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CROP PRODUCTION

Mathematical model for estimating the leaf area of cured dry tobacco using linear measurements

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ABSTRACT: Leaf area of dried Flue-cured tobacco is a reflection of climate and stage of growth of fresh tobacco in field; it also serves as the foundation for calculating a number of significant physical properties of tobacco. So the purpose of this paper was to establish a model to estimate the leaf area of dried Flue-cured tobacco in China from linear dimensions. Three Hundred eight tobacco leaves from different growing area and stalk position were sampled randomly and separated for model selection among linear, proportional and power model type and external evaluation individually. Results showed that there was a significant and strong correlation between leaf area and length×width , The equation LA = 0.495(L×W), where LA is the leaf area and L×W is the product of leaf length and width, was optimum and adequate for the estimation of leaf area of dried tobacco in China examined by Fisher's test, Akaike delta information criterion (AIC) and Bayesian information criterion (BIC). Growing area and stalk position had minor effect on the parameter before (L×W). The equation can sufficiently predict the area of leaf for external evaluation.

Key words: proportional model, leaf length, leaf width, growing area, stalk position.

Modelo matemático para estimar a área foliar do tabaco seco curado por meio de medidas lineares

RESUMO: A área foliar do tabaco curado pelo Flue seco é um reflexo do clima e do estágio de crescimento do tabaco fresco no campo; também serve como base para o cálculo de várias propriedades físicas significativas do tabaco. Portanto, o objetivo deste artigo foi estabelecer um modelo para estimar a área foliar do tabaco curado por Flue seco na China a partir de dimensões lineares. Foram amostradas aleatoriamente 308 folhas de tabaco de diferentes áreas de cultivo e posição do colmo e separadas para seleção de modelos entre tipo linear, proporcional e de potência e avaliação externa individualmente. Os resultados mostraram que houve uma correlação significativa e forte entre área foliar e comprimento×largura. A equação LA = 0,495 (L×W), em que LA é a área foliar e L×W é o produto do comprimento e largura da folha, ótima e adequada para a estimativa da área foliar de tabaco seco na China examinada pelo teste de Fisher, critério de informação delta de Akaike (AIC) e critério de informação bayesiana (BIC). A área de cultivo e a posição do caule tiveram efeito menor no parâmetro antes (C×L). A equação pode prever suficientemente a área da folha para avaliação externa.

Palavras-chave: modelo proporcional, comprimento da folha, largura da folha, área de crescimento, posição talo.

INTRODUCTION

Flue-cured tobacco is an important industrial crop in China with a calculated annual yield of 1500,000 t on approximately 640,000 ha of land every year (TANG et al., 2020). It is the dried leaf after curing that is utilized in the tobacco industry. The quality of dried tobacco is of great concern (ZONG et al., 2022) and has a direct impact on the quality and the cost of final product. Leaf area is one of the significant qualitative indicators of dried Flue-cured tobacco among others. It reflects the environment and culture practice of fresh tobacco leaves during the growth season and constructs the basis for measurements of some meaningful structural and productive characteristics of dried tobacco leaves, such as leaf mass per area.

Leaf area can be measured by an automatic surface meter (FALLOVO et al., 2008) or from tracing (MOUSTAKAS & NTZANIS, 1998) and photography (KOUBOURIS et al., 2018). Most of the measurements were performed in the laboratory. A time-consuming process of digital transformation and mathematical calculation was required during the measurement of leaf area. In order to develop a convenient and non-destructive method that can be used in non- laboratory environment to obtain the leaf area, many researchers managed to build a relationship between leaf area and leaf dimensions which can be measured easily on different species (POTDAR &

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PAWAR, 1991; WILLIAMS & MARTINSON, 2003; ROUPHAEL et al., 2006; SERDAR & DEMIRSOY, 2006; PEKSEN, 2007; FALLOVO et al., 2008; KOUBOURIS et al., 2018; FERNANDES et al., 2020). Independent variable selection and model type choice were two of the emphasis in these literatures: Leaf length (L), leaf width (W), and some secondary combinations derived from L and W including L/W and L×W were chosen as independent variables for model development, L×W was preferential in a lot of research (POTDAR & PAWAR, 1991; PEKSEN, 2007; FALLOVO et al., 2008; KOUBOURIS et al., 2018); Linear and power models were two basics in the estimation of leaf area from leaf dimensions, more complicated models which were combinations of linear and power models were also developed for more accurate prediction.

