



Nonlinear mixed effect models to describe the dry matter accumulation in garlic plants

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ABSTRACT: Given the importance of describing the accumulation of total dry matter in garlic accessions and the advantage of Nonlinear Mixed Effect Models (NLME) in this process, the present work research compared four nonlinear equations (Gompertz, Logistic, Richards, and von Bertalanffy) in the fit of accumulation of total dry matter per plant of 30 garlic accessions. The objective was also to identify the best accessions according to each growth parameter by estimating the random effects around the mean through the best among the models. The analysis was carried out using the R software. The best model was the Logistic according to the criteria used for comparison (AIC, BIC, R_{adj}^2 , MSE and MAE), presenting estimates closer to the actual observed values. According to the random effects estimated by this model, which represent deviations from the mean, the accessions that showed the highest asymptotic weight were 4505, 4826 and 4500, while accessions 4826, 4837 and 4491 took longer to reach the inflection point of the curve. The NLME approach used one fit per equation to obtain information on all individuals in the sample, efficiently adjusting the accumulated total dry matter and identifying the best accessions according to the estimated random effects of its parameters.

Key words: *Allium sativum*, fixed effects, growth, random effects, statistical modeling.

Modelos não lineares mistos na descrição do acúmulo de matéria seca em plantas de alho

RESUMO: Diante da importância de descrever o acúmulo de matéria seca total em acessos de alho e a vantagem dos Modelos Não Lineares Mistos (MNLM) nesse processo, o presente trabalho teve como objetivo comparar quatro equações não lineares (Gompertz, Logístico, Richards e von Bertalanffy) no ajuste do acúmulo de matéria seca total por planta de 30 acessos de alho. O objetivo também foi identificar os melhores acessos de acordo com cada parâmetro de crescimento, estimando os efeitos aleatórios em torno da média através do melhor entre os modelos. A análise foi realizada com o uso do software R. O melhor modelo foi o Logístico segundo os critérios utilizados para comparação (AIC, BIC, R_{adj}^2 , EQM e EAM), apresentando estimativas mais próximas dos valores reais observados. De acordo com os efeitos aleatórios estimados por este modelo, que representam desvios da média, os acessos que apresentaram maior peso assintótico foram 4505, 4826 e 4500, enquanto os acessos 4826, 4837 e 4491 demoraram mais para atingir o ponto de inflexão da curva. A abordagem NLME utilizou apenas um ajuste por equação para obter informações sobre todos os indivíduos da amostra, ajustando eficientemente a matéria seca total acumulada e identificando os melhores acessos de acordo com os efeitos aleatórios estimados de seus parâmetros.

Palavras-chave: *Allium sativum*, efeitos fixos, curvas de crescimento, efeitos aleatórios, modelagem estatística.

INTRODUCTION

According to REIS et al. (2014), to make a good management decision and to detect possible problems in the development of the garlic crop (*Allium sativum*), it is recommended to study the accumulation of dry mass in its different parts. Thus, the need for studies to identify this characteristics' behavior becomes relevant. Dry matter analysis in garlic accessions plays a fundamental role in evaluating productivity and identifying relevant genetic characteristics, contributing to advances in genetic improvement and agricultural efficiency.

The growth curves of accumulated dry matter over time are usually described by fitting

Nonlinear Models (NLM). This approach is suitable to describe these curves since their parameters have biological interpretation and processes such as growth vary with time (MAZZINI et al., 2005). This can be corroborated by studies in several areas of knowledge involving the growth of dry matter of cassava (SILVA et al., 2014), garlic (PUIATTI et al., 2018) and lettuce (CARINI et al., 2020).

