

# Estimation and Validation of the Leaf Areas of Five June-bearing Strawberry (*Fragaria × ananassa*) Cultivars using Non-destructive Methods

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**Abstract.** Non-destructive estimation of leaf area is a more efficient and convenient method than leaf excision. Thus, several models predicting leaf area have been developed for various horticultural crops. However, there are limited studies on estimating the leaf area of strawberry plants. In this study, we predicted the leaf areas via nonlinear regression analysis using the leaf lengths and widths of three-compound leaves in five domestic strawberry cultivars ('Arihyang', 'Jukhyang', 'Geumsil', 'Maehyang', and 'Seolhyang'). The coefficient of determination ( $R^2$ ) between the actual and estimated leaf areas varied from 0.923 to 0.973. The  $R^2$  value varied for each cultivar; thus, leaf area estimation models must be developed for each cultivar. The leaf areas of the three cultivars 'Jukhyang', 'Seolhyang', and 'Maehyang' could be non-destructively predicted using the model developed in this study, as they had  $R^2$  values over 0.96. The cultivars 'Arihyang' and 'Geumsil' had slightly low  $R^2$  values, 0.938 and 0.923, respectively. The leaf area estimation model for each cultivar was coded in Python and is provided in this manuscript. The estimation models developed in this study could be used extensively in other strawberry-related studies.

**Additional key words :** leaf area estimation, non-destructive measurement, python

## Introduction

The most commonly cultivated strawberry *Fragaria × ananassa* is an octoploid with 56 chromosomes (Hancock, 2020). Strawberries are cultivated worldwide in an area of 384,668 ha, with a harvest of 23,036 kg/ha (FAOSTAT, 2022). The cultivation area in Korea is 5,683 ha, with 163,646 tons of strawberries production (KOSIS, 2021). The 'Seolhyang' cultivar is ranked first in the domestic strawberry market (84.5%), followed by the 'Geumsil', 'Jukhyang', and 'Maehyang' cultivars. In 2020, Korean strawberry exports amounted to 4,823 tons, with the primary cultivars being 'Geumsil', 'Maehyang', and 'Arihyang' (RDA, 2022).

Strawberry plants are perennial herbs with leaves, roots, stems, and a crown. Leaves, roots, runners, and flower buds grow from the crown. The leaves of strawberry plants are

trifoliate and appear spirally from the crown. The main functions of the leaves in plants are to absorb light for photosynthesis and assist with transpiration and respiration. The factors resulting in a reduction in the amount of light available to the plant can affect photosynthesis, the evapotranspiration rate, and water regulation. These functions are affected by the leaf area indices of the plants (Blanco and Folegatti, 2005; Syvertsen et al., 2003). Several studies have demonstrated the importance of leaf area in estimating crop growth, development rate, harvest potential, light utilization efficiency, and water and nutrient uptake (Bhatt and Chanda, 2003; Keramatlou et al., 2015; Olivera and Santos, 1995; Williams, 1987). Furthermore, studies have shown that there is a close relationship between the leaf area and fruit quality or maturity of fruit crops (Torri et al., 2009; Usenik et al., 2008). Thus, measurement of the leaf area is essential for the evaluation of plant growth, fruit yield, and fruit quality.

Leaf area is usually measured using different approaches, including photography, image analysis, and electronic leaf

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area meters. However, these methods are destructive, as they require leaf excision. Destructive methods are also costly and time-consuming. Recently, non-destructive measuring devices, such as hand scanners, have been developed. However, these are expensive and challenging to use on certain crop types (Yun et al., 2016). The advantage of using non-destructive leaf area measurement is that it can be performed continuously and repeatedly for the same plant, reducing the variability and damage caused by leaf excision (NeSmith, 1991). Therefore, a simple, fast, reliable, cost-effective, and non-destructive method for measuring the leaf area is necessary. A simple linear equation to predict the leaf area of a horticultural plant non-destructively can be performed without expensive leaf area measuring equipment (Olfati et al., 2010; Salerno et al., 2005). The high accuracy and precision of non-destructive methods based on linear measurements have been demonstrated for several crops (Astegiano et al., 2001; Guo and Sun, 2001; Silva et al., 2015; Salerno et al., 2005; Tsiatas and Maslaris, 2005). However, there are few studies using non-linear equations for estimating leaf area, and studies on estimating leaf area of strawberries have not been conducted since 2005 (Demirsoy et al., 2005). As well, current research on the estimation of leaf area in domestic strawberry cultivars is limited.

