -2.0169 + 1.4464L + 0.1517L^2$	0.93	Cargnelutti Filho <i>et al.</i> (2015)
06	$AF = 1.0579W^{2.0383}$	0.97	Cargnelutti Filho <i>et al.</i> (2015)
07	$AF = -48.6371 + 7.3031L$	0.89	Cargnelutti Filho <i>et al.</i> (2015)
08	$AF = -75.6346 + 20.1473W$	0.92	Cargnelutti Filho <i>et al.</i> (2015)
09	$AF = 1.4154W^{1.9096}$	0.96	Cargnelutti Filho <i>et al.</i> (2015)
10	$AF = 0.7425CxW^{0.9167}$	0.97	Cargnelutti Filho <i>et al.</i> (2015)
11	$AF = 9.34484W$	0.76	Tartaglia <i>et al.</i> (2016)

Table 3 - Linear (*a*) and angular (*b*) coefficients of the regression equation between the measured leaf area and that estimated by the equations for six sets of data: the complete set of data (TD), the complete set of data minus the data from the extreme N treatments (10, 20 and 160 kg ha⁻¹) in 2013 and 2014 (TD_{MN}), all the Hyola 61 (H61) genotype data, all the Hyola 61 genotype data, minus the data from the extreme nitrogen treatments (10, 20 and 160 kg ha⁻¹) in 2013 and 2014 (H61_{MN}), the Diamond (Diam) genotype and the ALHTM6 (M6) genotype

Equation Number	Data set					
	TD	TD _{MN}	H61	H61 _{MN}	Diam	M6
Linear coefficient - <i>a</i>						
01	-0.19 ^{ns}	-1.92 ^{ns}	2.03 ^{ns}	-0.16 ^{ns}	-1.55 ^{ns}	-4.45*
02	-1.37 ^{ns}	-3.03*	0.59 ^{ns}	-1.57 ^{ns}	-2.25 ^{ns}	-5.17*
03	-8.41*	-7.79*	-12.45*	-14.48*	-5.59*	0.07 ^{ns}
04	-5.16*	-5.01*	-8.14*	-10.73*	-3.96*	1.96 ^{ns}
05	-3.56 ^{ns}	-2.92 ^{ns}	-8.22 ^{ns}	-11.54*	-7.31*	4.53 ^{ns}
06	3.37*	1.46 ^{ns}	6.19*	3.87*	1.01 ^{ns}	-2.07 ^{ns}
07	-8.01*	-5.03 ^{ns}	-18.48*	-16.92*	-0.48 ^{ns}	4.28 ^{ns}
08	-3.98*	-1.45 ^{ns}	-12.06*	-9.58*	5.41*	7.40*
09	-2.52*	-4.12*	-0.77 ^{ns}	-2.89 ^{ns}	-3.05 ^{ns}	-5.93*
10	-10.64*	-9.81*	-15.17*	-16.97*	-7.12*	-1.44 ^{ns}
11	7.04 ^{ns}	-2.71 ^{ns}	31.62*	7.02 ^{ns}	20.53 ^{ns}	-7.60 ^{ns}
Angular coefficient - <i>b</i>						
01	0.96 ^{ns}	0.99*	0.95 ^{ns}	0.98*	0.97*	1.02*
02	0.99*	1.02*	0.98*	1.01*	0.99*	1.04 ^{ns}
03	1.25 ^{ns}	1.22 ^{ns}	1.30 ^{ns}	1.32 ^{ns}	1.19 ^{ns}	1.01*
04	1.24 ^{ns}	1.22 ^{ns}	1.29 ^{ns}	1.31 ^{ns}	1.22 ^{ns}	1.01*
05	1.12 ^{ns}	1.05*	1.22 ^{ns}	1.21 ^{ns}	1.12 ^{ns}	0.80 ^{ns}
06	0.90 ^{ns}	0.93 ^{ns}	0.89 ^{ns}	0.91 ^{ns}	0.92 ^{ns}	0.97*
07	1.17 ^{ns}	1.08 ^{ns}	1.30 ^{ns}	1.24 ^{ns}	0.97*	0.87 ^{ns}
08	1.01*	0.99*	1.08 ^{ns}	1.07 ^{ns}	0.83 ^{ns}	0.88 ^{ns}
09	0.98 ^{ns}	1.00*	0.97 ^{ns}	0.99*	0.97*	1.02*
10	1.21 ^{ns}	1.18 ^{ns}	1.27 ^{ns}	1.28 ^{ns}	1.14 ^{ns}	0.98*
11	1.07*	1.08*	0.90*	1.07*	0.58 ^{ns}	1.05*

^{ns}Linear coefficient does not differ from 0 by t-test at 5% probability of error; * Angular coefficient does not differ from 1 by t-test at 5% probability of error

Table 4 - Bias Index (BIAS), concordance index (*d*), mean absolute error (MAE), mean relative error (MRE), random mean squared error (MSE_r) and systematic mean squared error (MSE_s) associated with evaluation of the equations for estimating leaf area in rapeseed (*Brassica napus* L.) for six sets of data of differing variability

