Photoresponse Curve Model Research and Correlation Analysis of *Euphorbia marginata*

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Abstract
To explore the best model for the research of Euphorbia marginata photosynthetic characteristics and the physiological factors which affect net photosynthetic rate .By the comparison of light response curves obtained by the five models and the contrast between the measured values and several parameters of photosynthetic characteristics, also analyze the correlation between net photosynthetic rate of Euphorbia marginata and the main ecological factors.The result shows:(1)The fitting decision coefficient R2 of modified rectangular hyperbola model is 0.999,and the fitting and predicted MAE and MSE were both smaller. It's saturated light intensity and maximum net photosynthetic rate were 1723.896 μmol·m-2 ·s-1 and 26.726 μmol·m-2 ·s-1respectively.In addition, they were both closest to the measured value. On the whole, the fitting effect was better than exponential mode, nonrectangular hyperbola model, rectangular hyperbola model and the modified exponential model. Modified rectangular hyperbola model is the most suitable model for the photosynthetic characteristics research of Euphorbia marginata. (2) Stomatal conductance. Intercellular CO_2 concentration and transpiration rate etc. have a big influence on net photosynthetic rate of Euphorbia marginata. They all showed a very significant ($P < 0.01$) correlation with it.

Keywords: Euphorbia marginata, Light-response curve models, Net photosynthetic rate.

Introduction
Euphorbia marginata is an annual herbaceous plant of *Euphorbia*, *Euphorbiaceae*. It is planted in yard and parks in China. As an important medicinal plant, the whole *Euphorbia marginata* plant could be used as medicines and has good curative effect in irregular menstruation, innominate inflanunatory of unknown origin and traumatic injuries[1]. Existing researches on *Euphorbia marginata* only focus on auxanology and cytology[2-3], but few has studied physiology. No research on applicability of photoresponse curve model of *Euphorbia marginata* has been reported yet in China. Since growth and organic matter accumulation of plants depend on photosynthesis, studying photosynthetic characteristics of *Euphorbia marginata* is of important significance. Except for non-rectangle hyperbola model, rectangle hyperbola model and exponential mode, photoresponse models which are widely used include improved rectangle hyperbola model and improved exponential mode which are developed by Ye et al[4,5] based on C_3 photosynthetic characteristics of plants[6]. Due to different plant species and ecological factors, plants have different physiological properties. Above five models have different photosynthesis parameters and are applicable to different fields[7].

Research has pointed out that the improved rectangle hyperbola model has the best fitting effect of *Blumea balsamifera* (a medicinal plant)[8]; the non-rectangle hyperbola model is more reasonable to test results of winter wheat[9]; the improved exponential model is more applicable to study C4 plants like *sorghum* and *amaranth*; and the improved rectangle hyperbola model has higher degree of fitting to *Pinellia ternate* which is a C3 plant[6]. In this paper, these five models were applied for fitting of *Euphorbia marginata*. Fitting results were compared and the optimal model to study photosynthetic characteristics of *Euphorbia marginata* was screened. The correlation analysis on net photosynthetic rate develops an important way for further culture of a high-quality and high-output *Euphorbia marginata* variety.

Material and Methods
Study area: This test was carried out in the experimental base of College of Life Science, China West Normal University, Sichuan (30°48′N and 106°03′ E). The study area is hot and rainy in summer, warm and wet in winter, and belongs to subtropical monsoon climate. The annual average temperature and annual average rainfall are 17.6℃ and 980-1100 mm[10]. The altitude is 294m and purple soil is the dominant soil.

Test materials and methods: *Euphorbia marginata* samples that grew well (under natural sunshine conditions) and had no artificial destruction in test field in the experimental base were collected as test materials. They had green leaves with white sides, thin roots, single stem and abundant branches[11]. In the flowering phase of *Euphorbia marginata*, photosynthesis was measured by a LI-6400XT photosynthetic instrument. The measurement process is: healthy complete leaves were collected from middle and upper sections of 3 *Euphorbia marginata* strains with good and uniform growth (3 leaves from each strain).

