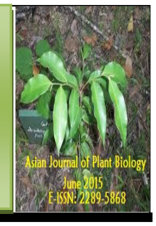
HIBISCUS

ASIAN JOURNAL OF PLANT BIOLOGY

http://journal.hibiscuspublisher.com/index.php/AJPB



Short Communication

Test of Randomness of Residuals for Modified Gompertz Model used for Modelling the Growth of Callus Cultures from *Glycine wightii* (Wight & Arn.) Verdc.

Shukor, M.S.[1], Masdor, N.A.[2], Halmi, M.I.E.[3], Ahmad, S.A.[4] and Shukor, M.Y.[1,4*]

[1]Snoc International Sdn Bhd, Lot 343, Jalan 7/16 Kawasan Perindustrian Nilai 7, Inland Port, 71800, Negeri Sembilan, Malaysia.
[2]Biotechnology Research Centre, MARDI, P. O. Box 12301, 50774 Kuala Lumpur, Malaysia
[3]Department of Chemical Engineering and Process, Faculty of Engineering and Build Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor.
[4]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Associate Prof. Dr. Mohd. Yunus Abd. Shukor
Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
Email: yunus.upm@gmail.com
Tel: +603-89466722
Fax: +603-89430913

HISTORY

Received: 1st May 2015
Received in revised form: 17th of June 2015
Accepted: 5th of July 2015

KEYWORDS

Glycine wightii
callus growth
modified Gompertz model
least square method
runs test

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. 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. Formerly, we model callus growth of *Glycine wightii* from published literature to obtain vital growth constants. We discovered that the modified Gompertz model via nonlinear regression utilizing the least square method was the best to explain the growth curve. Nevertheless, an important thing to consider, that has not been stated more than enough, is the residual of the model needs to be random. To make sure that randomness being fulfilled we carry out the Wald-Wolfowitz runs test. The results demonstrated that the number of runs was 5, and the expected number of runs within the assumption of randomness was 5, suggesting the series of residuals had perfect runs. The p-value obtained was higher than 0.05, hence the null hypothesis is not rejected suggesting no persuading proof of non-randomness of the residuals plus they do stand for noise.

INTRODUCTION

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 [1,2]. *Glycine wightii* species is native to Brazil and Africa. It is often known as an important climbing vine-like perennial soybean [3]. *Glycine wightii* falls under the family of Leguminosae. It is within the sub-family Papilionoideae, under the genus Glycine and with the sun-genus Bracteata. *In vitro* culture of *Glycine wightii* species has been produced from leaves [4], cotyledons and hypocotyls [5]. Callus culture 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]. It is often found that callus growth curve is sigmoidal in features. Regularly, plant scientists studying callus growth disregard the use of mathematical growth that are beneficial in finding important growth constants such as lag period, maximum specific growth rate and maximum growth or asymptote. All these constants are useful for further modelling.

We have utilized several growth models (manuscript in preparation) to model the growth of *glycine wightii* callus from a published literature [7]. We found out that the modified Gompertz model via nonlinear regression making use of the least square method was the most effective model to explain the growth curve (manuscript in preparation). The method of mathematically fitting nonlinear curve while using ordinary least squares method depends heavily on the residuals for the curve to be normally distributed, of equal variance (homoscedastic), as well as doesn't display autocorrelation [8–10]. Apart from this, an essential thing to consider which has not been pointed out enough would be that the residuals must be random. To ensure that randomness to be satisfied we carry out the Wald–Wolfowitz runs test [11] statistical diagnosis tests.

The runs test is an important tool in nonlinear regression to detect nonrandomness of the residuals [12]. The runs test could identify systematic difference of the curve for example over or under evaluation of the sections when utilizing a particular model. The runs test considers the sequence of the residuals, which are generally positive and negative. A good runs is generally represents by alternating or a balance number of positive and negative residual values. The number of runs of sign is frequently portrayed by means of a percentage of the maximum number attainable[11].

METHODOLOGY

In order to process the data, the graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [13] which helps to digitize scanned plots into table of data with good enough precision [14]. Data were acquired from the works of Silva et al. [7] from Figure 1 and then replotted (**Fig. 1,** with permission) (Shukor, M.S., Masdor, N.A., Shamaan, N.A., Wan Johari, W.L. and Shukor, M.Y 2015. Modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Manuscript in preparation).

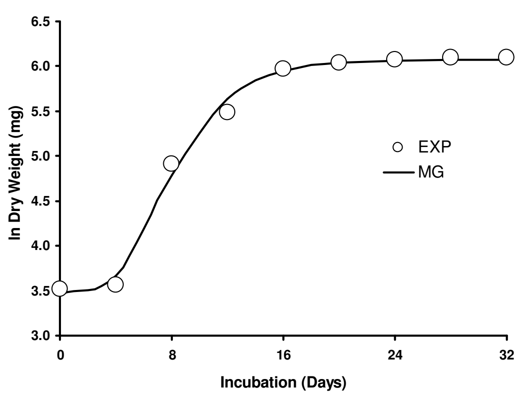


Fig. 1. Growth curves of *Glycine wightii* callus modelled using the modified Gompertz (MG) model.

Runs test

The runs test [12] was carried out to the residuals of the regression in order to detect nonrandomness. The runs test calculates the probability for the presence of too many or too few runs of sign. The presence of too many of a run sign could indicate the presence of negative serial correlation whilst the presence of too few runs could indicate a clustering of residuals with the same sign or the presence of systematic bias.