Statistic	Equation number										
	1	2	3	4	5	6	7	8	9	10	11
Total data (TD)											
BIAS index	0.04	0.02	-0.12	-0.15	-0.07	0.06	-0.07	0.03	0.05	-0.07	-0.13
d	0.99	0.99	0.96	0.96	0.92	0.99	0.91	0.98	0.99	0.97	0.58
MAE	10.1	9.7	14.0	15.0	20.2	11.1	22.1	15.0	10.6	12.6	46.7
MRE	15.4	15.2	13.2	13.9	23.7	14.9	35.0	43.2	17.5	14.3	86.0
MSE _r	208	194	232	231	804	245	744	355	199	250	854
MSE _s	13	9	482	522	620	58	700	44	23	337	3666

Continued Table 4

TD minus the extreme nitrogen treatment (TD _{MN})											
BIAS index	0.04	0.02	-0.10	-0.12	-0.01	0.06	-0.01	0.03	0.05	-0.04	-0.20
d	0.99	0.99	0.96	0.96	0.93	0.99	0.92	0.98	0.99	0.97	0.50
MAE	9.2	9.0	10.7	11.5	16.2	9.6	18.3	15.1	9.8	10.1	46.8
MRE	17.3	17.2	12.7	13.1	24.6	16.2	38.6	52.9	19.8	14.9	101.4
MSE _r	176	164	211	207	761	205	728	384	169	229	1170
MSE _s	11	16	323	351	342	25	394	32	24	225	3811
Data for the Hyola 61 genotype (H61)											
BIAS index	0.03	0.01	-0.15	-0.17	-0.12	0.06	-0.10	0.03	0.04	-0.10	-0.21
d	0.99	0.99	0.94	0.94	0.88	0.99	0.87	0.98	0.99	0.95	0.52
MAE	11.8	11.3	18.8	20.0	26.2	13.4	28.4	15.5	12.1	16.7	55.2
MRE	13.1	12.7	14.1	15.0	23.5	13.4	30.0	28.6	14.4	14.2	66.3
MSE _r	272	253	274	275	843	323	759	337	258	294	964
MSE _s	14	5	822	869	1236	93	1412	121	21	605	5066
H61 minus the extreme nitrogen treatment (H61 _{MN})											
BIAS index	0.02	0.01	-0.13	-0.15	-0.07	0.05	-0.05	0.02	0.04	-0.08	-0.39
d	0.99	0.99	0.95	0.95	0.89	0.99	0.88	0.98	0.99	0.96	0.48
MAE	11.1	10.9	15.5	16.3	22.3	12.0	25.0	16.0	11.6	14.3	61.3
MRE	15.3	15.1	13.7	14.0	25.1	15.0	33.9	38.4	17.1	15.2	84.3
MSE _r	255	237	267	260	853	302	837	401	242	290	1764
MSE _s	8	15	725	756	1018	40	1177	133	19	554	6262
Diamond genotype (Diam)											
BIAS index	0.06	0.05	-0.08	-0.12	0.00	0.06	0.04	0.10	0.09	-0.02	0.10
d	0.99	0.99	0.98	0.98	0.97	0.98	0.97	0.97	0.98	0.99	0.52
MAE	6.9	6.6	6.9	8.1	8.5	7.0	10.6	12.5	7.6	5.9	32.2
MRE	16.8	16.7	12.4	13.5	19.8	15.6	32.5	46.5	19.8	13.6	110.2
MSE _r	92	87	27	25	110	104	188	177	90	30	537
MSE _s	13	11	79	117	61	15	18	60	26	38	1255
ALHTM6 genotype (M6)											
BIAS index	0.06	0.05	-0.01	-0.04	0.15	0.07	0.07	-0.01	0.08	0.05	0.06
d	0.98	0.98	0.99	0.98	0.95	0.98	0.96	0.95	0.98	0.99	0.52
MAE	7.9	7.9	5.4	5.7	12.5	7.5	13.5	15.9	8.7	6.2	35.2
MRE	21.4	21.5	10.9	10.9	28.3	19.0	53.6	86.6	25.1	15.5	122.2
MSE _r	107	103	92	101	444	115	326	368	109	96	675
MSE _s	20	26	6	10	141	15	28	4	31	8	1828

significantly different from 0 and 1 (data not shown), and were therefore not suitable.

Most of the selected equations (Table 3) have *W* as an independent variable, while the others have *LxW* (2 equations) or *L* (2 equations). These results indicate that *W* is the most suitable variable for estimating LA in rapeseed, and equations that use this independent variable therefore tend to have better performance, as previously pointed out by Cargnelutti Filho *et al.* (2015) and Tartaglia

et al. (2016). Leaf *W* was also the best performing variable in estimating LA in the sunflower (MALDANER *et al.*, 2009), *Bergenias purpurascens* (ZHANG; LIU, 2010) and Italian courgette (FIALHO *et al.*, 2011), possibly because *W* maintains constant shape during LA growth and development (MISLE *et al.*, 2013).