Impurities like dust on leaves were removed gently with a brush. CO_2 concentration and flow rate were controlled at 400μmol·mol^{-1} and 500μmol·s^{-1} by the CO_2 steel cylinder and a built-in control system for CO_2 concentration adjustment. Leaf temperature and relative humidity of the sample room were set 25℃ and 65%±5%, respectively.

Light intensity was controlled by red-blue light sources and the photosynthetically active radiation (PAR) gradients were set 1800, 1500, 1200, 1000, 800, 500, 200,180, 150,120, 100, 80, 50, 20 and 0μmol·m-^{2} ·s^{-1}. Combined with measurement method of Xu et al.[12], light intensity was set 1000μmol·m^{-2} ·s^{-1} before the measurement to induce test leaves for 20min. Next, test leaves entered into automatic measurement program. The longest and shortest waiting time were set 240s and 120s, respectively. Attentions were paid to automatic matching in the measurement. Measurement results were input into computer from the photosynthetic instrument. Measurement data were analyzed by SPSS 22 after preliminary processing and graphing by EXCEL.

Models

Non-rectangle hyperbola model:

$$Pn = \frac{\alpha I + \mathrm{Pmax} - \sqrt{\left(\alpha I + \mathrm{Pmax}\right)^2 - 4\alpha Ik\mathrm{Pmax}}}{2k} - \mathrm{Rd} \qquad (1)$$

where Pn is net photosynthetic rate when PAR=I; α is the initial slope of curve when I=0 (intrinsic quantum yield. The higher α, the higher potentials of optical energy transformation of plants and the stronger photosynthesis. So intrinsic quantum yield could be distinguished from apparent quantum yield (AQY)[6]); Pmax is the maximum net photosynthetic rate; k (0<k<1) is a parameter that reflects bending degree of the curve; and Rd is dark respiration rate of plants[13-14].

Rectangle hyperbola model:

$$Pn = \frac{\alpha \mathrm{IPmax}}{\alpha \mathrm{I} + \mathrm{Pmax}} - \mathrm{Rd} \qquad (2)$$

where Pn, α, Pmax, I and Rd are same as those in equation (1). When Pn=0, the PARi is corresponding to the light compensation point (Ic)[13-14]:

$$Ic = \frac{Rd \times Pmax}{\alpha \times (Pmax - Rd)}$$

Improved rectangle hyperbola model:
The model expression is:

$$Pn = \alpha \frac{1-\beta I}{1+\gamma I}(I - \mathrm{Ic}) \qquad (3)$$

where Ic[14] is light compensation point, that is, net photosynthetic rate when PAR=0. β is a correction factor and γ is ratio between initial slope of the curve and Pmax (γ= α / Pmax). Rest parameters have same meaning with those in equation (1). The light saturation point (LSP) and Pmax formulas are:

$$I_s = \frac{\sqrt{(\beta+\gamma)/\beta} - 1}{\gamma} \qquad Pmax = \alpha \times \left(\frac{\sqrt{(\beta+\gamma)} - \sqrt{\beta}}{\gamma}\right)^2 - Rd$$

Subtract $\alpha\beta I^2/(1+\gamma I)$ based on equation (3) and it could get:

$$Pn = \alpha \frac{1-\beta I}{1+\gamma I} I - Rd$$

Exponential model:
The fitting equation[15] is:

$$Pn = Pmax \times \left[1 - e^{\frac{-\alpha I}{Pmax}}\right] - Rd \qquad (4)$$

α, Rd and Pmax could be calculated from fitting. Parameters have same meaning with those in equation (1). Let Pn=0, it can get:

$$\mathrm{Ic} = \frac{-\mathrm{Pmax}}{\alpha \times \ln\left(1 - \frac{\mathrm{Rd}}{\mathrm{Pmax}}\right)}$$

Improved exponential model:

$$\mathrm{Pn} = \alpha e^{(-\beta \mathrm{I})} - \gamma e^{(-\varepsilon \mathrm{I})} \qquad (5)$$

where Pn[6,16] is the net photosynthetic rate corresponding to PARi. Rd=α-γ when I=0. When Pn=0, $Ic = \frac{\ln(\frac{\alpha}{\gamma})}{\beta - \varepsilon}$.