As mentioned above, many models with various measuring independent variables were also adopted for the estimation of the leaf area of fresh tobacco. The effects of variety (BOZHINOVA, 2006), stalk position (MAW & MULLINIX, 1992), irrigation rate (SUGGS et al., 1960), and nitrogen treatment (MOUSTAKAS & NTZANIS, 1998) on estimating models were investigated. But to our best knowledge, no research was focused on the leaf area estimation of dried Flue-cured tobacco with linear dimensions. This research developed and evaluated mathematical models to estimate the leaf area of dry cured tobacco through linear measurements and evaluated the effect of local cultivation, variety and stem position on the relationship of linear measurements with leaf area.

MATERIALS AND METHODS

A total of 308 tobacco (*Nicotiana tabacum L.*) leaves were sampled randomly from the volume products purchased by China Tobacco Anhui Industrial CO., LTD in the year 2021. To ensure the robustness and applicability of the estimation model to establish, samples were extracted from different growing areas, varieties, and stalk position listed in table 1.

The referring growing areas Lijian (LJ, 26.68°N, 100.25°E), Liangshan (LS, 27.90°N, 102.27°E), Zhangjiajie (ZJJ，29.18°N, 110.48°E), Zunyi (ZY, 27.71°N, 106.94°E) belong to Yunnan province, Sichuan province, Hunan province and Guizhou province respectively that cover a majority of ecological regions of tobacco production in China. The varieties listed in table 1 were planted widely in the corresponding growing areas. So samples in this research represented the practical tobacco production of China.

Samples from volume products were placed at temperate 25 °C, humidity 65% for 48 hrs, and then pressed under a metal plate weighting about 5 kg for another 48 hrs to flatten the tobacco, the prepared sample was illustrated in figure 1. The length (L) and width (W) of tobacco which had the same definition as SCHLÖSSER et al. (2020) were measured by ruler to the nearest 1 mm. Leaf areas were measured according to the method of Georgios Koubouris et al. (2018) with minor modification, sample images were photographed in 750 dpi with a scale of 10 cm. leaf area of tobacco was calculated using ImagJ software from the sample image.

Five leaves of each entry in table 1 were extracted for the generation of external evaluation dataset. The rest of samples were combined as model development dataset. The correlation among length (L), width (W), length×width (L×W) and real leaf area (LA) were studied in the research. Data for model development were fitted by linear model with equation of LA = a×(L×W) + b, by proportional model

Table 1 - number of leaves sampled from different growing area, variety and stalk position.

Growing area	Variety	Stalk position	Number of leaves
Lijian (LJ)	Yunyan 87	Upper	41
		Middle	32
		Lower	31
Liangshan (LS)	HongDa	Upper	30
		Middle	25
		Lower	24
Zhangjiajie (ZJJ)	K326	Upper	26
		Middle	27
		Lower	24
Zunyi (ZY)	Yunyan 87	Upper	23
		Middle	25

Figure 1 - Tobacco sample after moisture adjustment and flatting treatment.

with equation of LA = a×(L×W), and by power model with equation of LA = a×(L×W)^b separately, a and b were parameter constants in each model. Growing area-specific and stalk position-specific models were also developed using samples from different growing areas and different stalk positions listed in table 1 individually in order to investigate factors affecting the estimation of leaf area. The built models were tested by external evaluation dataset: the estimated leaf areas of samples in external evaluation dataset were calculated by the fitting equations of models and compared to real leaf areas by t-test. All the data fitting were carried out on Origin 2018 software and statistical analyses were executed on IBM SPSS statistics 24.

RESULTS AND DISCUSSIONS

Leaf variables in table 2 ranged from 45.93 cm to 80.82 cm with a CV of 10.67% for length, 10.39 cm to 26.48 cm with a CV of 17.46% for width, 623.11 cm^2 to 1904.46 cm^2 with a CV of 19.09% for length×width, 309.98 cm^2 to 968.99 cm^2 with a CV of 19.01% for real leaf area, respectively. Dried Flue-cured tobacco in China presented moderate leaf dimensions variation in contrast to fresh Burley tobacco 'Dbh 2252' which had a CV from 15.73% to 78.85% (TOEBE et al., 2020), or fresh Flue-cured tobacco McN-944 of which leaf area ranged between 78 cm^2 and 1100 cm^2 (MOUSTAKAS & NTZANIS, 1998). Flue-cured tobacco was harvested and cured during the mature stage when leaves were fully developed, the absence of small tobacco leaves often occurring in the vegetative growth stage may be the reason for small variations of dimension in dried Flue-cured tobacco.