Another important aspect found in evaluating the *a* and *b* coefficients was the suitability of the equations for the Diamond and ALHTM6 genotypes, despite having

been obtained for the Hyola 61, Hyola 76 and Hyola 433 genotypes, or even for oval-shaped leaves (Table 3). In the case of the ALHTM6 genotype, the number of suitable equations was greater than for the other genotypes, or when all the data were used together. However, for the Diamond genotype, the number of equations was equal to that of the Hyola 61 genotype, with most of the equations being the same (equations 02, 05 and 09). Furthermore, it was found that for the TD_{MN} and $H61_{MN}$ data sets, the number of equations was higher than for TD and H61 (Table 3). This response indicates that management factors may affect the estimation of LA in rapeseed, as they affect leaf morphology. On the other hand, since there were equations that fit each set of data (TD, TD_{MN} , H61, $H61_{MN}$, Diam and M6), they prove the hypothesis of more general equations for estimating LA in rapeseed, without the specificity indicated by Cargnelutti Filho *et al.* (2015) and/or by Tartaglia *et al.* (2016) being necessary.

Additional analysis for data dispersion relative to the 1:1 line suggested by Piñeiro *et al.* (2008), was only carried out for equations that met the significance of the *a* and *b* coefficients (01, 02, 03, 04, 05, 06, 07, 08, 09, 10 and 11) for at least one set of evaluated data (Table 4). This strategy was adopted, since the other equations are susceptible to significant error.

In general, the selected equations also presented other favourable evaluation statistics, except for equation 11, which had the most distant values in relation to the other equations (Table 4). This equation will be presented and discussed separately.

Equations 01, 02, 03, 04, 05, 06, 07, 08, 09 and 10 presented values for BIAS close to zero in the different sets of data under evaluation ($-0.04 \leq \text{BIAS} \leq 0.09$), indicating that there were no under or overestimates. The accuracy of these equations is also confirmed by the *d* index, where the majority had a value equal to 0.99 ($0.93 \leq d \leq 0.99$). Such a condition indicates good similarity between the measured and estimated values for LA (Table 4).

The MAE associated with the LA estimates ranged from 5.4 cm² to 16.2 cm², and were within the range of errors found by Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016) when obtaining the equations for other environments, management conditions and/or genotypes. The similarity of the values for MAE in the present study, in relation to those found by Cargnelutti Filho *et al.* (2015) and Tartaglia *et al.* (2016), is another good indicator of the estimating power of the selected equations for LA in rapeseed under different environmental and management conditions. When the analysis was carried out by comparing the sets of data, it was found that although the number of equations was greater for the TD_{MN} and $H61_{MN}$ data sets, on average, the MAE increased (TD_{MN}) or did

not change ($H61_{MN}$) in relation to the TD and H61 data sets respectively. Overall, however, the MRE increased by 16.4 and 3.1 percentage points respectively under this condition (Table 4).

Another important aspect to be noted in this study is the suitability of the equations for the Diamond and ALHTM6 genotypes (Table 4). In addition to there being a greater number of suitable equations for these two genotypes compared to the other sets of data, the values for MAE were generally less than those seen for H61, $H61_{MN}$, TD and TD_{MN} ($5.4 \leq \text{MAE} \leq 11.6$). On the other hand, the mean value for MRE among the equations for the Diamond and ALHTM6 genotypes increased in relation to the equations evaluated for the TD, TD_{MN} , H61 and $H61_{MN}$ sets of data (Table 4), but not so as to compromise their application. The suitability of the equations generated for genotypes with a different genetic base (Diamond and ALHTM6) is a robust indicator, which helps in proving the hypothesis of this work.

A breakdown of the errors associated with the estimations made by the equations (01, 02, 03, 04, 05, 06, 07, 08, 09, 10) also corroborates their indication as the most suitable for estimating LA in rapeseed using L and W, or LxW (Table 4). As can be seen, all the selected equations showed the MSE_s as less than the MSE_r . This is a desired condition when evaluating equations, as it shows that the equation is free from any bias associated with the measured data and/or generation of the equations (WILLMOTT *et al.*, 1985).

In relation to equation 11, despite the *a* and *b* coefficients not being different to 0 and 1 respectively (Table 3), the equation did not meet the other statistics of performance evaluation (Table 4). This equation had the largest deviations between the selected equations for each data set to which it was applied, with the exception of M6. In addition, the *d* index was one of the lowest among all the equations (Table 4), with the MAE and MRE being among the highest. However, the statistic that made equation 11 unsuitable for estimating LA in rapeseed was the MSE_s , which was higher than the MSE_r for each set of data (Table 4). As such, the equations presented by Tartaglia *et al.* (2016) were not sufficiently suitable for estimating LA in rapeseed from the leaf dimensions of W and L or LxW, outside the conditions in which they were obtained.

In general, equations 02, 05 and 09 proved to be more suitable for estimating LA in rapeseed, and should be used, as they deal with variability related to genetic diversity and factors of crop management. As such, the hypothesis that LA in rapeseed can be estimated by general equations is confirmed, where the linear dimensions of W and L or LxW are taken as independent variables.

CONCLUSION

Leaf area in rapeseed is adequately estimated by general biometric equations for different genotypes, conditions of nitrogen cover and sowing times, as they do not maintain any specificity regarding genotype and/or leaf morphology.

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