Derivative of equation (5) under any light intensity is $\mathrm{Pn}' = -\alpha\beta e^{(-\beta \mathrm{I})} - \gamma\varepsilon e^{(-\varepsilon \mathrm{I})}$. When light intensity and PAR are 0, $\alpha_0 = \mathrm{Pn}'(\mathrm{I}=0) = \gamma\varepsilon\text{-}\alpha\beta$ [14,18]. When γε=αβ, Pn'＝0, indicating that there's a maximum net photosynthesis rate (Pmax).

Results and Analysis

Data processing: Pn means of output data under different PAR were calculated in Microsoft EXCEL. Next, Pn means under different light intensities were divided into two groups randomly. One group is Pn in the PAR range of 0-1000 μmol·m^{-2}·s^{-1}. Parameters were estimated by the nonlinear module of SPSS22 and Pn values calculated from five equations were fitted values. The coefficient of determination ($0<R^2<1$) was used to judge applicability of the model. The other group is tested values of Pn when PAR was 1200 μmol·m^{-2}·s^{-1}, 1500 μmol·m^{-2}·s^{-1} and 1800 μmol·m^{-2}·s^{-1}. Estimated parameters of different models were used in model expressions and Pn values under the light intensity could be calculated. The calculated Pn values were also called predicted values, which were used to estimate accuracy of five models[16]. To measure prediction and fitting accuracies more accurately, the following two parameters were defined:

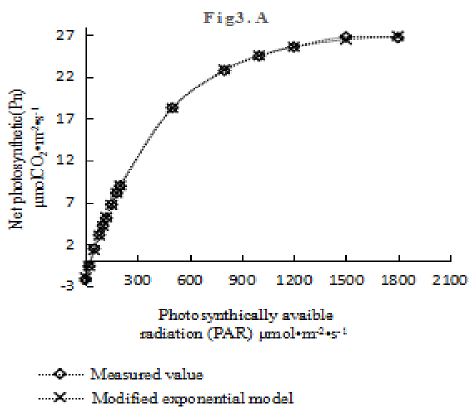
(1) Mean absolute error (MAE): $MAE = \frac{1}{n}\sum_{t=1}^{n}\left|y_t - \hat{y}_t\right|$

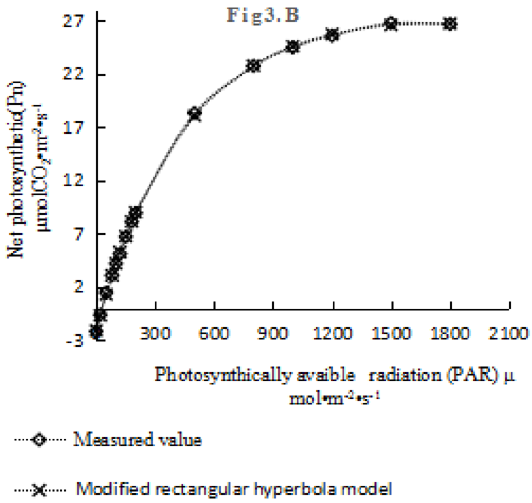
(2) Mean square error (MSE): $MSE = \frac{1}{n}\sum_{t=1}^{n}\left(y_t - \hat{y}\right)^2$

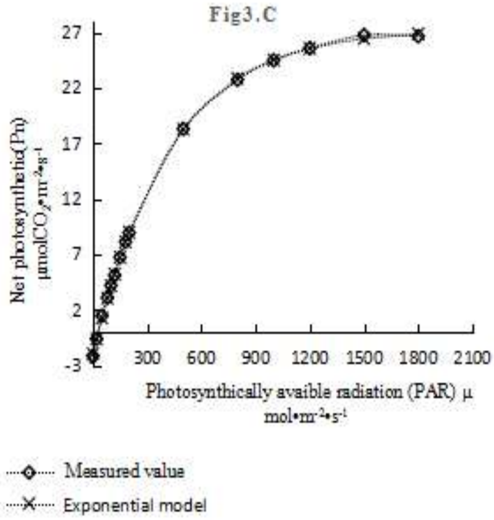
Y_t is tested value and $\hat{y}_t$ is fitted value or predicted value. The closer MAE and MSE to 0, the higher degree of coincidence between model fitted values and tested values[6]. Fitted results of LSP, LCP, Pmax and Rd of five models were compared with tested valued. Meanwhile, scatter diagrams of tested values, predicted values and fitted values of Pn were drawn and compared. A correlation analysis was carried out using SPSS22.

