



Analysis of leaf photosynthetic rates of hydroponically-grown paprika (*Capsicum annuum* L.) plants according to vertical position with multivariable photosynthesis models

Dae Ho Jung¹ · Inha Hwang¹ · Jiyong Shin¹ · Jung Eek Son¹

Received: 12 June 2020 / Revised: 13 August 2020 / Accepted: 31 August 2020 / Published online: 3 November 2020
© Korean Society for Horticultural Science 2020

Abstract

The photosynthetic rates of leaves depend on the vertical position and cultivation conditions. However, few models have been proposed to express photosynthesis according to leaf position, and there was a lack of quantitative analysis between physiological indicators and model parameters. The objectives of this study were to analyze the leaf photosynthetic characteristics of paprika plants according to leaf vertical position using photosynthesis models, and to analyze the relationship between the total nitrogen content and the photosynthetic model parameters. Leaf photosynthetic rates at different vertical positions were measured under varying light intensities and CO₂ concentrations in triplicate. Rectangular hyperbola and FvCB (Farquhar, von Caemmerer, and Berry) models were selected, calibrated, and validated as multivariable photosynthesis models. Total nitrogen contents and SPAD values were measured at each leaf position and the coefficients of the photosynthetic rate models were compared. The R² values for the rectangular hyperbola and FvCB models were 0.86 and 0.91, and the RMSE values were 4.651 and 2.104, respectively. Total nitrogen content linearly increased with increasing vertical leaf position and it was linearly related to the maximum carboxylation capacity and maximum electron transport rate, estimated in the FvCB model. In this study, the FvCB model was considered more suitable for expressing the relationship between total nitrogen contents and plant's physiological responses according to the vertical position of leaves. The vertical leaf photosynthetic rate models established in this study will contribute to determining optimal environmental conditions for maximizing crop photosynthesis and establish the criteria for precise CO₂ enrichment in greenhouses.

Keywords CO₂ concentration · Hydroponics · Leaf photosynthesis model · Light intensity · Nitrogen content

1 Introduction

The photosynthetic rates of leaves depend on the vertical position and cultivation conditions. In order to estimate the overall photosynthetic response of a crop, it is important to understand how different photosynthetic characteristics depend on the vertical position (Jung et al. 2018). The light intensities at the bottom of densely-planted crops are greatly reduced by shading effects among adjacent plants (Chen

et al. 1999). A decrease in light intensity will impair crop growth and consequently reduce the photosynthetic rate and crop production (Aminifard et al. 2012). In addition, nutrient and pigment contents such as nitrogen and chlorophyll affects the photosynthesis of crops. In order to increase the production of fruit vegetable crops, quantitative methods for assessing crop photosynthesis under various environmental and physiological conditions are necessary. Recently, modeling technique has attracted attention as a method to quantify environmental factors affecting photosynthesis (Medina-Ruíz et al. 2011; Noe and Giersch 2004).

In the past, simple photosynthesis models using a single variable were widely used, but recently, the use of complex multivariate models are increasing. Typically, simple multiplication models were used to quantify the photosynthetic rate for a single environmental factor (Jones et al. 1991; Park et al. 2016), but these models do not reflect the physiological characteristics, such as electron transfer rates of crops. This

Communicated by Young Yeol Cho, Ph.D.

✉ Jung Eek Son
sjeenv@snu.ac.kr

¹ Department of Agriculture, Forestry and Bioresources (Horticultural Science and Biotechnology Major), Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea

has led to an increasing demand for improved models. Rectangular hyperbola or negative exponential model has been mainly used to express photosynthetic rates for light intensity and CO₂ concentration (Baker and Allen 1993; Valladares et al. 1997; Thornley 1974) developed a simple model reflecting the chemical reactions that occur in light and dark reactions of photosynthesis. The Farquhar, von Caemmerer, and Berry model (FvCB model) is the most widely used in recent years (Farquhar et al. 1980; Yin et al. 2009). The FvCB model uses complex expressions corresponding to the physiological response of crops, but is considered to be the most suitable for determining changes in photosynthetic rate due to various environmental factors (Kim et al. 2016; Qian et al. 2012). Previous studies have identified photosynthesis changes with leaf age (Constable and Rawson 1980), but using leaf position is practically convenient because paprika leaves occur regularly along the phyllotaxis. Photosynthetic rate models have not been accurately validated and compared for each vertical leaf position in the crop.

According to Shin et al. (2011), the whole-plant photosynthetic rate was 30 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under 3000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ of light intensity in the case of ‘Fiesta’ paprika. To reflect the photosynthetic rate of the whole plant, affected by the vertical position of the leaves, an adequate model incorporating environmental factors with photosynthetic rate is required. Kim et al. (2016) measured the photosynthetic rate for each leaf position, but no comparison was performed between photosynthesis models. In addition, nitrogen affects the production of biochemical substances such as proteins, amino acids, nucleic acids, enzymes, and chlorophyll in plants (Suharja and Sutarno 2009), and the nitrogen content in the plants depends on the light distribution pattern of the plant canopy (Ellsworth and Reich 1993). With recent advances in three-dimensional plant modeling, the vertical light distribution and subsequent physiological changes of plants have been studied (Le Roux et al. 1999a; Sinoquet et al. 2001). In this case, an appropriate photosynthesis model for each position is required. In addition, indicators, such as nitrogen content, express the physiological response of plants. Thus, the relationship between physiological indicator and model parameter can be analyzed to determine the crop growth condition. However, few attempts have been made to interpret physiological response of paprika by comparing several photosynthesis models.

The objectives of this study were to analyze the leaf photosynthetic characteristics of paprika plants according to the vertical position of leaves using photosynthesis models, and to analyze the relationship between the total nitrogen content and the photosynthetic model parameters.

2 Materials and methods

2.1 Cultivation conditions

To investigate the leaf photosynthetic rate of paprika plants (*Capsicum annuum* L. ‘Scirocco’), experiments were conducted in Venlo-type greenhouses of the Protected Horticulture Research Institute, National Institute of Horticultural and Herbal Sciences (RDA), Haman, Korea (35.2°N, 128.4°E) for two cultivation periods. The setting temperatures for ventilation during the day and heating at night in the greenhouse were 30 °C and to 21 °C, respectively. The plants were sown on a tray on February 08, 2018 and May 06, 2019, and transferred to cubes on March 05, 2018 and June 07, 2019, respectively. During the seedling period, the electrical conductivity (EC) of PBG paprika nutrient solutions was initially set to 0.8 dS m^{-1} , gradually increased by 0.2 dS m^{-1} per week, and maintained at 2.5 dS m^{-1} at the end. After raising seedlings, the plants were transplanted on the slabs with a planting density of 2 plants/m on April 06, 2018 and July 10, 2019. Four cubes were planted on each slab. After transplanting, nutrient solutions with EC 2.5 dS m^{-1} and pH 6.0 were supplied 14 times a day at 33 mL per plant by drip irrigation.