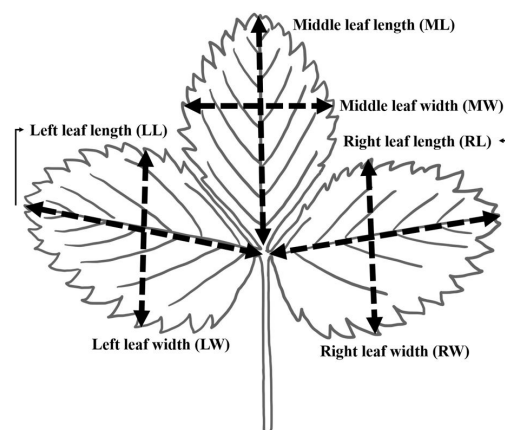
In this study, nonlinear regression analysis was performed to develop a method to estimate the trifoliate leaf area based on the lengths and widths of three leaflets in the following five domestic strawberry cultivars: ‘Arihyang’, ‘Jukhyang’, ‘Keumsil’, ‘Maehyang’, and ‘Seollhyang’. The Python code used to develop the model will be made available to other researchers in the field.

## Materials and Methods

In this study, five domestic strawberry cultivars (‘Arihyang’, ‘Jukhyang’, ‘Keumsil’, ‘Maehyang’, and ‘Seollhyang’) were used to develop an equation to estimate the leaf area. Two of the strawberry cultivar seedlings, ‘Maehyang’ and ‘Seollhyang’, were purchased from the Hamyang-gun seedling company in Gyeongsangnam-do (35°39'N, 127°40'E, elevation 448 m). ‘Keumsil’ strawberry seedlings were purchased from the Jinju strawberry farm in Gyeongsangnam-do (35°13'N 127°57'E, elevation 41 m). ‘Arihyang’ strawberry seedlings

were obtained from the National Institute of Horticulture and Herbal Science (35°49'N 127°01'E, elevation 30 m), and ‘Jukhyang’ strawberry transplants were obtained from Damyang-gun’s agricultural extension (35°18'N 126°58'E, elevation 44 m). In a tunnel-type greenhouse (25 × 7 m<sup>2</sup>), located at an experimental farm of the Kyungpook National University (35°52'N 128°35'E, elevation 30 m), strawberry transplants of the five cultivars were planted in two rows at 20 cm intervals on September 10, 2021.

Sampling of the leaves involved randomly collecting five leaves of each cultivar at intervals of 3 to 7 days from November 1, 2021 to January 24, 2022. The length, width, and area of each three-compound leaf were measured using the collected leaves. Leaf width (cm) was measured at the widest part of the leaf, and leaf length (cm) was measured from the tip of the leaf to the point of petiole intersection (Fig. 1). The actual area of the leaf was measured using an LI-3100 meter (Licor Biosciences, Lincoln, NE, USA). The leaf area was estimated, using 80 leaves for each cultivar, through nonlinear regression analysis (3D, Paraboloid) of Sigma plot 14.5 (SigmaPlot 14.5, SPSS Inc., USA). The independent variables, including the length and width of the middle leaf (ML and MW, respectively), ML and width of the left leaf (LW), ML and width of the right leaf (RW), MW and length of the left leaf (LL), and MW and length of the right leaf (RL), were combined. The model developed in this study was coded using Python in a Jupyter notebook-based environment.

