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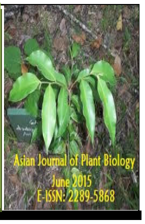

Modelling the Growth of Callus Cultures from *Glycine wightii* (Wight & Arn.) Verdc.

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ABSTRACT

One of the most important preliminary investigations of callus attributes is the growth characteristics. Most often than not, callus growth curve is sigmoidal in characteristics. In this work, we model callus growth from *Glycine wightii* from published literature to acquire essential growth constants. These growth constants are only able to be precisely extracted from mathematical modelling of the growth curves using numerous readily available primary models for example logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang models. Statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion) were utilized to find the best model. The best model was modified Gompertz. The growth constants obtained obtained such as lag, Y_{max}, μ_{max} were 3.78 d, 6.07 mg, and 0.318 d^{-1}, respectively, respectively. Growth parameter constants extracted from the modelling exercise will be helpful for additional secondary modelling implicating the consequence of media conditions as well as other factors on the growth of callus from this plant.

INTRODUCTION

Glycine wightii species is native to Brazil and Africa. It is often known as an important climbing vine-like perennial soybean [1]. It is in the family of Leguminosae, within the sub-family Papilionoideae, under the genus Glycine and with the sun-genus Bracteata. Tissue culture of *in vitro* cells, tissues and organs of *Glycine wightii* can yield efficient means in the genetics of breeding genetics, understanding the physiology and biochemistry of legumes. In addition it can be utilized in the production of plant biomass, plant improvement, as a mean for studying protein synthesis, and production of secondary metabolites [2,3].

In vitro culture of *Glycine wightii* species has been documented from leaves [4] and cotyledons and hypocotyls [5]. The production of callus is an important tool to study plant regulation, biosynthesis and biochemistry [6]. One of the most important preliminary investigation of callus attributes is the growth characteristics [7]. Most often than not, callus growth curve is sigmoidal in characteristics. Frequently, plant scientists studying callus growth neglect the utilization of mathematical growth that are useful in obtaining important growth constants such as lag period, maximum specific growth rate and maximum growth or asymptote. All these constants are useful for further modelling.

Modelling of the growth curves can yield important parameters that can be used for further optimisation works for callus such as determination of specific growth rate, lag period and maximum callus formation. Various mathematical functions such as Baranyi-Roberts [8] and Logistic, Gompertz, Richards, Schnute [9,10], Von Bertalanffy [11,12], Buchanan three-phase [13] and more recently the Huang model [14] can be utilized to model callus growth curve. Besides exhibiting predictive ability and internal uniformity, which is a must, the potency of a model ought to be looked at by its mathematical straightforwardness, flexibility, the number of its adjustable parameters and, where appropriate, if they have intuitive meaning. In this work, we model the callus growth curve from *Glycine wightii* utilizing various primary growth models.

MATERIALS AND METHOD

Acquisition of Data

In order to process the data, graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [15]. The software helps to digitize scanned plots into table of data with good enough precision [16]. Data were acquired from a published work [7] from Figure 1 and then replotted.

Fitting of the data

To find out regardless of whether there is a statistically substantial distinction between models with many amount of parameters, according to the quality of fit, data was statistically examined by way of several methods such as the root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion) [17].

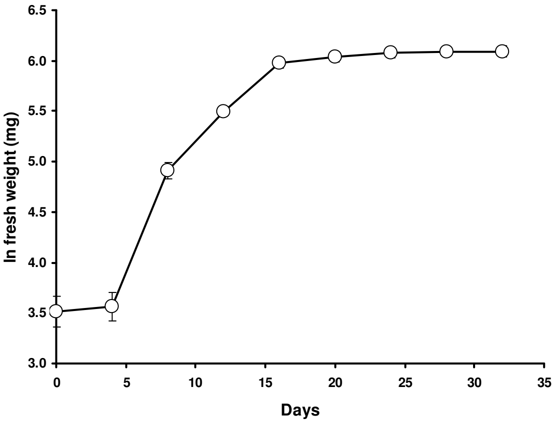

Fig. 1. Refitting of the callus growth curve of *Glycine wightii*.