2.2 Measurements of leaf photosynthetic rate, SPAD value, and leaf total nitrogen content

Leaf photosynthetic rates were measured twice over two years for model establishment and verification. On July 04, 2018 and October 07, 2019, the first measurements were conducted from 10:00 to 15:00 to avoid photosynthesis afternoon depression (Kim et al. 2016; Qian et al. 2012). Leaf photosynthetic rates were measured using a portable photosynthesis measuring device (LI-6400, LI-Cor. Inc., Lincoln, NE, USA) with a 6400-02B LED light source chamber. Light intensities were set to 0, 50, 100, 200, 400, 900, 1500 and 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, and CO₂ concentrations in the chamber were set to 50, 100, 400, 800, and 1200 $\mu\text{mol mol}^{-1}$, as previously employed by Schaffer et al. (1997). A light response curve at 50 $\mu\text{mol mol}^{-1}$ CO₂ level was derived and the same curve derived sequentially at the next CO₂ level. Block temperature, relative humidity and flow rate of an infrared CO₂ gas analyzer (6400-02B, LI-Cor. Inc., Lincoln, NE, USA) were controlled at 25 °C, 65–85%, and 500 $\mu\text{mol s}^{-1}$, respectively. To determine specifically how the photosynthetic rate model depends on each vertical leaf position, measurements of photosynthetic rate were made for eight levels of light intensities and five levels of CO₂ concentrations for each vertical leaf

position. The leaves used for the measurements were fully-expanded and the plants were in the reproductive phase. Measurements were taken in triplicate on different leaves of paprika at heights of 0, 25, 50, 75, 100, 125, and 150 cm as shown in Fig. 1.

The second measurements were conducted from 10:00 to 15:00 on July 05, 2018 and October 11, 2019, and the leaf photosynthetic rate was obtained to verify the model established in the first measurement. Light intensities were set to 0, 100, 400, 800, and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and CO_2 concentrations were set to 100, 400, 800, and 1200 $\mu\text{mol mol}^{-1}$. The measurement method was the same as the first, and measurements were made on five levels of light intensity and four levels of CO_2 concentration for each position of the leaves. Each measurement was performed in triplicate in different leaves.

The SPAD values were measured with a chlorophyll meter (SPAD-502, Minolta, Osaka, Japan) and recorded as a mean of 10 measurements for each individual leaf. Measurements were taken along the edge of the leaf and were measured three times on different leaves at each position. Leaves were sampled at each position and finely ground through

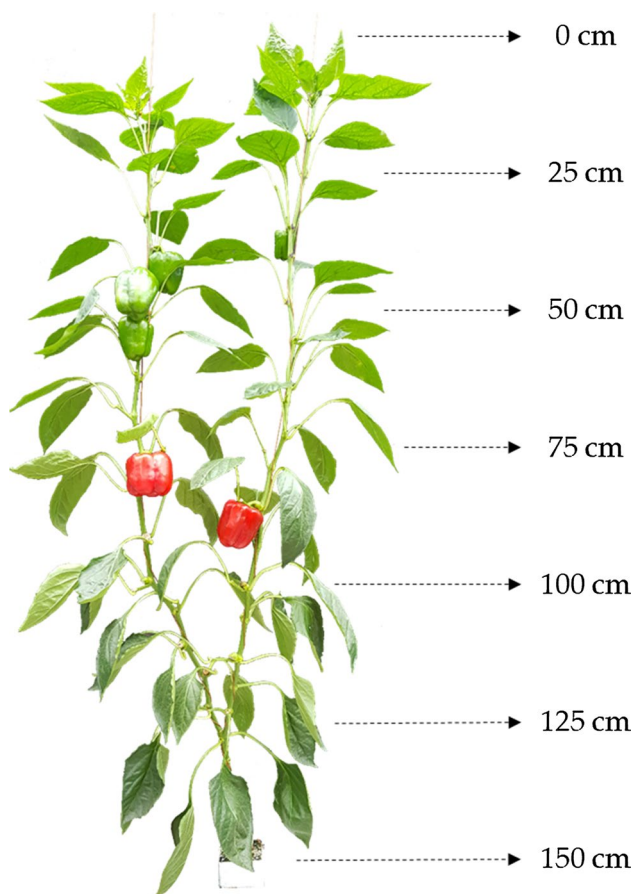


Fig. 1 Vertical positions of leaf photosynthetic rate measurement in hydroponically-grown paprika plants

a mill after freeze-drying. Leaf total nitrogen content was analyzed using the Kjeldahl method (Bremner 1960).

2.3 Estimation of intercellular CO_2 concentration

In order to express the A/C_i curve (where A is the net CO_2 assimilation rate and C_i is intercellular CO_2 concentration) and to calculate leaf photosynthetic rate with the FvCB model using the measured atmospheric CO_2 concentration, the relationship between the atmospheric and intercellular CO_2 concentrations was regressed. Eight models are available that express the CO_2 exchange between the atmosphere and the leaves of plants. In this study, the widely used Ball-Berry model was selected because of its simple equation (Katul et al. 2000):

$$\frac{C_i}{C_a} = 1 - \frac{1}{m} * \frac{1}{RH} \quad (1)$$

where C_i and C_a are the intercellular and atmospheric CO_2 concentrations ($\mu\text{mol mol}^{-1}$), respectively, m is an empirical parameter, and RH is the relative humidity. In other plant species m ranges from 3 to 10, but it has not been reported for paprika (Leuning 1995). To estimate the m value for paprika, C_i , C_a , and RH values were measured using the portable photosynthesis measuring device, and regression analysis was conducted using Eq. (1). Measured C_a and RH values were used for all C_i calculations through this experiment.

2.4 Leaf photosynthetic rate models

The first model for expressing leaf photosynthetic rates with varying light intensity and CO_2 concentration was a rectangular hyperbola model established by Kaitala et al. (1982). The rectangular hyperbola model used in previous studies was expressed in the following equation:

$$P = \left(\frac{\alpha * PPFD * \beta * C_i}{\alpha * PPFD + \beta * C_i} \right) - R \quad (2)$$

where P is the leaf photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), α is the photochemical efficiency ($\mu\text{mol mol}^{-1}$), $PPFD$ is the photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), β is the carboxylation conductance (s^{-1}), C_i is the intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$), and R is the respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

Since it is difficult to reflect the effect of temperature change on leaf photosynthetic rate in the rectangular hyperbola model, a modified rectangular hyperbola model with temperature variable was used, which is based on empirical equations to express the change in photochemical efficiency and carboxylation conductance (Jung et al. 2017). In this study, an exponential equation was selected rather than

quadratic equation because the leaf temperature variation was small. The photochemical efficiency and carboxylation conductance used in the rectangular hyperbola model are expressed in the following equations:

$$\alpha = e^{-a*T_l} \quad (3)$$

$$\beta = e^{-b*T_l} \quad (4)$$

where T_l is the leaf temperature ($^{\circ}\text{C}$), and a and b are regression coefficients. The rectangular hyperbola model used in the analysis is expressed in the following equation:

$$P = \left(\frac{e^{-a*T_l} * PPF D * e^{-b*T_l} * C_i}{e^{-a*T_l} * PPF D + e^{-b*T_l} * C_i} \right) - R \quad (5)$$

where a and b are regression coefficients, $PPFD$ is the photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$), C_i is the intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$), and R is the respiration ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$).