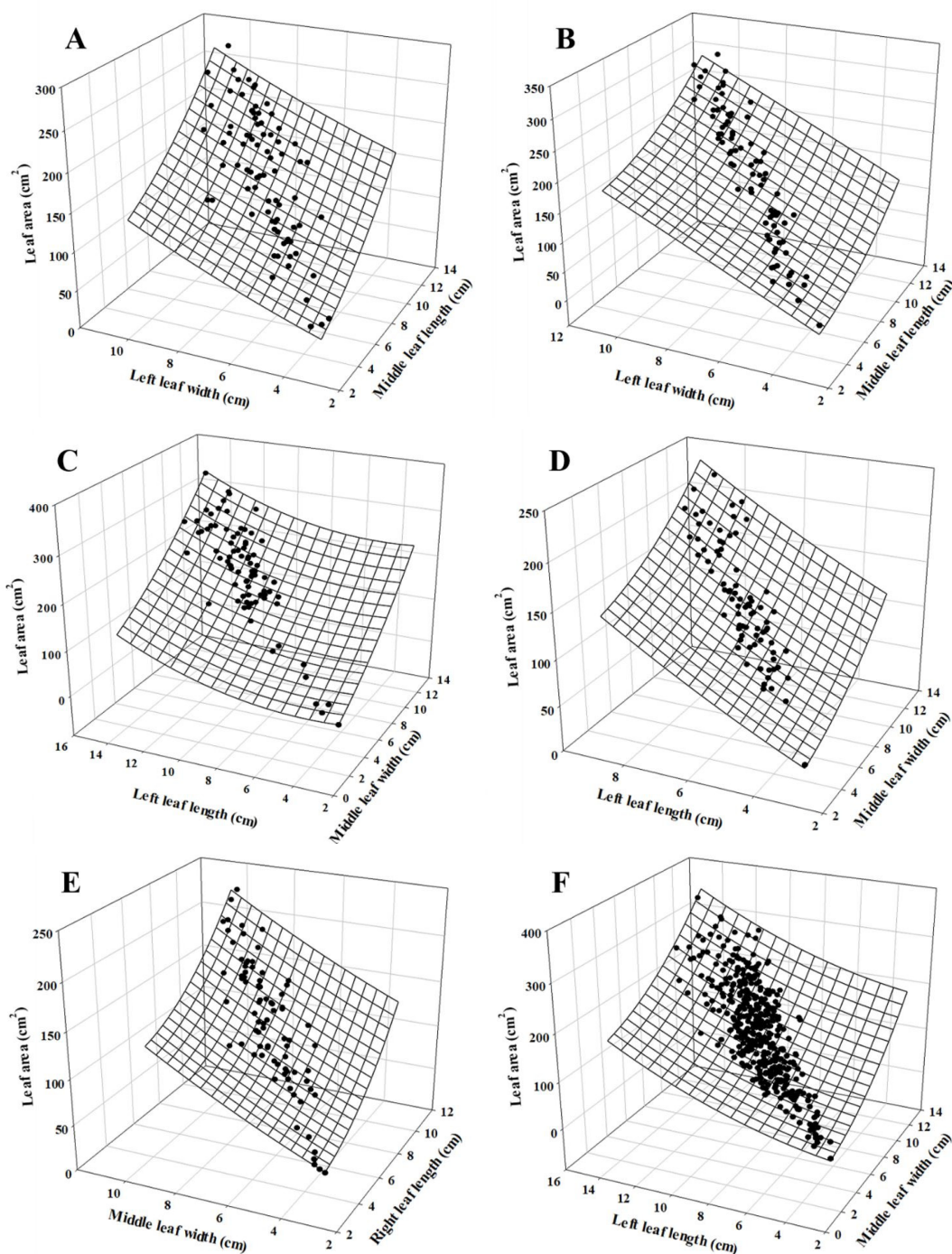


**Fig. 1.** Diagram of the strawberry three-compound leaf showing the measurement positions for leaf length and leaf width used to predict the leaf area.

## Results and Discussion

In this study, several nonlinear equations were evaluated to estimate the leaf areas of five strawberry cultivars. Leaf area was estimated using the lengths and widths of individual

leaves from each variety as independent variables (Fig. 2). In the case of ‘Seolhyang’, the independent variables MW and RL showed the highest coefficient of determination ( $R^2 = 0.964$ ). ‘Maehyang’ and ‘Keumsil’, which have upright leaves, showed the highest  $R^2$  values (0.960 and 0.923,