The test statistic is

H_0= the sequence was produced randomly
H_a= the sequence was not produced randomly

$$Z=\frac{R-\bar{R}}{sR} \quad (1)$$

Where Z is the test statistic, $\bar{R}$ is the expected number of runs, R is the observed number of runs and sR is the standard deviation of the runs. The computation of the values of $\bar{R}$ and sR (n_1 is positive while n_2 is negative signs) is as follows;

$$\bar{R}=\frac{2n_1.n_2}{n_1+n_2}+1 \quad (2)$$

$$s^2R=\frac{2n_1.n_2(2n_1.n_2-n_1-n_2)}{(n_1+n_2)^2(n_1+n_2-1)} \quad (3)$$

As an example;

Test statistic: $Z = 3.0$
Significance level: $\alpha = 0.05$
Critical value (upper tail): $Z_{1-\alpha/2} = 1.96$
Critical region: Reject H_0 if $|Z| > 1.96$
Since the test statistic value (Z) is larger than the critical value then the null hypothesis is rejected at the 0.05 significance level or the sequence was produced in a nonrandom manner.

RESULTS

From **Table** 2, the number of runs was 5 (the expected number of runs under the assumption of randomness was 5). This indicates that the series of residuals had perfect runs. The Z-value implies that the number of standard errors for the observed number of runs is below the predicted number of runs, the related p-value indicate how extreme this z-value is. The interpretation is the same like other 0-values statistics. If the p-value is less than 0.05 then the null hypothesis that the residuals are indeed random can be rejected. Since the p-value was greater than 0.05, therefore the null hypothesis is not rejected, indicating no convincing evidence of non-randomness of the residuals and they do represent noise.

Table 2. Runs test for randomness for the modified Gompertz model.

Runs test	Residual data set
observations	5
below mean	3
above mean	6
no of runs	9
E(R)	5.00
var(R)	1.50
stdev(R)	1.22
Z-value	0.00
p-value	0.50

Although the runs test has also used as a test for autocorrelation in time-series regression models, simulation studies using Monte Carlo have indicated that the runs test produces asymmetrical error rates in the two tails [15]. The investigation is carried out to analyse the empirical properties of the runs test utilizing (a) sample sizes of between 12 and 100 (b) using non-intervention and intervention regression models, (c) utilizing directional and nondirectional tests (d) with three levels of α, and (e) with 19 levels of autocorrelation among the errors. In addition, both directional and nondirectional tests produce no satisfactory results with respect to Type I error. The increment of the ratio of degrees of freedom with respect to sample size to a value as high as 0.98 fails to remedy the situation. Henceforth, only the Durbin-Watson method should be the better choice to assess autocorrelation.

To conclude, the runs test utilized in this work has shown that the use of the modified Gompertz model in fitting of the growth curve of *Glycine wightii* is adequate. The use of runs test has been neglected by many publications, and this is worrying as the data may be nonrandom. Randomness is an important requirement for all of the parametric statistical evaluation methods. In the event that a trend is suggested by diagnostic tests, various treatments such as nonparametric analysis or changing to a different model should be used to remedy the problem.

ACKNOWLEDGEMENT

Snoc International Sdn Bhd. financially supported this project.

REFERENCES

[1] Ibrahim R, Hussein S, Noordin N, Azlan E, Manan MA, Adrian H, et al. Advanced cell culture technology for essential oil production and microarray studies leading to discovery of genes for fragrance compounds in *Michelia alba* (Cempaka putih). Acta Hortic. 2008;765:95–100.

[2] Hussein S, Ling APK, Ng TH, Ibrahim R, Paek KY. Adventitious roots induction of recalcitrant tropical woody plant, *Eurycoma longifolia*. Romanian Biotechnol Lett. 2012;17(1):7026–35.

[3] Tokita N, Shimojo M, Masuda Y. Amino acid profiles of tropical legumes, cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*) and Siratro (*Macroptilium atropurpureum*), at pre-blooming and blooming stages. Asian-Australas J Anim Sci. 2006;19(5):651–4.

[4] Hammatt N, Nelson RS, Davey MR. Plant regeneration from seedling explants of perennial Glycine species. Plant Cell Tissue Organ Cult. 1987;11(1):3–11.

[5] Pandey P, Bansal YK. Plant regeneration from leaf and hypocotyl explants of *Glycine wightii* (W. and A.) Verdc. var longicauda. Jpn J Breed Tokio. 1992;42:1–5.

[6] Kiong ALP, Then CN, Hussein S. Callus induction from leaf explants of *Ficus deltoidea* Jack. Int J Agric Res. 2007;2(5):468–75.

[7] Silva ALC da, Caruso CS, Moreira R de A, Horta ACG. Growth characteristics and dynamics of protein synthesis in callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Ciênc E Agrotecnologia. 2005;29(6):1161–6.

[8] Razali NM, Wah YB. Power comparisons of Shapiro–Wilk, Kolmogorov– Smirnov, Lilliefors and Anderson–Darling tests. J Stat Model Anal. 2011;2:21–3.

[9] Jarque CM, Bera AK. Efficient tests for normality, homoscedasticity and serial independence of regression residuals: Monte Carlo evidence. Econ Lett. 1981;7(4):313–8.

[10] Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames Iowa: Iowa State University Press; 1980.

[11] Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987;1(5):365–74.

[12] Draper NR, Smith H. Applied Regression Analysis. Wiley, New York; 1981.

[13] Rohatgi, A. WebPlotDigitizer.http://arohatgi.info/WebPlotDigitizer/app/ Accessed June 2 2014.;

[14] Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.

[15] Huitema BE, McKean JW, Zhao J. The runs test for autocorrelated errors: unacceptable properties. J Educ Behav Stat. 1996;21(4):390–404.