The FvCB model was expressed in the following equations:

$$P = \min(A_c, A_j) \quad (6)$$

$$A_c = \left(\frac{V_c * (C_i - \Gamma^*)}{C_i + K_c * (1 + O/K_o)} \right) - R \quad (7)$$

$$V_c = V_{cmax} * \left(\frac{31 + (69/(1 + e^{-0.009*(PPFD-500)}))}{100} \right) \quad (8)$$

$$A_j = \left(\frac{J * (C_i - \Gamma^*)}{4C_i + 8\Gamma^*} \right) - R \quad (9)$$

$$J = \left(\frac{\alpha * PPF D + J_{max} - \sqrt{(\alpha * PPF D + J_{max})^2 - 4\theta * J_{max} * \alpha * PPF D}}{2\theta} \right) \quad (10)$$

where P is the leaf photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), A_c is the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation limited photosynthesis rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), A_j is the ribulose-1,5-bisphosphate (RuBP) regeneration limited photosynthesis rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), V_c is the carboxylation capacity at a certain light intensity ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), C_i is the intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$), Γ^* is the CO_2 compensation point ($\mu\text{mol mol}^{-1}$), K_c is the Michaelis–Menten constant of Rubisco for CO_2 ($\mu\text{mol mol}^{-1}$), O is the O_2 concentration ($210 \text{ mmol mol}^{-1}$), K_o is the Michaelis–Menten constant of Rubisco for O_2 ($\mu\text{mol mol}^{-1}$),

V_{cmax} is the maximum carboxylation capacity ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), $PPFD$ is the photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$), J is the electron transport rate at a certain light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$), J_{max} is the maximum electron transport rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), α is the efficiency of light energy conversion on an incident light basis ($0.42 \text{ mol e}^- \text{mol}^{-1} \text{ photon}$), and θ is the curvature of the light response of J (0.25 dimensionless) (Qian et al. 2012). The Michaelis–Menten constants of Rubisco for CO_2 and O_2 in the model were calculated based on Arrhenius function.

Regression analysis was performed on each model using the SPSS statistical package (IBM, New York, NY, USA). The light intensity, leaf temperature, relative humidity, and CO_2 concentration were set as input data in the model expression, and the measured photosynthetic rates were set as dependent variables to conduct a non-linear regression. All analyses were performed for each of the measured results for each vertical leaf position of the paprika.

2.5 Validation of leaf photosynthetic rate models

In the leaf photosynthetic rate model determined by regression analysis, the calculated leaf photosynthetic rate was compared with the measured leaf photosynthetic rate in the secondary measurement, which was different from the data used to establish the models under the specific light intensity and CO_2 concentration. A regression analysis was performed on a 1:1 line using the SPSS statistical package (IBM), and a graph of the model was drawn using Sigmaplot (Systat Software, San Jose, CA, USA).

2.6 Analyses of photosynthetic parameters

The relationship between the SPAD value and the total nitrogen content measured by leaf positions was analyzed through

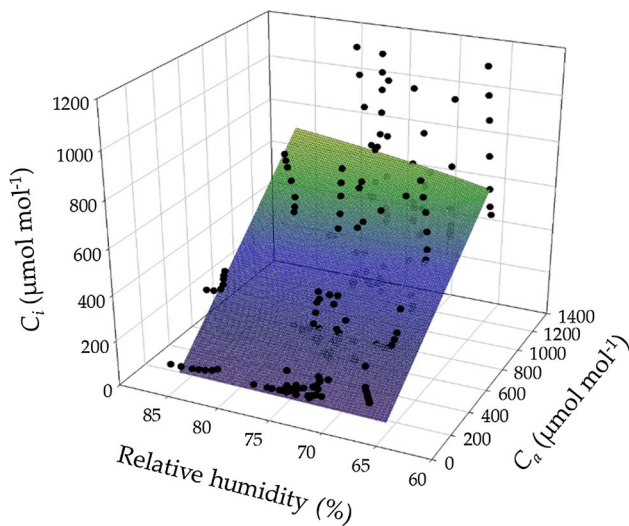


Fig. 2 Relationship between atmospheric CO₂ concentration (C_a), relative humidity (RH), and intercellular CO₂ concentration (C_i) using the Ball–Berry model in hydroponically-grown paprika plants

3 Results

3.1 Intercellular CO₂ concentration

The intercellular CO₂ concentration increased linearly with increasing atmospheric CO₂ concentration (Fig. 2). As the atmospheric CO₂ concentration increased from 100 to 1200 μmol mol⁻¹, the intercellular CO₂ concentration increased from 100 to 1100 μmol mol⁻¹. The m value in Eq. (1) was estimated to be 3.177 at a relative humidity of 74%. The R^2 and root mean square error (RMSE) values in the Ball–Berry model were estimated to be 0.63 and 0.539, respectively.

3.2 Vertical SPAD values and leaf total nitrogen content

The leaf SPAD values over 75 cm in the vertical position decreased linearly, while those under 75 cm showed no tendency (Fig. 3a). The maximum and minimum SPAD values measured were 48.5 at 0 cm and 72.2 at 125 cm, respectively. Leaf total nitrogen content linearly increased with increasing vertical leaf position (Fig. 3b). The maximum and minimum total nitrogen contents measured were 5.46% at 0 cm and 2.43% at 150 cm, respectively.

3.3 Regression analyses of rectangular hyperbola and FvCB models

For the rectangular hyperbola model, the regression coefficients of a , b , and R included in Eq. (5) were analyzed (Table 1). a showed a low value at 150 cm, and a decreasing tendency at other heights. As the height increased,

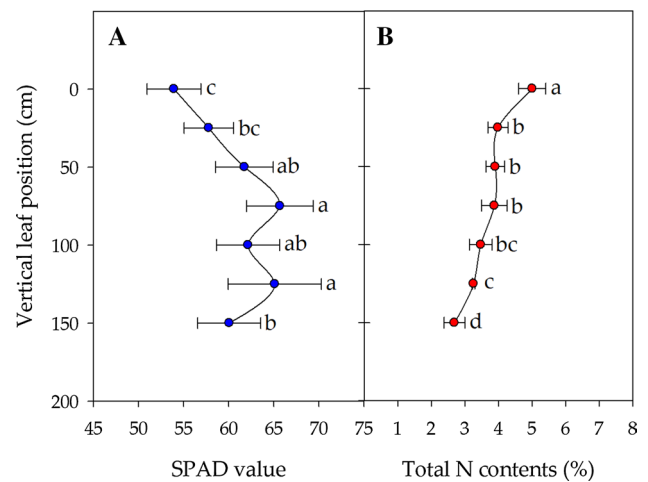


Fig. 3 Vertical distributions in SPAD value (a) and total nitrogen content (b) of hydroponically-grown paprika plants. Bars represent mean \pm SD ($n=3$). Each value followed by the same letter are not significantly different by Duncan’s multiple range test at $p \leq 0.05$

b values decreased, and the result obtained at 0 cm was about 75% of the result obtained at 150 cm. R showed a low value at 150 cm, and a decreasing tendency at other heights. The results were substituted for Eq. 5 showing the leaf photosynthetic rate in three-dimensional space with light intensity and CO₂ concentration on the X and Y axes (Fig. 4). The rectangular hyperbola model showed over-estimated values at 25 and 50 cm under light intensity and high CO₂ concentration conditions. The photosynthetic rates over 50 cm increased in the form of saturation curves with increasing light intensity and CO₂ concentrations. The photosynthetic rates under 100 cm also increased in the form of saturation curves with increasing light intensity. With increasing CO₂ concentration, however, the leaf photosynthetic rates increased linearly, without showing the form of a saturation curve.