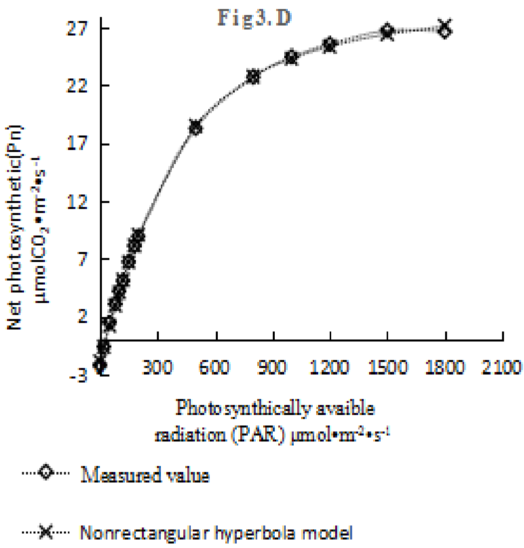
Comparison and Analysis

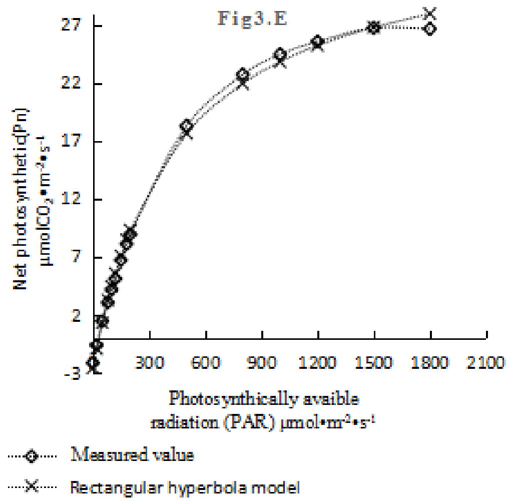
Fitting and comparison of photoresponse curve of *Euphorbia marginata:* Fig.3 (A-E) shows that all five models can represent photoresponse curves of *Euphorbia marginata* well. The correlation coefficient of degree of fitting (R^2) of the rectangle hyperbola model is the lowest (0.997), but R^2 of rest four models is as high as 0.999, indicating that all five model could get satisfying degree of fitting to *Euphorbia marginata*. According to TABLE 1, MSE and MAE between tested values and fitted values are smaller than those between predicted values and tested values in all five models. In other words, Fitted MSE<Tested MSE and Fitted MAE<Tested MAE, indicating that five models have better tested fitting results to photoresponse curve of *Euphorbia marginata* than predicted fitting results. Tested and fitted MSEs and MAEs of five models were compared, finding that rectangle hyperbola model and non-rectangle hyperbola model had great differences between tested values and fitted values in, while rest three models presented small differences. The bottom-to-up order of tested and fitted MSEs and MAEs is modified rectangle hyperbola model < modified exponential model < exponential model < non-rectangle hyperbola model < rectangle hyperbola model.











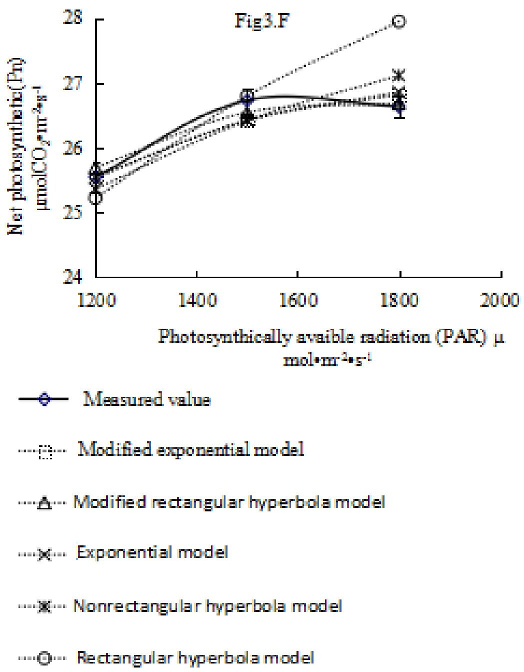


Fig. 3(A-E): *Euphorbia marginata* light-response curves which five models fitted F. The comparison between tested values and predicted values which five models' equations fitted