No significant asymmetry of data distribution was observed in leaf width, length×width and real leaf area, but a negative asymmetry were revealed in leaf length, indicating a small occupation of long leaves in the whole tobacco samples. Long leaves usually reflect the over-nutrition of tobacco that was unfavorable in market of China and can easily cause curing problem. Large leaves were naturally discarded in tobacco grading and rarely appeared in randomly chosen samples in this research. The normality of data distribution of variables like width, length×width and real leaf area guaranteed the validity of subsequent model development.

There were significant correlations between linear dimension and leaf area and among linear dimensions, except length versus width of tobacco, however, a strong linear correlation was observed only in the relationship of leaf area with length×width (L×W) (Table 3), so length×width (L×W) should be chosen as an optimum independent variable to construct the linear model.

Three models so-called Linear (LA = a×(L×W) + b), Proportional (LA = a×(L×W)), and Power (LA = a×(L×W)^b) were utilized in the estimation of leaf area using length×width (L×W) as an independent variable, three corresponding equations simulated on the model development dataset by the above models were obtained (Figure 2). The data were fitted well by the three equations because of the significance of the models examined by the F test. Parameter a in all three models and Parameter b in the Power model were extremely

Table 2 - Descriptive statistics of variables used in model development for estimating Flue cured tobacco leaf area in China.

Variable	---Descriptive statistics---					
	Min	Max	Mean	SD	CV (%)	Skew	Kurt
L(cm)	45.93	80.82	65.55	7.03	10.67	-0.38*	-0.11^{ns}
W(cm)	10.39	26.48	18.79	3.29	17.46	-0.19^{ns}	-0.46^{ns}
L×W(cm^2)	623.11	1904.46	1229.29	239.57	19.09	-0.04^{ns}	-0.09^{ns}
LA(cm^2)	309.98	968.99	608.73	118.17	19.01	0.00^{ns}	0.05^{ns}

[(a)]L = length, W = width, L×W = length×width, LA = real leaf area; [(b)] Min = minimum, Max = maximum, Mean = mean, SD = standard deviation, CV = coefficient of variation, Skew = skewness, Kurt = kurtosis; [(c)] * = significant different from zero at 95% probability level, ns = not significant.

Table 3 - Correlation analysis between linear dimension and leaf area and among leaf dimensions of Flue-cured tobacco in China.

		L	W	L×W	LA
L	CC	1	-0.11	0.46	0.45
	p	--	0.08	<0.01	<0.01
W	CC	-0.11	1	0.83	0.83
	p	0.08	--	<0.01	<0.01
L×W	CC	0.46	0.83	1	0.99
	p	<0.01	<0.01	--	<0.01
LA	CC	0.45	0.83	0.99	1
	p	<0.01	<0.01	<0.01	--

[a]L = length, W = width, L×W = length×width, LA = real leaf area; [b] CC = correlation coefficient, P = P value.

significant, but Parameter b in the Linear model was not significant. The Proportional should be considered as the optimum among the three models based on the Akaike delta information criterion (AIC) and Bayesian information criterion (BIC) listed in table 4.

The Proportional model was adopted by many researchers (SUGGS et al., 1960; MOUSTAKAS & NTZANIS, 1998; BOZHINOVA, 2006; TOEBE et al., 2020) to investigate the relationship between real leaf area and length×width (L×W) of fresh leaves from different tobacco type and variety. Though the effects of model fitting were good, the parameters of different tobacco types were different, Burley type had a coefficient of nearly 0.70, compared to 0.63-0.65 for Flue-cured tobacco. But the parameter of dried Flue-cured tobacco illustrated in figure 2 was 0.495, much lower than its fresh counterpart. The parameter has physical meaning (SUGGS et al., 1960). It can be denoted as the ratio of the area occupied by tobacco leaf from its circum-rectangle, so the decrease of parameter suggested a shrink of fresh tobacco surface during drying process that is a common understanding to tobacco worker.