For the FvCB model, V_{cmax} and J_{max} included in Eqs. 8 and 10 were analyzed (Table 2). From 0 to 150 cm in the vertical position of the leaf, those two values showed decreasing tendencies, with higher accuracy in regression analysis at 0 cm. The results were substituted for Eqs. 6–10, showing the leaf photosynthetic rate in three-dimensional space with light intensity and CO₂ concentration on the X and Y axes (Fig. 5). Over 50 cm, all light intensity conditions were shown to be the RuBP regeneration-limited zone under low CO₂ concentration conditions. In the high CO₂ concentration conditions, the light intensity within 600–1000 μmol m⁻² s⁻¹ was found to be the RuBP regeneration-limited zone. Similar patterns were observed under 75 cm, but the Rubisco carboxylation limited zone was found to be wider than the RuBP regeneration-limited zone.

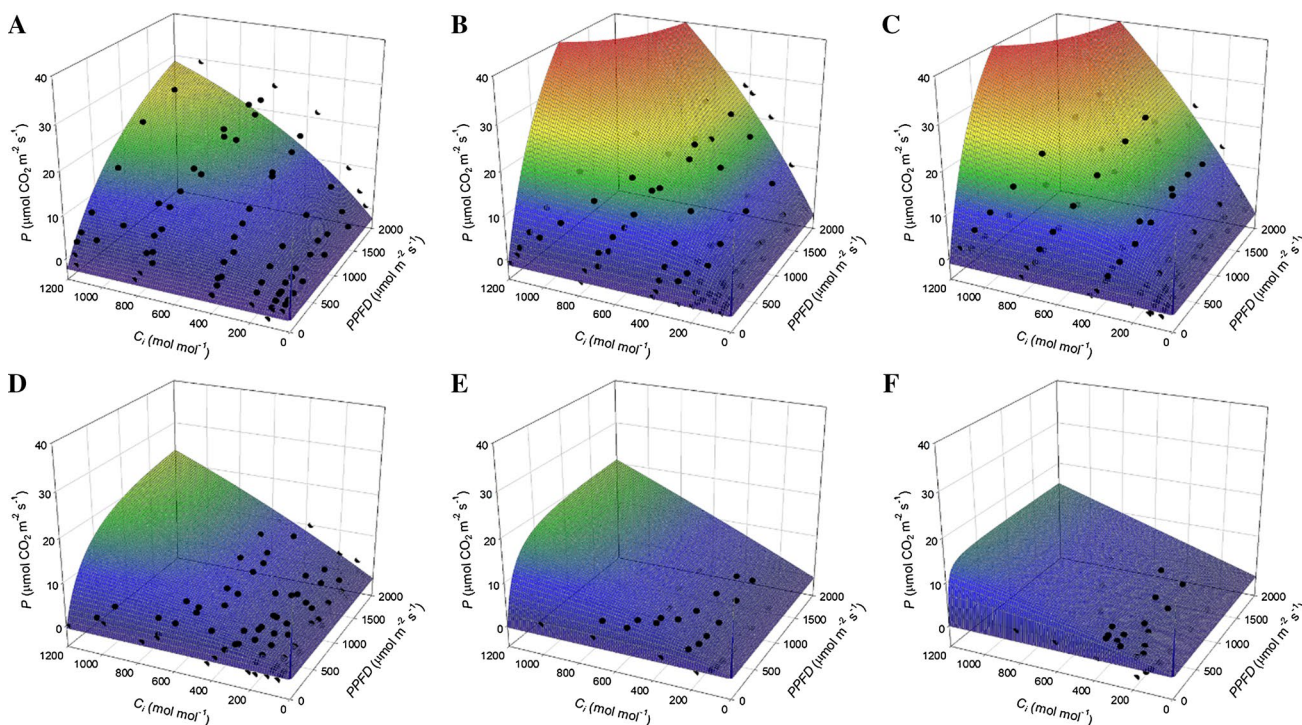


Fig. 4 Leaf photosynthetic rates (P) of hydroponically-grown paprika plants estimated using the rectangular hyperbola model with light intensity ($PPFD$) and intercellular CO_2 concentration (C_i) at vertical leaf positions of 0 (a), 25 (b), 50 (c), 75 (d), 125 (e), and 150 cm

(f). The leaf temperature was constant at 25 °C. White dots are measured values of leaf photosynthetic rates and curved meshes are the regressed estimates from the rectangular hyperbola model

Table 1 Regression coefficients, R^2 , and RMSE values of the rectangular hyperbola model for hydroponically-grown paprika plants according to vertical leaf position

Leaf position (cm)	a	b	R	R^2	RMSE
0	0.126	0.120	2.506	0.71	5.956
25	0.097	0.101	1.568	0.89	2.968
50	0.090	0.111	1.198	0.85	3.363
75	0.099	0.141	1.039	0.62	3.294
100	0.090	0.127	0.602	0.51	2.511
125	0.070	0.150	0.423	0.68	1.683
150	0.015	0.160	0.099	0.64	1.625

3.4 Validation of leaf photosynthetic rate models

Rectangular hyperbola and FvCB model-estimated leaf photosynthetic rates for each height were compared to measured rates against a 1:1 regression line to evaluate model performance (Fig. 6). The R^2 values and the RMSE

values were 0.86 and 0.90, and 4.651 and 2.104 in the rectangular hyperbola and FvCB models, respectively.

3.5 Relationship among photosynthetic parameter, vertical SPAD value, and leaf total nitrogen content

There was a small negative relationship between the SPAD values and the total nitrogen contents, showing a negative slope as -0.1010 (Fig. 7). The linear regression results for the parameters in the equation showed a low correlation with $R^2=0.33$. The α and β value estimated by the rectangular hyperbola model indicated a negative correlation with SPAD values, however, β value indicated a positive correlation with total nitrogen content. Similarly, the V_{cmax} and J_{max} values estimated by the FvCB model indicated a negative correlation with SPAD value and a positive correlation with total nitrogen content (Fig. 8). Estimating photosynthetic model parameters from the SPAD values resulted in low accuracy across all regressions. The regression results of the relationship between total nitrogen content and photosynthetic rate model parameters were generally more accurate than using SPAD values, but the maximum R^2 value was as low as 0.61.