RMSE

The calculation of RMSE was according to Eq. (1). *Pdi* is the values predicted by the model, and *Obi* is the experimental data, *n* is the number of experimental data, and *p* is the number of parameters of the model being assessed. (Eqn. **1**).

$$RMSE=\sqrt{\frac{\sum_{i=1}^{n}(Pd_i-Ob_i)^2}{n-p}}$$

Although the coefficient of determination or R^2 is used to assess the quality of fit of a modelin linear regression models, in nonlinear regression , the use of the method does not readily provides comparable analysis since the number of parameters amongst models vary. In order to solve this, an adjusted R^2 is used to calculate the quality of nonlinear models. The formulas are shown below (Eqns. **2** and **3**). RMS is Residual Mean Square and s_y^2 is the total variance of the y-variable.

$$Adjusted\left(R^2\right)=1-\frac{RMS}{s_Y^2} \qquad \textbf{(2)}$$

$$Adjusted\left(R^2\right)=1-\frac{\left(1-R^2\right)(n-1)}{(n-p-1)} \qquad \textbf{(3)}$$

Akaike information criterion with correction (AICc)

AIC is established on information theory. The Akaike information criterion (AIC) supplies a path for model selection through calculating the comparative quality of a given statistical model for a given number of experimental data [18]. If the number of points is not many times greater than the number of parameters of a model, the model with the greater number of parameters can sometimes be chosen as the best. In order to remedy this situation, anotehr version of AIC, the Akaike information criterion (AIC) with correction or AICc is employed. The AICc is computed for each and every data set for each model, and incorporates a penalty for extra parameters (Eqn. **4**);

$$AICc=2p+n\ln\left(\frac{RSS}{n}\right)+2(p+1)+\frac{2(p+1)(p+2)}{n-p-2} \qquad \textbf{(4)}$$

Where *p* is the number of parameters of the model and *n* is the number of data points. In general, the model having the smallest AICc value is extremely likely correct [18].

Accuracy Factor (AF) and Bias Factor (BF)

Ross was the first to utilize the Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models [19]. A Bias Factor equal to 1 indicates a perfect match between predicted and observed values. For microbial growth curves or degradation studies, a bias factor with values < 1 indicates a fail-dangerous model while a bias factor with values > 1indicates a fail-safe model. The Accuracy Factor is always ≥ 1, and higher AF values indicate less precise prediction (Eqns. **5** and **6**). These statistical tests do not incorporate penalty for extra number of parameters.

$$\text{Bias factor}=10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \qquad \textbf{(5)}$$

$$\text{Accuracy factor}=10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i/Ob_i)|}{n}\right)} \qquad \textbf{(6)}$$

RESULTS AND DISCUSSION

Probably the most essential area of the curve fitting exercise is the ability to employ a growth model, which has an excellent fundamental mechanistic function as outlined by good theoretical knowledge of the system. To obtained the best model, eight different growth models had been utilized (**Table 1**) for this study to suit the experimental data.

Table 1. Growth models used in this study.

Model	n	Equation
Modified Logistic	3	$y=\dfrac{A}{\left\{1+\exp\left[\dfrac{4\mu_{\max}}{A}(\lambda-t)+2\right]\right\}}$
Modified Gompertz	3	$y=A\exp\left\{-\exp\left[\dfrac{\mu_{\max}e}{A}(\lambda-t)+1\right]\right\}$
Modified Richards	4	$y=A\left\{1+v\exp(1+v)\exp\left[\dfrac{\mu_{\max}}{A}(1+v)\left(1+\dfrac{1}{v}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y=\left(\mu_{\max}\dfrac{(1-\beta)}{\alpha}\right)\left[\dfrac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y=A+\mu_{\max}x+\dfrac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x}+e^{-h_0}-e^{-\mu_{\max}x-h_o}\right)$ $-\ln\left(1+\dfrac{e^{\mu_{\max}x+\frac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x}+e^{-h_0}-e^{-\mu_{\max}x-h_0}\right)}-1}{e^{\left(y_{\max}-A\right)}}\right)$
Von Bertalanffy	3	$y=K\left[1-\left[1-\left(\dfrac{A}{K}\right)^3\right]\exp^{-\left(rx/3K^{\frac{1}{3}}\right)}\right]^3$
Huang	4	$y=A+y_{\max}-\ln\left(e^A+\left(e^{Y_{\max}}-e^A\right)e^{-\mu_{\max}B(x)}\right)$ $B(x)=x+\dfrac{1}{\alpha}\ln\dfrac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-Phase Linear Mode	3	y = A, if x < lag y=A + k(x−λ), if $\lambda \le x \ge x_{max}$ y = $y_{\max}$, if $x \ge x_{max}$