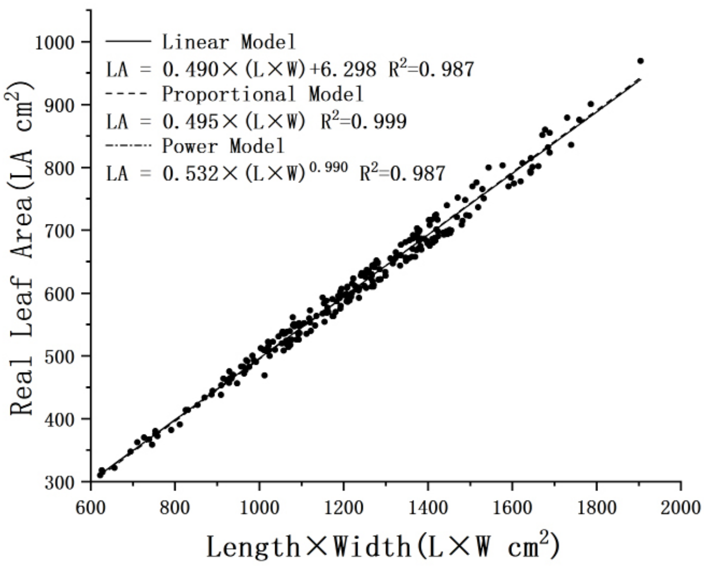

Figure 2 - Linear, Proportional and Power model for estimating real leaf area (LA) in relation to length×width (L×W) based on universal model development dataset and coefficient of determination on each model for dried Flue cured tobacco in China.

Table 4 - Model and parameters evaluation among Linear, Proportional and Power equations for estimating real leaf area (LA) in relation to length×width (L×W).

Model	Ta	Tb	MMS	RMSE	F value	AIC	BIC
Linear	138.21**	1.42ns	3.46	181.14	19101.09**	1314.20	1324.79
Proportional	729.71**	_	9.68	181.87	532470.30**	1314.22	1321.28
Power	18.50**	131.37**	4.84	181.33	267023.47**	1314.47	1325.06

[a]Ta = t value for Parameter a, Tb = t value for Parameter b, MMS = mean of sum of square from the model, RMSE = mean of sum of square from residual error, AIC = Akaike delta information criterion, BIC = Bayesian information criterion; [b] ** = extremely significant, ns = not significant.

The model development dataset were split into four parts according to growing area, four parts of data were fitted by the Proportional model separately and four growing area-specific equations were obtained and illustrated in figure 3. Growing area-specific equations can appropriately expressed the relationship between real leaf area and length×width (L×W) of tobacco leaves from different growing area. The coefficients of determination (R^2) in each equation were more than 0.95 which ensured the validity of equations, but less than that derived from whole model development dataset (Universal equation). The decrease of R^2 can be attributed to the decline of sample numbers used in the growing area-specific model simulation.

The model development dataset was segmented based on stalk position in figure 4 and three stalk position-specific equations were established. The Proportional model still works on the simulation of real leaf area if data of Flue-cured tobacco from different stalk positions were used separately. A decrease in coefficient of determination in stalk position-specific