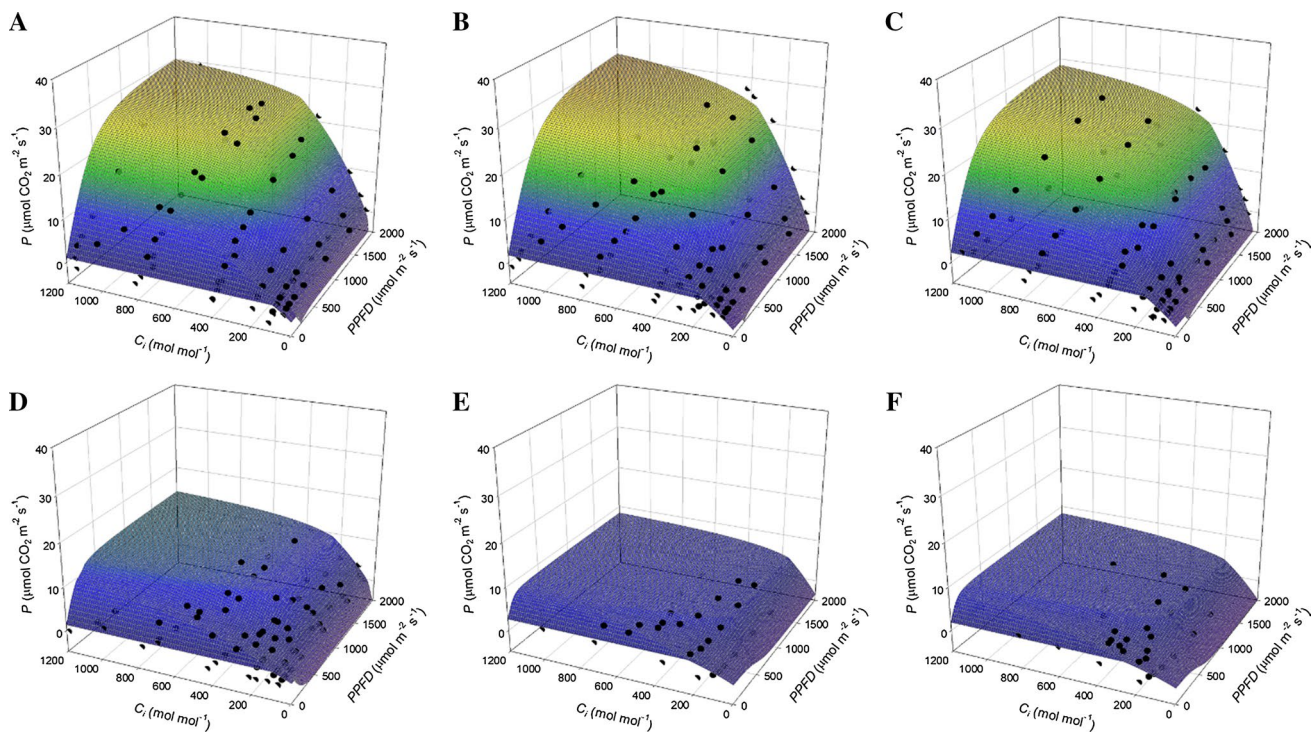


Fig. 5 Leaf photosynthetic rates (P) of hydroponically-grown paprika plants estimated using the FvCB model with light intensity ($PPFD$) and intercellular CO_2 concentration (C_i) at vertical leaf positions of 0 (a), 25 (b), 50 (c), 75 (d), 125 (e), and 150 cm (f). The leaf tempera-

ture was constant at 25 °C. White dots are measured values of leaf photosynthetic rates and curved meshes are the regressed estimates from the FvCB model

Table 2 Maximum carboxylation capacity (V_{cmax}) and maximum electron transport rate (J_{max}), R^2 , and RMSE values of the FvCB model for hydroponically-grown paprika plants according to vertical leaf position

Leaf position (cm)	V_{cmax}	R^2	RMSE	J_{max}	R^2	RMSE
0	74.818	0.82	2.722	136.392	0.95	2.237
25	88.693	0.83	3.072	144.382	0.95	2.086
50	87.206	0.84	2.361	127.002	0.87	3.323
75	42.909	0.60	2.376	58.521	0.89	1.482
100	39.813	0.50	2.978	36.106	0.47	2.631
125	39.701	0.56	2.125	37.612	0.76	1.184
150	34.776	0.64	1.567	32.808	0.80	0.999

4 Discussion

For the rectangular hyperbola model, the photochemical efficiency and carboxylation conductance were used to express plant physiological reactions as regressing parameters in the model. The photochemical efficiency increased toward the bottom leaves of the plants, but the bottom leaves showed low leaf photosynthetic rates. In the previous study, photochemical efficiency and carboxylation conductance of willow trees were $0.00028 \mu\text{mol}^{-1} \text{m}^2$ and $0.001053 \text{ kg CO}_2 \text{ m}^{-3} \text{ s}^{-1}$, respectively (Kaitala et al. 1982). In addition, the photochemical efficiency measured

in orache plants was known to be $0.177 \mu\text{mol CO}_2 \text{ mol}^{-1}$ (Marshall and Biscoe 1980). This value was similar to the photochemical efficiency of leaf positions over 50 cm estimated in the rectangular hyperbola model in this study. Thus, the rectangular hyperbola model was suitably established through regression analysis. However, the rectangular hyperbola model showed overestimated values under high light intensity and CO_2 concentration conditions especially at the positions at 25 and 50 cm (Fig. 4b, c). The lack of accuracy in the position revealed that the most active photosynthetic responses is a factor that reduces the reliability of the model. In addition, a modified rectangular hyperbola model with temperature variables was also

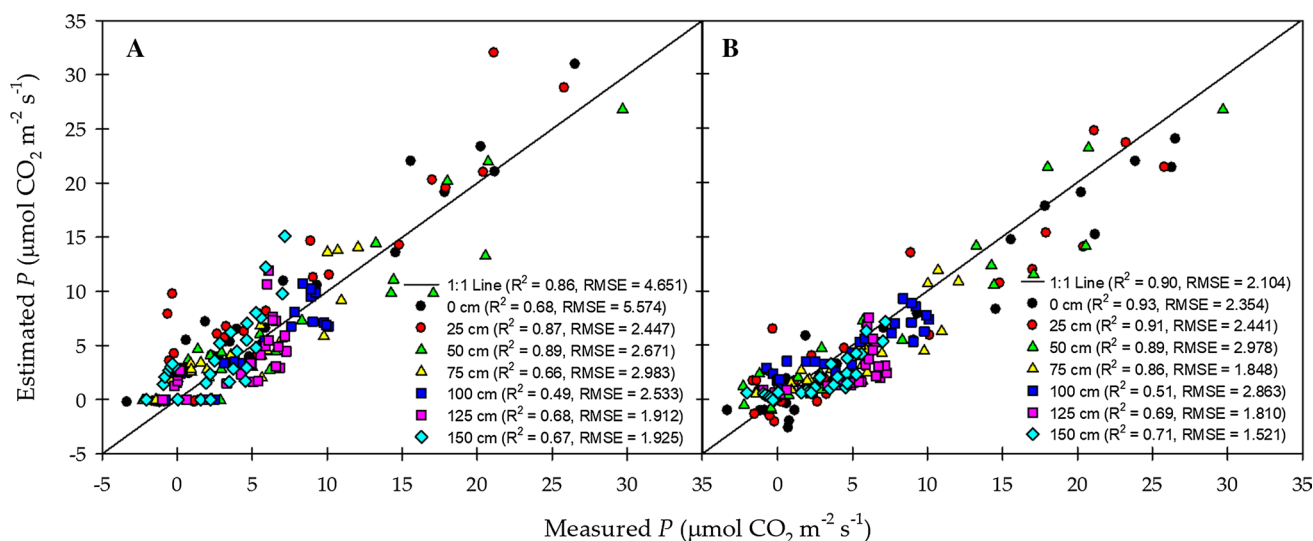


Fig. 6 Validation results of leaf photosynthetic rates of hydroponically-grown paprika plants estimated by the rectangular hyperbola (a) and FvCB (b) models