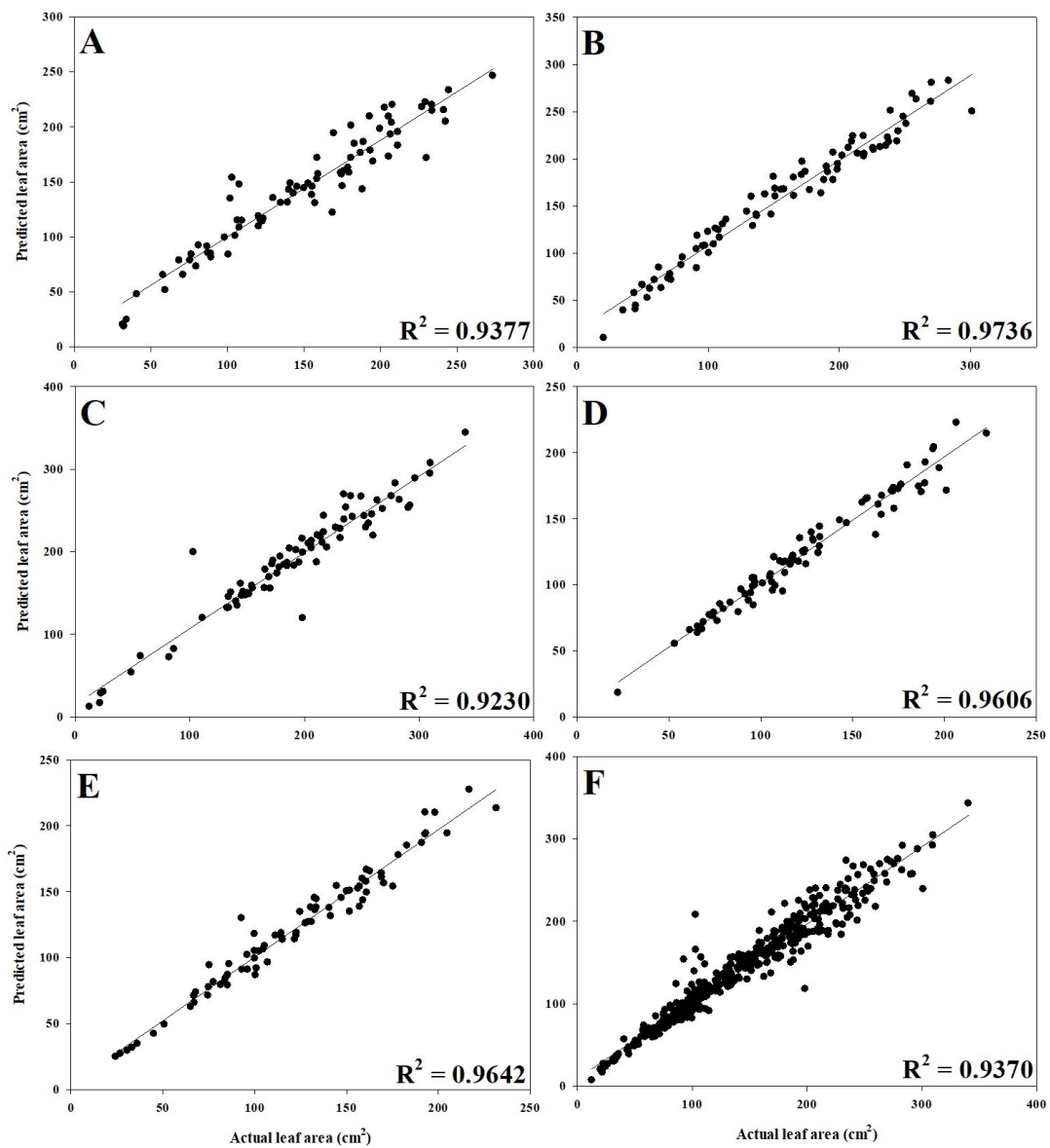


**Fig. 2.** Leaf area estimation model established using 3D nonlinear regression analysis (A: ‘Arihyang,’ B: ‘Jukhyang,’ C: ‘Keumsil,’ D: ‘Maehyang,’ E: ‘Seolhyang,’ F: Total leaves; n = 80).

**Table 1.** Relationship between the actual strawberry leaf area of each cultivar and the independent variable used in the non-destructive estimation method.

Cultivar	Regression equation	x	y	y0	a	b	c	d	Standard error	R <sup>2</sup>
Arihyang		CL	LW	-46.8247	2.9588	8.6907	0.8706	0.3442	14.79	0.9377
Jukhyang		CL	LW	-56.7406	-3.1558	20.7433	0.9916	-0.033	11.89	0.9736
Keumsil	$LA = y_0 + a \times x + b \times y + c \times x^2 + d \times y^2$	CW	LL	-3.7433	11.9907	-7.9649	0.8282	0.9531	20.02	0.9230
Maehyang		CW	LL	-33.7759	13.141	-0.1245	0.4744	0.6415	8.70	0.9606
Seollhyang		RL	CW	-3.7635	-7.7361	9.3429	1.5336	0.3409	9.19	0.9642
Total leaves		CW	LL	-12.8336	2.0435	2.0115	1.1497	0.6503	16.04	0.9370

LA, leaf area; ML, middle leaf length; MW, middle leaf width; LL, left leaf length; LW, left leaf width; RL, right leaf length.