Note:
A= bacterial lower asymptote;
n= no of parameters
μ_{max}= maximum specific growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
y_{max}= bacterial upper asymptote;
e = exponent (2.718281828)
t = sampling time
α,β, k = curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the cells. the lag time (day^{-1}) can be calculated as $h_0=\mu_{max}$

The ensuing fitting illustrates visually sufficient fitting for the models of Huang, modified Gompertz, modified logistics, Von Bertalanffy, Baranyi-Roberts and Buchanan-3-models (**Figs. 2-9**).

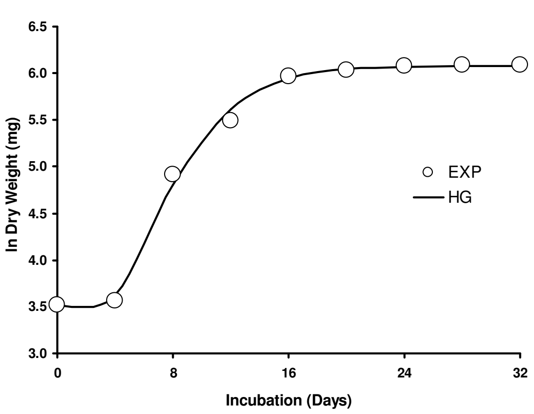

Fig. 2. Growth curves of *Glycine wightii* fitted by the Huang growth model.

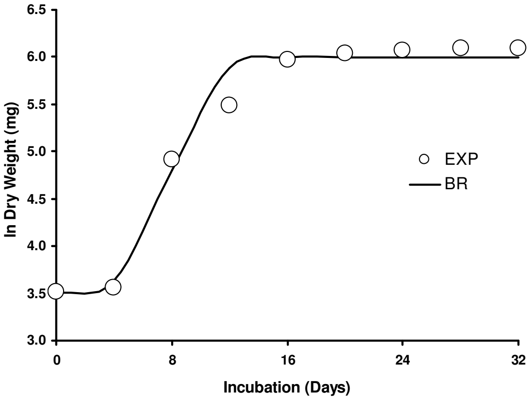

Fig. 3. Growth curves of *Glycine wightii* fitted by the Baranyi-Roberts growth model.

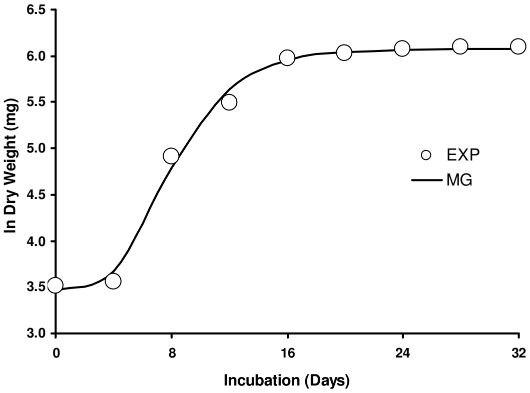

Fig. 4. Growth curves of *Glycine wightii* fitted by the modified Gompertz growth model.

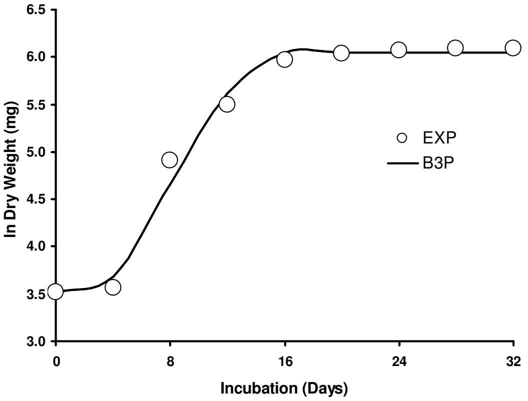

Fig. 5. Growth curves of *Glycine wightii* fitted by the Buchanan-3-phase growth model.