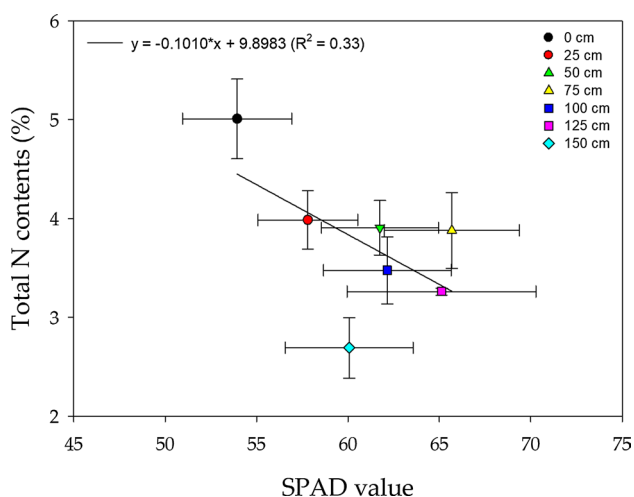


Fig. 7 Relationship between SPAD value and total nitrogen content of hydroponically-grown paprika plants according to vertical leaf position. The solid line indicates the regressed primary linear equation. Bars represent mean \pm SD ($n=3$)

developed, but empirical equations were used to express the change in photochemical efficiency and carboxylation conductance (Jung et al. 2017). However, the temperature changes occurred during photosynthesis measurements in this experiment were too small to use the quadratic empirical models as used in the previous study.

For the FvCB models, the Michaelis–Menten constants for CO_2 and O_2 concentrations were used to express photosynthetic reactions that vary with temperature (Qian et al. 2012). In terms of reflecting temperature, the FvCB model can more accurately represent the leaf photosynthetic rate

compared to the rectangular hyperbola model. Our results showed the lowest R^2 value with the FvCB model (Fig. 6), but the R^2 value was more than 0.90, indicating that the model itself is reliable. For shaded leaves at the bottom of the canopy in various crops, decreases in Rubisco content and RuBP regeneration capacity were reported (Baker and McKiernan 1988; Evans 1993; Osborne et al. 1998). As previously reported, this study also showed a tendency for the maximum carboxylation capacity and the maximum electron transport rate to decrease from the upper leaf to the lower leaf (Table 2). At leaf positions of 0–50 cm, where photosynthesis actively occurs, the FvCB model was more accurate than the rectangular hyperbola model under high light intensity and CO_2 concentration conditions.

Regressions using measured leaf photosynthetic rates were often inaccurate in the middle and bottom leaves of the plant canopy. By using a 3D plant model and simulations, Sinoquet et al. (1998) reported that the light intensity on the middle leaves of plants varied significantly. More adequate leaf photosynthetic rate models need to be applied to the top or outer leaves at high light intensity and the inner leaves at low light intensity. The bottom leaves showed smaller differences in light distribution compared to the middle and top leaves. Therefore, the leaf photosynthetic rate did not significantly change with changing environmental factors in the bottom leaves (Léchaudel et al. 2013). Photosynthesis varies depending on the leaf position due to changes in physiological and anatomical characteristics, such as leaf cell structure and chlorophyll content, according to environmental conditions (Larbi et al. 2015). In general, shaded leaves at the bottom of plants have low photosynthetic capacity and nitrogen content, resulting in insufficient photosynthesis

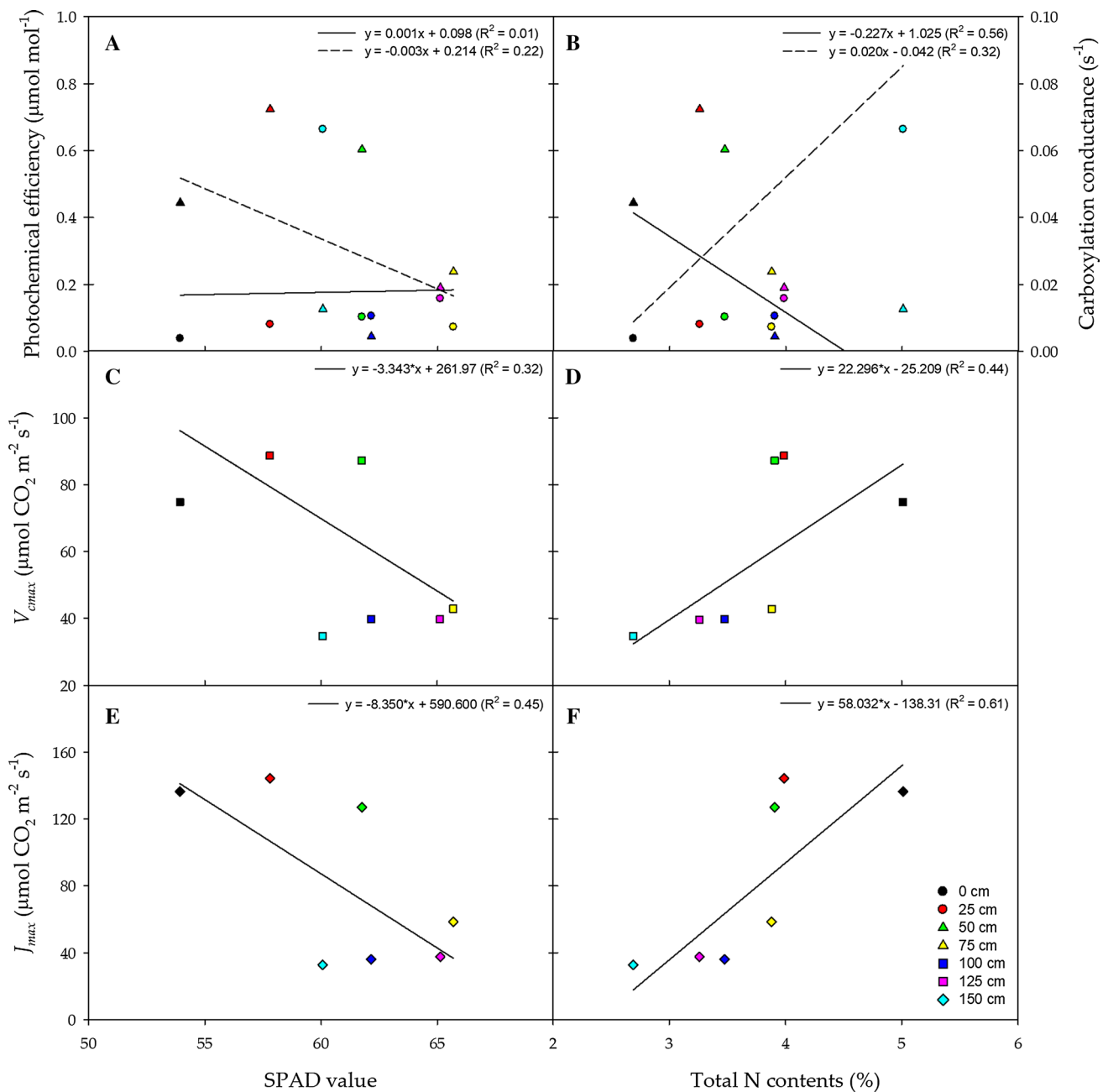


Fig. 8 Relationships between the estimated values of α and β (rectangular hyperbola model, **a** and **b**), V_{cmax} (FvCB model, **c** and **d**), J_{max} (FvCB model, **e** and **f**), and the measured SPAD values (**a**, **c**, and **e**)

and total nitrogen content (**b**, **d**, and **f**), respectively, of hydroponically-grown paprika plants. The solid line indicates the regressed primary linear equation

even with increased CO_2 concentrations (Del Pozo et al. 2007). In order to develop a more accurate model, it is necessary to identify the relationship between anatomical leaf structure and physiological indicators according to the vertical positions.