Remark: Fig 3.F, the measured values and predicted values which five models' equations fitted correspond to 3 values of PAR,1200μmol·m^{-2}·s^{-1}, 1500μmol·m^{-2}·s^{-1} and 1800μmol·m^{-2}·s^{-1} respectively. Error bars: mean±Standard Error, indicating the positive and negative deviation of the 3 measured mean of Pn on each plant blades.

It can be seen from Fig.3 (A-F) that 0≦PAR≦1500μmol·m^{-2}·s^{-1}, Pn is positively correlated with PAR. When 1500<PAR<1800μmol·m^{-2}·s^{-1}, only the curve of the improved rectangle hyperbola model tends to be stable with the increase of PAR, which is similar with slow reduction of tested values under high light intensity. This reflects that the fitted Pn of the improved rectangle hyperbola model and tested Pn reach light saturation under the same light intensity and this fitted Pn is very close to tested Pn. By contrast, fitted Pn of rest four models keeps increasing with PAR. Particularly, the final curve of the rectangle hyperbola model changes the most violently.

Parameter comparison of photoresponse curves of *Euphorbia marginata:* Table 2 reveals that fitted LCP and Pmax of all five models are very close to tested values (LCP ≈ 30μmol·m^{-2} ·s^{-1} and Pmax ≈ 26.733μmol·m^{-2} ·s^{-1}). Fitted LCP and Pmax of the improved rectangle hyperbola model are the closest to tested values (LCP ≈ 30.034μmol·m^{-2} ·s^{-1} and Pmax ≈ 26.726μmol·m^{-2} ·s^{-1}). Tested LSP of *Euphorbia marginata* is about 1600μmol·m^{-2} ·s^{-1}, while fitted LSP of the improved exponential model and improved rectangle hyperbola model are 1723.896 μmol·m^{-2} ·s^{-1} and 2578.214 μmol·m^{-2} ·s^{-1}, respectively. Despite that fitted Rd of the rectangle hyperbola model is slightly higher than the tested values, fitted Rd of rest four models are smaller than the tested values to different extents. Fitted Rd of the rectangle hyperbola model is very close to the test value. For these five models, AQY of four models falls in or very close to the normal ranges (maximum theoretical range: 0.08-0.125μmol CO_2·μmol^{-1} photons) despite that AQY of the rectangle hyperbola model is 0.086.By comparing fitted photosynthetic parameters of different models with tested values, the fitted values of the improved rectangle hyperbola model are closer to tested values than other models.

Correlation analysis between Pn of *Euphorbia marginata* and some key physiological and ecological factors: Correlation coefficients of Pn of test leaves with PARi, relative humidity (RH), atmospheric temperature (Ta), transpiration rate (Tr), intercellular CO_2 concentration (Ci) and stomatal conductance (Gs) are listed in Table 3. Correlation coefficients between Pn of *Euphorbia marginata* leaves and Gs, Tr, Ta and PARi are 0.933, 0.964, 0.812 and 0.925, showing extremely significant ($p<0.01$) positive correlation. Pn has extremely significant negative correlation with Gs and RH, showing correlation coefficients of 0.958 and 0.731. To sum up, all these 6 physiological and ecological factors are closely related with Pn of *Euphorbia marginata*. The correlation order is: Tr>Ci>Gs>PARi>Ta>RH.

Discussion

Based on comparison of photoresponse curves of *Euphorbia marginata* of five models and comparison of several photosynthetic parameters, it is easy to discover that: (1) viewed from fitted MSE and MAE as well as predicted MSE and MAE, the improved rectangle hyperbola model is superior to other four models in representing photoresponse curve of *Euphorbia marginata*.