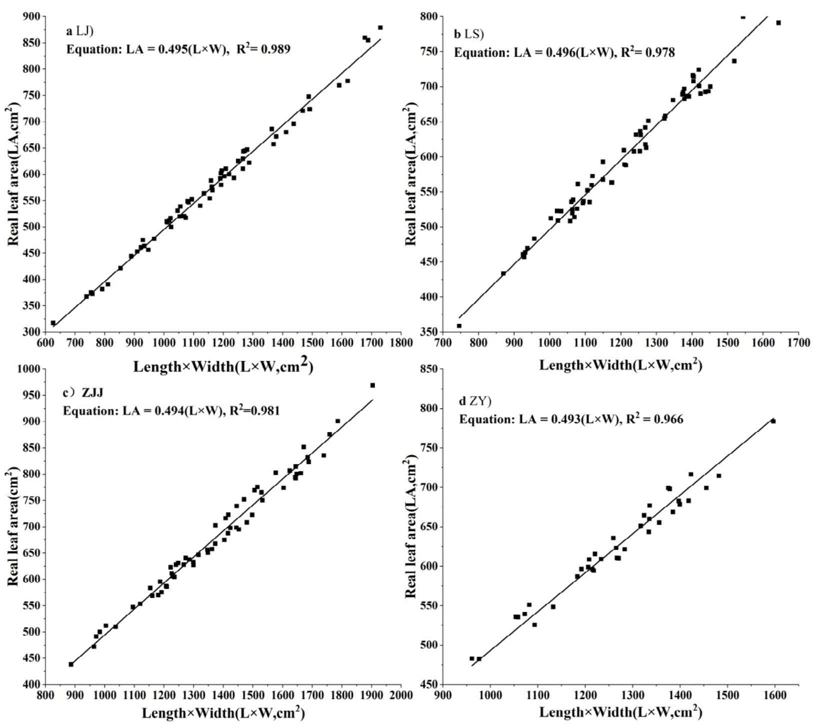

Figure 3 - Equations simulated by Proportional model between real leaf area(LA) and length×width (L×W) of dried flue-cured tobacco leaves from different locations in China: a) Lijian (LJ), b) Liangshan (LS), c) Zhangjiajie (ZJJ) and d) Zunyi (ZY).

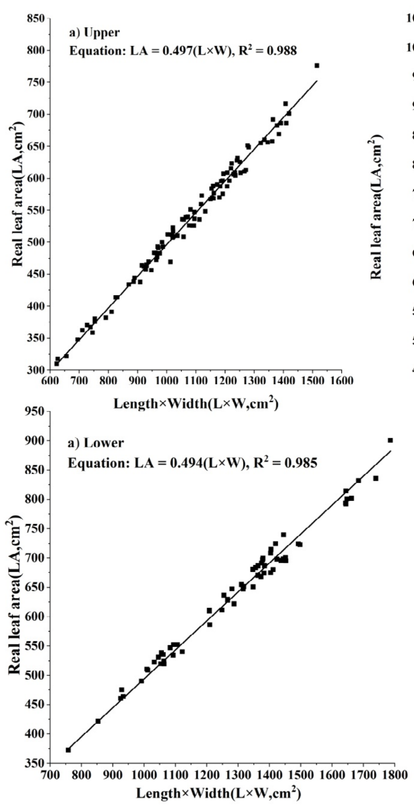

Figure 4 - Equations simulated by Proportional model between real leaf area (LA) and length×width (L×W) using dried Flue cured tobacco in China from different stalk positions: a) Upper leaves, b) Middle leaves and c) Lower leaves.

equations relative to the Universal equation was also shown in figure 4, proving again that a small sample size led to the decline of R^2.

The growing area-specific equations and stalk position-specific equations had similar parameters to universal equations, altering only at the 0.001 level. In other words, the coefficient of equation in the research was not affected by growing area, stalk position, and even variety if considering the fact that three cultivars were planted in the growing areas.

A generalized linear model was employed to examine the effects of growing area and stalk position on the estimation of leaf area from length×weight, the statistical results listed in table 5 proved again that growing areas and stalk positions had no significant effects on the Proportional model, however, length×weight had a significant effect on the model.

The impacts of variety, stalk position and culture practice on parameter of fresh tobacco were controversial: the Flue-cured tobacco cultivar NC 2326 had different parameters ranged from 0.54 to 0.69 in various stalk positions (MAW & MULLINIX, 1992); In BOZHINOVA et al. (2006)'s study, it was concluded that individual parameters should be used in different Burley varieties, but in two of the three studied cultivars, leaf position did not affect the value of parameters; SCHLÖSSER et al. (2020) stated that measurements of the leaf area of tobacco *Nicotiana tabacum L* can be estimated from a single model; MOUSTAKAS et al. (1998) calculated a coefficient of 0.653 on estimating Flue-cured tobacco area from linear measurements under Mediterranean conditions, nitrogen treatment and growth stage had no effect on the value of parameter; McKee et, al. (1970) studied the parameters for estimating the leaf area of 3 varieties of Type 41, Pennsylvania Broadleaf , 0.64 could be used for the calculation of leaf area at±1% accuracy, but different parameters ranging from 0.61 to 0.65 should be used in each variety for greater precision.

SUGGS, et al. (1960) developed an ellipse model to depict the shape of tobacco and explained from a theoretical point of view the stability of parameter against varieties and irrigation treatments. But RAPER et al. (1974) argued that the ellipse model should not be used directly in the calculation of parameter

Table 5 - Parameters estimation of Proportional model using growing areas and stalk positions as dummy variable.