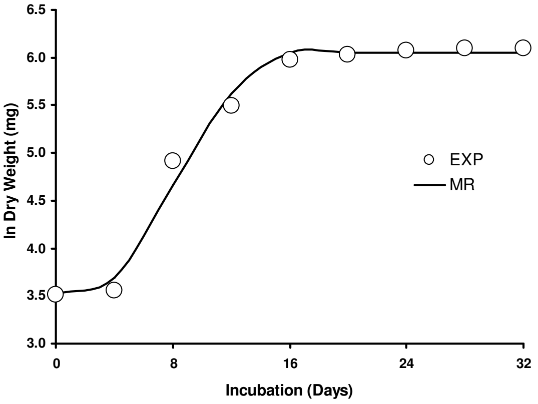

Fig. 6. Growth curves of *Glycine wightii* fitted by the modified Richard growth model.

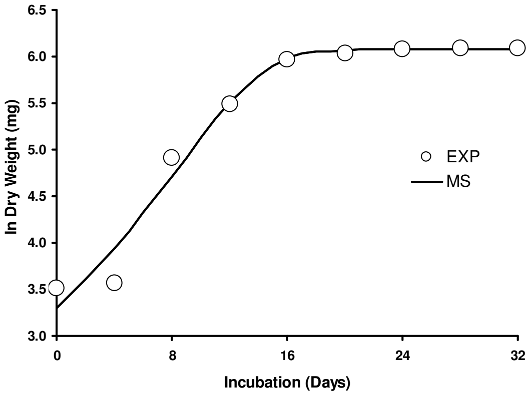

Fig. 7. Growth curves of *Glycine wightii* fitted by the modified Schnute growth model.

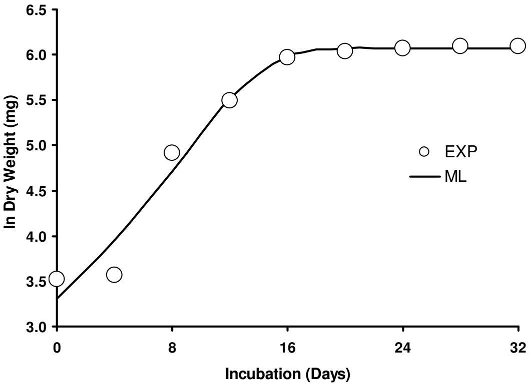

Fig. 8. Growth curves of *Glycine wightii* fitted by the modified logistics growth model.

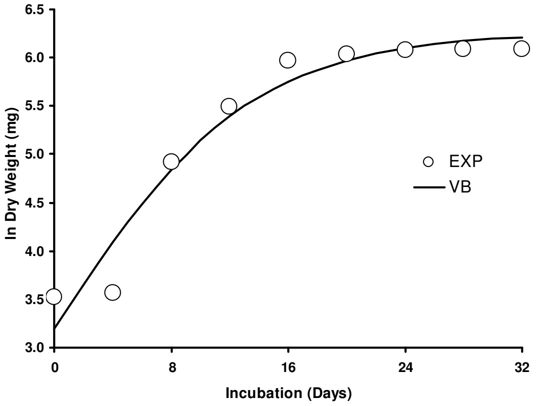

Fig. 9. Growth curves of *Glycine wightii* fitted by the von Bertalanffy growth model.

The statistical analysis results (**Table 2**) indicated that the modified Gompertz model was the best with highest adjusted R^2, lowest RMSE and AICc values, and Bias and Accuracy Factor values closest to unity. The method provides a relative approximation of the information lost for each and every time a particular model is utilized to indicate the method which produces the information or data. For virtually any output of a collection of predicted models, the most accepted model is the model displaying the minimum value for AIC. This value is often a negative value, with for example; an AICc value of -100 more preferred than the one with -50. The formula includes a number of parameters punishment, the greater the parameters, the less favoured the end result or the greater the AIC value. Therefore, AIC does not simply returns goodness of fit, but additionally, it doesn't encourage utilizing more complex model (overfitting). The modified Gompertz model was fitted to the data and the resultant fitted values obtained such as lag, Y_{max}, μ_{max} were 3.78 d, 6.07 mg, and 0.318 d^{-1}, respectively (**Table 3**).

Table 2. Statistical analysis of the various fitting models.