Table 1
The accuracy of five models through the values of mean absolute error (MAE) and mean square error (MSE)

Model name	Fitted MSE	Fitted MAE	Tested MSE	Tested MAE
Modified exponential model	0.009	0.072	0.043	0.168
Modified rectangular hyperbola model	0.011	0.077	0.020	0.132
Exponential model	0.008	0.068	0.048	0.178
Non-rectangular hyperbola model	0.020	0.113	0.119	0.323
Rectangular hyperbola model	0.205	0.413	0.608	0.567

Table 2
The comparison of the measured values and the photosynthetic parameters calculated by the 5 models

Model Name	LCP	LSP	Pmax	Rd	AQY	k	R^2
Measured value	≈30	≈1600	26.733	2.144	--	--	--
Modified exponential model	29.673	2578.214	27.067	1.964	0.069	--	0.999
Exponential model	29.798	--	29.266	1.980	0.069	--	0.999
Non-rectangular hyperbola model	29.873	--	32.263	1.877	0.064	0.664	0.999
Rectangular hyperbola model	32.051	--	37.985	2.583	0.086	--	0.997

Remark: LCP, light compensation point; LSP, light saturation point; Rd, rate of dark respiration;Pmax , maximum net photosynthetic rate; AQY, The apparent quantum yield; k, curvature of light response curve;

Table 3
The correlation coefficient between net photosynthetic rate and the main physiological and ecological factors

Parameter	Pn	Gs	Ci	Tr	Ta	RH
Ci	0.933**					
Gs	-0.958**	-0.801**				
Tr	0.964**	0.994**	-0.853**			
Ta	0.812**	0.579*	-0.936**	0.655**		
RH	-0.731**	-0.4780	0.849**	-0.566*	-0.950**	
PARi	0.925**	0.998**	-0.784**	0.990**	0.547*	-0.4480

Remark: Significantly correlated at 0.01 and 0.05 levels (bilateral),respectively. Pn:Net photosynthetic rate; Gs:Stomatal Conductance; Ci:IntercellularCO_2 concentration; Tr:Transpiration rate; Ta:Atmosphere temperature; RH:Relative humidity of Atmosphere; PARi:Light intensity in the leaf chamber.

It can reflect actual physiological and growth states of *Euphorbia marginata* more accurately. (2) Compared to other models, the improved rectangle hyperbola model can better reflect photoinhibition of *Euphorbia marginata* which is represented by slow reduction of Pn against increase of PAR (under this circumstance, PAR is called LSP of the plant). Therefore, LSP and Pmax of *Euphorbia marginata* could be calculated directly from the improved rectangle hyperbola model.

In this paper, fitted LCP, LSP, Pmax and Rd of the improved rectangle hyperbola model are 30.034 μmol·m^{-2} ·s^{-1}, 1723.896 μmol·m^{-2} ·s^{-1}, 26.726 μmolCO_2·m^{-2}·s^{-1} and 2.079 μmolCO_2·m^{-2}·s^{-1}, which are close to tested values. References [18] and [19] pointed out that LCP and LSP ranges are 0-10μmol·m^{-2} ·s^{-1} and 500-1000 μmol·m^{-2} ·s^{-1} with respect to shade plants, and 50-100μmol·m^{-2}·s^{-1} and 1500-2000 μmol·m^{-2} ·s^{-1} or higher for heliophytes. Leng et al.[20] believed that plants with high LSP but low LCP could make photosynthesis under low light intensity and grow well under high light intensity. AQY range of plants under good growth condition in natural environment is 0.04~0.07[21]. The stronger light conversion efficiency of leaves, the higher AQY[22,23]. In this test, AQY of *Euphorbia marginata* was calculated 0.072 from the improved rectangle hyperbola model, indicating that although *Euphorbia marginata* is not a typical heliophyte, but still can grow well in environment without adequate light. It has a larger adaptation range to light and better adaptability to environment compared to typical shade plants (low LSP and LCP).

Conclusion

The improved rectangle hyperbola model is the best model to study photoresponse curve of *Euphorbia marginata*. The correlation analyses between Pn of *Euphorbia marginata* and some major environmental factors reflect that transpiration rate, intercellular CO_2 concentration and stomatal conductance are key influencing factors of photosynthesis. *Euphorbia marginata* has strong utilization of weak light. However, high light intensity shall be avoided appropriately in artificial plantation for the sake of photosynthesis.

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