In this study, the nitrogen content at the bottom leaves was lower (Fig. 3b). According to Mavengahama et al. (2006), the optimum level of leaf nitrogen content for 6-weeks old paprika was approximately 3.7%. The total nitrogen content

in this experiment was up to 5%, but there was no stress responses. The vertical distribution of total nitrogen content in paprika found herein was consistent with the measurements obtained in deciduous forests for species such as maple, oak, and walnut (Ellsworth and Reich 1993; Le Roux et al. 1999b). It is interpreted that the nitrogen is allocated to the top of the plant due to the need for photosynthesis-related enzymes. The distribution and allocation of nitrogen is a contentious topic, but it is generally known to be related

with light intensity (Thornley 2004). Models having physiological characteristics with nitrogen distribution will be useful for farmers growing paprika at a high planting density.

According to Sun et al. (2019), SPAD values measured in tomatoes were 31.46–60.90, similar to those of paprika in this study. Pestana et al. (2001) observed that chlorophyll contents and SPAD values represent exponential relationship through orange trees. Díaz-Pérez (2013) also reported that chlorophyll contents in paprika were not correlated with leaf nitrogen content. The bottom leaves adapted to low light intensity had larger chloroplast size and chlorophyll contents than the top leaves adapted to high light intensity. In this study, the SPAD values according to leaf position was consistent with the previous studies (Figs. 3, 7). In addition, the SPAD values were less accurate in estimating parameters of photosynthetic rate models (Fig. 8). Considering photosynthetic mechanism, total nitrogen contents is more suitable for expressing the plant physiological responses than SPAD values. The SPAD values and nitrogen contents according to leaf position are proportional to accumulated light intensity (Thornley 2004; Yu et al. 2016). Because the light intensity exponentially decreases in crop canopy, it is likely that the SPAD values and nitrogen contents also tend to be the same. Thus, the relationship between the light intensity and SPAD value or nitrogen content of each leaf can be analyzed first with an exponential model, which can improve the accuracy rather than using only the parameters of the photosynthesis model.

5 Conclusions

Leaf photosynthetic rates of hydroponically-grown paprika plants were analyzed according to vertical position using two different multivariable photosynthetic rate models. The validation results showed that the R^2 values of rectangular hyperbola and FvCB models were as high as 0.86 and 0.90 with RMSE values of 4.651 and 2.104, respectively. However, the R^2 values of SPAD values and total nitrogen contents at the maximum electron transport rate in the FvCB model were as low as 0.45 and 0.61, respectively. Total nitrogen content linearly increased with increasing vertical leaf position and has a close relation with the maximum carboxylation capacity and maximum electron transport rate in the FvCB model. Compare to the rectangular hyperbola model, the FvCB model showed reliable values under high light intensity and CO_2 concentration conditions at a position where photosynthesis was very active. It is desirable to use the FvCB model that expresses the relationship between total nitrogen contents and plant's physiological responses according to the vertical position of leaves. The vertical leaf photosynthetic rate models established in this study will contribute to determine optimal environmental conditions

for maximizing crop photosynthesis in greenhouses and to establish the criteria for precise CO_2 enrichment.

Acknowledgements This research was supported by the MSIT (Ministry of Science and ICT), Korea, under the Grand Information Technology Research Center support program (IITP-2020-0-01489) supervised by the IITP (Institute for Information & communications Technology Planning & Evaluation).

Author contributions Conceptualization, methodology, and investigation, DHJ, IH, JYS and JES; resources and data curation, DHJ, IH and JYS; writing and editing, DHJ and JES; funding acquisition, JES.

Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Aminifard MH, Aroiee H, Ameri A, Fatemi H (2012) Effect of plant density and nitrogen fertilizer on growth, yield, and fruit quality of sweet pepper (*Capsicum annuum* L.). Afr J Agric Res 7:859–866. <https://doi.org/10.5897/ajar10.505>
- Baker JT, Allen LH Jr (1993) Contrasting crop species responses to CO_2 and temperature: rice, soybean, and citrus. Vegetatio 104:239–260. https://doi.org/10.1007/978-94-011-1797-5_16
- Baker NR, McKiernan M (1988) Modifications to the photosynthetic apparatus of higher plants in response to changes in the light environment. Biol J Linn Soc 34:193–203. <https://doi.org/10.1111/j.1095-8312.1988.tb01958.x>
- Bremner JM (1960) Determination of nitrogen in soil by the Kjeldahl method. J Agric Sci 55:11–33. <https://doi.org/10.1017/S0021859600021572>
- Chen JM, Liu J, Cihlar J, Goulden ML (1999) Daily canopy photosynthesis model through temporal and spatial scaling for remote sensing applications. Ecol Model 124:99–119. [https://doi.org/10.1016/s0304-3800\(99\)00156-8](https://doi.org/10.1016/s0304-3800(99)00156-8)
- Constable GA, Rawson HM (1980) Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. Funct Plant Biol 7:89–100. <https://doi.org/10.1071/pp9800089>
- Del Pozo A, Perez P, Gutierrez D, Alonso A, Morcuende R, Martinez-Carrasco R (2007) Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. Environ Exp Bot 59:371–380. <https://doi.org/10.1016/j.envexpbot.2006.04.009>
- Díaz-Pérez JC (2013) Bell pepper (*Capsicum annuum* L.) crop as affected by shade level: microenvironment, plant growth, leaf gas exchange, and leaf mineral nutrient concentration. HortScience 48:175–182. <https://doi.org/10.21273/hortsci.48.2.175>
- Ellsworth DS, Reich PB (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. Oecologia 96:169–178. <https://doi.org/10.1007/bf00317729>
- Evans JR (1993) Photosynthetic acclimation and nitrogen partitioning within a *Lucerne* canopy. I. canopy characteristics. Aust J Plant Physiol 20:55–67. <https://doi.org/10.1071/pp9930055>

- Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149:78–90. <https://doi.org/10.1007/bf00386231>
- Jones JW, Dayan E, Allen LH, van Keulen H, Challa H (1991) A dynamic tomato growth and yield model (TOMGRO). *Trans Am Soc Agric Eng* 34:663–672. <https://doi.org/10.13031/2013.31715>
- Jung DH, Lee JW, Kang WH, Hwang IH, Son JE (2018) Estimation of whole plant photosynthetic rate of Irwin mango under artificial and natural lights using three-dimensional plant model and ray-tracing. *Int J Mol Sci* 19:152. <https://doi.org/10.3390/ijms19010152>
- Jung DH, Yoon HI, Son JE (2017) Development of a three-variable canopy photosynthetic rate model of romaine lettuce (*Lactuca sativa* L.) grown in plant factory modules using light intensity, temperature, and growth stage. *Protec Hortic Plant Fact* 26:268–275. <https://doi.org/10.12791/ksbec.2017.26.4.268>
- Kaitala V, Hari P, Vapaavuori E, Salminen R (1982) A dynamic model for photosynthesis. *Ann Bot* 50:385–396. <https://doi.org/10.1093/oxfordjournals.aob.a086378>
- Katul GG, Ellsworth DS, Lai CT (2000) Modeling assimilation and intercellular CO₂ from measured conductance: a synthesis of approaches. *Plant Cell Environ* 23:1313–1328. <https://doi.org/10.1046/j.1365-3040.2000.00641.x>
- Kim JH, Lee JW, Ahn TI, Shin JH, Park KS, Son JE (2016) Sweet pepper (*Capsicum annuum* L.) canopy photosynthesis modeling using 3D plant architecture and light ray-tracing. *Front Plant Sci* 7:1321. <https://doi.org/10.3389/fpls.2016.01321>
- Larbi A, Vázquez S, El-Jendoubi H, Msallem M, Abadía J, Abadía A, Morales F (2015) Canopy light heterogeneity drives leaf anatomical, eco-physiological, and photosynthetic changes in olive trees grown in a high-density plantation. *Photosyn Res* 123:141–155. <https://doi.org/10.1007/s1120-014-0052-2>
- Le Roux X, Grand S, Dreyer E, Daudet FA (1999a) Parameterization and testing of a biochemically based photosynthesis model for walnut (*Juglans regia*) trees and seedlings. *Tree Physiol* 19:481–492. <https://doi.org/10.1093/treephys/19.8.481>
- Le Roux X, Sinoquet H, Vandame M (1999b) Spatial distribution of leaf dry weight per area and leaf nitrogen concentration in relation to local radiation regime within an isolated tree crown. *Tree Physiol* 19:181–188. <https://doi.org/10.1093/treephys/19.3.181>
- Leuning R (1995) A critical appraisal of a combined stomatal-photosynthesis model for C₃ plants. *Plant Cell Environ* 18:339–355. <https://doi.org/10.1111/j.1365-3040.1995.tb00370.x>
- Léchaudel M, Lopez-Lauri F, Vidal V, Sallanon H, Joas J (2013) Response of the physiological parameters of mango fruit (transpiration, water relations, and antioxidant system) to its light and temperature environment. *J Plant Physiol* 170:567–576. <https://doi.org/10.1016/j.jplph.2012.11.009>
- Marshall B, Biscoe PV (1980) A model for C₃ leaves describing the dependence of net photosynthesis on irradiance. *J Exp Bot* 31:29–39. <https://doi.org/10.1093/jxb/31.1.29>
- Mavengahama S, Ogunlela VB, Mariga IK (2006) Response of paprika (*Capsicum annuum* L.) to basal fertilizer application and ammonium nitrate topdressing in semi-arid Zimbabwe. *Crop Res* 32:421–429
- Medina-Ruiz CA, Mercado-Luna IA, Soto-Zarazúa GM, Torres-Pacheco I, Rico-García E (2011) Mathematical modeling on tomato plants: a review. *Afr J Agric Res* 6:6745–6749. <https://doi.org/10.5897/ajarx11.001>
- Noe SM, Giersch C (2004) A simple dynamic model of photosynthesis in oak leaves: coupling leaf conductance and photosynthetic carbon fixation by a variable intercellular CO₂ pool. *Funct Plant Biol* 31:1195–1204. <https://doi.org/10.1071/fp03251>
- Osborne CP, la Roche J, García RL, Kimball BA, Wall GW, Pinter PJ, la Morte RL, Hendrey GR, Long SP (1998) Does leaf position within a canopy affect acclimation of photosynthesis to elevated CO₂? *Plant Physiol* 117:1037–1045. <https://doi.org/10.1104/pp.117.3.1037>
- Park KS, Bekhzod K, Kwon JK, Son JE (2016) Development of a coupled photosynthetic model of basil hydroponically grown in plant factories. *Hortic Environ Biotechnol* 57:20–26. <https://doi.org/10.1007/s13580-016-0019-7>
- Pestana M, David M, de Varennes A, Abadía J, Faria EA (2001) Responses of “Newhall” orange trees to iron deficiency in hydroponics: effects on leaf chlorophyll, photosynthetic efficiency, and root ferric chelate reductase activity. *J Plant Nutr* 24:1609–1620. <https://doi.org/10.1081/pln-100106024>
- Qian T, Elings A, Dieleman JA, Gort G, Marcelis LFM (2012) Estimation of photosynthesis parameters for a modified Farquhar-von Caemmerer-Berry model using simultaneous estimation method and nonlinear mixed effects model. *Environ Exp Bot* 82:66–73. <https://doi.org/10.1016/j.envexpbot.2016.05.016>
- Schaffer B, Whitley AW, Searle C, Nissen RJ (1997) Leaf gas exchange, dry matter partitioning, and mineral element concentrations in mangos as influenced by elevated atmospheric carbon dioxide and root restriction. *J Am Soc Hortic Sci* 122:849–855. <https://doi.org/10.21273/jashs.122.6.849>
- Shin JH, Ahn TI, Son JE (2011) Quantitative measurement of carbon dioxide consumption of a whole paprika plant (*Capsicum annuum* L.) using a large sealed chamber. *Kor J Hortic Sci Technol* 29:211–214
- Sinoquet H, le Roux X, Adam B, Ameglio T, Daudet FA (2001) RATP: a model for simulating the spatial distribution of radiation absorption, transpiration and photosynthesis within canopies: application to an isolated tree crown. *Plant Cell Environ* 24:395–406. <https://doi.org/10.1046/j.1365-3040.2001.00694.x>
- Sinoquet H, Thanisawanyangkura S, Mabrouk H, Kasemsap P (1998) Characterization of the light environment in canopies using 3D digitising and image processing. *Ann Bot* 82:203–212. <https://doi.org/10.1006/anbo.1998.0665>
- Suharja S, Sutarno S (2009) Biomass, chlorophyll and nitrogen content of leaves of two chili pepper varieties (*Capsicum annuum*) in different fertilization treatments. *Nusantara Biosci* 1:9–16. <https://doi.org/10.13057/nusbiosci/n010102>
- Sun G, Wang X, Sun Y, Ding Y, Lu W (2019) Measurement method based on multispectral three-dimensional imaging for the chlorophyll contents of greenhouse tomato plants. *Sensors* 19:3345. <https://doi.org/10.3390/s19153345>
- Thornley JHM (1974) Light fluctuations and photosynthesis. *Ann Bot* 38:363–373. <https://doi.org/10.1093/oxfordjournals.aob.a084820>
- Thornley JHM (2004) Acclimation of photosynthesis to light and canopy nitrogen distribution: an interpretation. *Ann Bot* 93:473–475. <https://doi.org/10.1093/aob/mch051>
- Valladares F, Allen MT, Pearcy RW (1997) Photosynthetic responses to dynamic light under field conditions in six tropical rainforest shrubs occurring along a light gradient. *Oecologia* 111:505–514. <https://doi.org/10.1007/s004420050264>
- Yin X, Struik PC, Romero P, Harbinson J, Evers JB, van der Putten PEL, Vos J (2009) Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C₃ photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. *Plant Cell Environ* 32:448–464. <https://doi.org/10.1111/j.1365-3040.2009.01934.x>
- Yu KQ, Zhao YR, Zhu FL, Li XL, He Y (2016) Mapping of chlorophyll and SPAD distribution in pepper leaves during leaf senescence using visible and near-infrared hyperspectral imaging. *Trans ASABE* 59:13–24. <https://doi.org/10.13031/trans.59.10536>