Parameter	a	SE	-------95% Wald's CI-----		-----------------Hypothesis testing----------------		
			LCL	UCL	Wald's χ^2	DF	Sig
LJ	2.00	5.93	-9.62	13.63	0.11	1	0.74
LS	3.00	6.36	-9.47	15.48	0.22	1	0.64
ZJJ	1.09	7.05	-12.73	14.91	0.24	1	0.88
ZY	-0.60	6.91	-14.14	12.95	0.00	1	0.93
B	2.23	2.41	-2.49	6.95	0.86	1	0.35
C	0.43	2.27	-4.01	4.88	0.04	1	0.85
X	0.00	-	-	-	-	-	-
L×W	0.49	0.00	0.48	0.50	11136.37	1	0.00

(a)LJ = Lijian, LS = Liangshan, ZJJ = Zhangjiajie, ZY = Zunyi, B = upper leaves, C = middle leaves, X = lower leaves; (b) a = Parameter a in Proportional model, SE = standard error of Parameter a, 95% Wald's CI = 95% Wald's Confidence interval, LCL = lower confidence limit, UCL = upper confidence limit, DF = degree of freedom, Sig = significance.

due to the variation of leaf shape under different growth stages and the distortion of leaf surface, a correction factor was introduced to adjust the coefficient for better estimation of tobacco leaf area. Dried Flue-cured tobacco was studied in this manuscript instead of a fresh one, Flue-cured tobacco was harvested and dried at the mature stage when the leaf tissue was fully stretched and the variation of leaf shape was minor. The leaf was suppressed for 2 days to flatten the distortion on the surface as mentioned in MATERIALS AND METHODS section before measurements of leaf dimensions. So the leaf area of dried Flue-cured tobacco in China was estimated well by the Proportional equation LA = 0.495(L×W) after the elimination of risks hindering the application of the ellipse model on tobacco leaf area estimation.

To further examine the applicability of the universal equation established in the manuscript, leaf area in the external evaluation dataset was estimated using the universal equation, growing area-specific equation, or stalk position-specific equation individually. T-test was used to examine the difference among real leaf area (LA), universal equation estimated area (ULA), and growing area-specific equation estimated leaf area (GLA), or among real leaf area, universal equation estimated area (ULA), and stalk position-specific equation estimated leaf area (SLA) in table 6. No significant difference

Table 6 - T-test among real leaf area (LA), universal equation estimated area (ULA) and growing area-specific equation estimated leaf area (GLA) or stalk position-specific equation estimated leaf area (SLA) using external evaluation dataset.

----------t-test among LA, ULA and GLA----------			
growing area	----------t value----------		
	LA versus ULA	LA versus GLA	ULA versus GLA
LJ	-0.036^{ns}	-0.036^{ns}	0.000^{ns}
LS	-0.071^{ns}	-0.103^{ns}	-0.071^{ns}
ZJJ	-0.014^{ns}	0.026^{ns}	0.407^{ns}
ZY	-0.162^{ns}	-0.100^{ns}	-0.162^{ns}
----------t-test among LA, ULA and DLA----------			
stalk position	----------t value----------		
	LA versus ULA	LA versus SLA	ULA versus SLA
Upper	-0.014^{ns}	-0.016^{ns}	-0.081^{ns}
Middle	-0.159^{ns}	-0.159^{ns}	0.000^{ns}
Lower	0.023^{ns}	0.060^{ns}	0.023^{ns}

(a)LJ = Lijian, LS = Liangshan, ZJJ = Zhangjiajie, ZY = Zunyi; (b) LA = real leaf area, ULA = universal equation estimated area, GLA = growing area-specific equation estimated leaf area, SLA = stalk position-specific equation estimated leaf area; (c) Upper = upper leaf, Middle = middle leaf, Lower = lower leaf; (d) ns = not significant different from zero at 95% probability level.

was observed between real leaf areas and estimated leaf areas which were the same in statistics too. The universal equation LA = 0.495 (L×W) can predict the leaf area of dried Flue-cured tobacco in China perfectly.

CONCLUSION

A universal model equation LA = 0.495 (L×W) can be used in the estimation of leaf area (LA) of dried Flue-cured tobacco in China from leaf length×width (L×W), no matter which growing area, stalk position or variety the tobacco leaf belongs to.

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DECLARATION OF CONFLICT OF INTEREST

The authors declared no conflict of interest. The sponsor plays no role in experiment design, in data collection, analysis and interpretation, in manuscript compilation or in decision to publish.

AUTHORS' CONTRIBUTION

All authors took part equally in constructing the manuscript. All authors read the final version of the manuscript and agreed to publish it.

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