**Fig. 3.** Relationship between the actual leaf area measurements of strawberries and the leaf area estimated using the model proposed in Table 1 (A: ‘Arihyang,’ B: ‘Jukhyang,’ C: ‘Keumsil,’ D: ‘Maehyang,’ E: ‘Seollhyang,’ F: Total leaves; n = 80).

respectively) with MW and LL as independent variables. ‘Arihyang’ and ‘Jukhyang’, which have semi-open leaves, had the highest  $R^2$  values (0.9377, 0.9736 respectively) when ML and LW were used as independent variables (Table 1). Leaf shape affects the determination of the independent variables used for estimating leaf area. Analysis of the leaf shape ratio for each strawberry cultivar confirmed that the leaf shape ratios of ‘Keumsil’ and ‘Maehyang’ were 1.3 and 1.4, respectively. These ratios were higher than those of the other cultivars (data not shown). When all leaves of each cultivar were used, the  $R^2$  value (0.9370) was highest when MW and LL were used as variables. As shown in this study, estimated of leaf area for each cultivar showed a higher  $R^2$  value than that for estimated of combined each cultivar, and the  $R^2$  value was also very different for each cultivar. ‘Jukhyang’ showed the highest  $R^2$  value among all cultivars, with ‘Seolhyang’ and ‘Maehyang’ exhibiting the next highest  $R^2$  values. ‘Arihyang’ and ‘Geumsil’ had the lowest  $R^2$  values. A lower  $R^2$  value was possibly caused by poor seedling conditions initially observed in the ‘Arihyang’ cultivar, compared to those in the other cultivars, and due to excessive growth in the ‘Geumsil’ cultivar.

Several studies have reported a close relationship among leaf length, width, and area in horticultural crops (NeSmith, 1991; Robbins and Pharr, 1987; Uzun and Çelik, 1999). The leaf area estimation equation of the recently developed model for strawberries was “leaf area = 1.89 + 2.145 upper leaf length left leaf width,” resulting in a very high  $R^2$  value of 0.993 (Demirsoy et al., 2005). However, the strawberry cultivars used in the above-mentioned study were ‘sweet Charlie’ and ‘camarosa’, which are grown mainly in California and Florida. Applying Demirsoy’s model to our data, an  $R^2$  value of 0.9136 was obtained, which was lower than the  $R^2$  value obtained with the model developed in this study (data not shown). In addition, it was lower than the  $R^2$  values of the individual cultivars (Fig. 3). The reliability of the developed models may differ depending on the cultivar; thus, development of additional models suitable for a cultivar or improvement of the developed model is necessary.

Many growth and yield models developed recently are being coded in various languages and shared (Pradal et al., 2008). Programming languages include Python, Jullia, C,

and Java. Among them, Python can use various frameworks, and has the advantage of being open source and easy to use. Therefore, in this study, the leaf area estimation model developed using the Python language was coded.

The leaf area could be predicted non-destructively using the model developed in this study for ‘Jukhyang’, ‘Seolhyang’, and ‘Maehyang’ cultivars, with  $R^2$  values of 0.96 or higher. By measuring the leaf area using a non-destructive method, the functions of leaves, such as respiration, transpiration, and photosynthesis, can be evaluated. However, the ‘Arihyang’ and ‘Keumsil’ cultivars had low  $R^2$  values; hence, additional experiments or corrections are recommended. The leaf area estimation model for each cultivar developed in this study was also coded using Python and shared on github (VanRossum and Drake, 2010; GitHub address: skkim76/KNU-PHL-project (github.com)); it is available for use by other researchers.

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## 일계성 딸기 5품종의 비파괴적 방법을 사용한 엽면적 추정 및 검증

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**적 요.** 엽면적의 비파괴적 추정은 잎 절단에 의한 방법에 비해 간단하고 시간을 절약하므로 다양한 원예 작물에 대한 잎 면적 예측 모델이 개발되었습니다. 그러나 딸기 잎 면적 추정에 대한 연구는 제한적이다. 본 연구는 국내산 딸기 5품종(‘아리향’, ‘죽향’, ‘금실’, ‘매향’, ‘설향’)의 3복엽의 잎길기와 폭을 이용한 비선형회귀분석을 통해 잎면적을 예측하기 위해 수행되었다. 실제 잎 면적과 전개식을 통해 추정된 잎 면적의 관계에 대한 결정계수( $R^2$ )는 0.923에서 0.973까지 다양하였다.  $R^2$  값이 0.96 이상인 ‘죽향’, ‘설향’ 및 ‘매향’ 3 품종의 잎면적은 본 연구에서 개발한 모델을 사용하여 비파괴적으로 예측할 수 있습니다. 반면 묘의 상태가 좋지 못하고 과한 생장을 했던 ‘아리향’과 ‘금실’은 각각 0.938, 0.923으로 약간 낮은  $R^2$  값을 보였다. 본 연구에서 개발한 품종별 잎 면적 추정 모델을 Python으로 코딩하여 본 논문에 첨부하였다. 개발된 추정 모델은 많은 딸기 관련 연구에 광범위하게 사용될 수 있습니다.

**추가 주제어:** 잎 면적 추정, 비파괴적 측정, 파이썬