Model	p	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.08	1.00	0.99	1.01	1.00	-12.79
Baranyi-Roberts	4	0.20	0.98	0.96	1.02	1.00	3.73
modified Gompertz	3	0.12	0.99	0.99	1.02	1.00	-18.21
Buchanan-3-phase	3	0.13	0.99	0.98	1.02	1.00	-15.80
modified Richards	4	0.22	0.97	0.95	1.03	1.00	5.29
modified Schnute	3	0.25	0.97	0.93	1.03	1.00	7.67
modified Logistics	3	0.28	0.95	0.92	1.04	1.00	-2.52
von Bertalanffy	4	0.31	0.93	0.89	1.04	1.00	-0.64

Note:

RMSE	Root Mean Squared Error
R^2	Coefficient of Determination
adR^2	Adjusted Coefficient of Determination
AICc	Corrected Akaike Information Criterion
BF	Bias Factor
AF	Accuracy Factor
p	No of parameter

Table 3. Fitted growth parameters according to the modified Gompertz model.

Parameters	Value	Std-Error
lag (Days)	3.782	0.702
Y_{max} [ln dry weight (mg)]	6.072	0.052
μ_{max} (d^{-1})	0.318	0.038

The modified Gompertz model is a classical growth model that originates from the Verhulst [10,20]. The Gompertz function, was coined by Pierre François Verhulstis in 1844. The model is founded on an exponential relationship between specific growth rate and population density. The initial phase of growth is roughly exponential; after that, as saturation commences, the growth slows down, and at maturation, growth ceases. Gibson et al. [21] were the first group to make use of the Gompertz equation to suit microbial growth curves and also the equation was used successfully to explain the exponential and stationary stages of the microbial growth curves which is sigmoidal. Nevertheless, the model isn't sufficient to explain the lag phase. The model had been improved by Gibson et al. [21] to include the lag phase, and also have been used with in modelling several microbial growth curves so much that its popularity in mathematically modelling bacterial growth and product formation curves have been recognized [10,22,23]. The model is expressed as follows (Eqn 7):

$$N=D\exp\left(-\exp-B\left(t-M\right)\right)$$

$$\log\left(N\right)=A_g+D\exp\left(-\exp-B\left(t-M\right)\right) \qquad (7)$$

Where,

D = the difference in product concentration, optical density or log cfu/ml value of the upper and lower asymptotes
B = the maximum product concentration, death or growth rates at time M (h^{-1})
M = the time at which the absolute production, death or growth rates at maximum occur (h)
A_g = the lower asymptote value of the product concentration, optical density or log cfu/ml

The model has several main issues. For a start, in the static version, $N_{(t=0)}$ is not equal to N_o. In addition, an inflection point is the innate property of the sigmoidal curve resulting in the model to possess a systematic problem in explaining the exponential phase (Baranyi et al., 1993). Furthermore, the model tend to over-estimates its parameter values [24–26].

Compared to the logistics model, the asymmetrical sigmoidal property of the modified Gompertz could offer a greater flexibility. The main difference between sigmoidal models such as the logistic and Gompertz is at the point of inflection between the lower and the upper asymptotes. The logistics and Gompertz models show a distance of 1/2 and 1/e between the lower and the upper asymptotes, respectively [23]. Other growth models incorporate flexible slope function and variable point of inflection between these points. These functions are in fact either special or simpler cases of the original growth model. For instance the logistics, Gompertz or von Bertalanffy growth models fall under the Richard model [10,21,23].

The modified Gompertz being a three-parameter model is simpler than four or five-parameter models. A three-parameter model like the modified Gompertz is less complicated, the solution is a lot more stable, simpler to use, the estimations have more degrees of freedom and all of three parameters could be interpreted biologically. The additional fourth or fifth parameter in the four- or five-parameter models generally functions as shape parameter and in more often than not, they cannot be translated into biological meanings. In addition, the extra parameter functions in the four or five parameter models shows extremely large values under certain circumstances, reaching as high as 10,000. Current statistical methods only enforced a penalty based on number of parameters and not excessive parameter values. Hence, future statistical tests should incorporate penalty for these extreme values to discourage the use of these models. Another method to decrease these values is to increase the number of points [23].

The modified Gompertz model is widely used to model the growth of bacteria and bacterial secondary products production such as biohydrogen, methane, lactic acid, biofuel and bacterioricin [27–31]. It is also used to model plant growth [32–41], however, it is very rarely used in modelling of callus growth.

CONCLUSION

In conclusion, the various models used to fit the growth of *Glycine wightii* showed that the best model was modified Gompertz model based on statistical tests employed. The callus growth constants obtained from this work such as lag, Y_{max}, μ_{max} can be used in the further optimization works of the callus.

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