Even with good results in several applications reported in the literature, this methodology was improved by an approach known as Nonlinear Mixed-Effect Models (NLME). Among the advantages and improvements of traditional NLMs, we can highlight the possibility of including general fixed effects (e.g., variety, species, or the overall mean) and random effects (individual

deviations from the mean or the fixed effects included). In addition, it allows the joint modeling of the residual effects (SILVA et al., 2017), which eliminates the need for multiple adjustments (one per individual). In the context of growth curves, the fitting of the NLME method was used mainly in animal science, Guzerá breed cattle (ALVES et al., 2020) and dairy buffaloes (ARAUJO NETO et al., 2020). However, to the best of our knowledge, the application of this method for fitting total dry matter growth curves for garlic accessions is non-existent in the literature.

Given the above, this research has as objectives (1) compare different equations (Gompertz, Logistic, Richards and von Bertalanffy) using the mixed-effect models approach to describe the total accumulated dry matter of garlic accessions aiming to identify the best model and (2) identify the best accessions according to the model parameters, estimated by the random effects of the mixed models.

MATERIALS AND METHODS

The experiment was conducted in the period from March to November 2010 in the experimental area of the Department of Plant Science of the Federal University of Viçosa (UFV), Viçosa-MG, Zona da Mata of Minas Gerais, at the following geographical coordinates: 20°45' south latitude and 42°51' west longitude, with an altitude of 650m.

The experimental design was a complete randomized block design with four repetitions. The experimental units, also described by PUIATTI et al. (2018), consisted of four longitudinal rows (blocks) of 1m length, with a planting space of 0.25x0.10m, with a total of 40 plants, from which the plants of the two central rows were considered as valid. After being harvested, the plants were subjected to the field and shed curing process for 3 and 60 days, respectively, exposed to the sun and in a shed. After curing, the plants were groomed by cutting the aerial part 1.0 cm above the bulbs and removing the roots.

For each of the 30 accessions used, the total dry matter of the plant (TDMP), expressed in grams by plant, was evaluated in four periods, measured in days after planting (DAP): the initial period (60 DAP), the second period (90 DAP), the third period (120 DAP), and the final period (150 DAP). As 30 garlic accessions registered in the Horticultural Germplasm Bank (BHG/UFV)

were evaluated in these conditions, the TDMP was measured for the four periods described; the total sample was composed of 120 observations ($N = 120$). The mean and standard deviations for TDMP by period can be observed on table 1.

According to LINDSTROM & BATES (1990), the representation of the NLME can be understood as a hierarchical model. The general representation of the model considering four observations of i -th accession ($i = 1, \dots, 30$) can be denoted by:

$$\mathbf{y}_i = \boldsymbol{\eta}_i(\boldsymbol{\phi}_i) + \boldsymbol{\varepsilon}_i, \quad (1)$$

Where $\mathbf{y}_i = [y_{i1} \ y_{i2} \ y_{i3} \ y_{i4}]$ is the vector of observed values of i -th accession, $\boldsymbol{\varepsilon}_i = [\varepsilon_{i1} \ \varepsilon_{i2} \ \varepsilon_{i3} \ \varepsilon_{i4}]'$ express the i -th vector of residuals and is a vector representing a nonlinear function of the observation x_{ij} ($j = 1, \dots, 4$) and $\boldsymbol{\eta}_i(\boldsymbol{\phi}_i) = [f(\boldsymbol{\phi}_i, x_{i1}) \ f(\boldsymbol{\phi}_i, x_{i2}) \ f(\boldsymbol{\phi}_i, x_{i3}) \ f(\boldsymbol{\phi}_i, x_{i4})]'$ the vector of parameters $\boldsymbol{\phi}_i$, which can be decomposed in this case by:

$$\boldsymbol{\phi}_i = \mathbf{A}_i\boldsymbol{\beta} + \mathbf{B}_i\mathbf{b}_i = \mathbf{I}\boldsymbol{\beta} + \mathbf{I}\mathbf{b}_i, \quad (2)$$

Where $\mathbf{A}_i = \mathbf{B}_i = \mathbf{I}$ are the design matrices associated to the fixed and random effects. In this case, $\boldsymbol{\beta}$ is typical for all accessions/genotypes, and the random effects around the average (\mathbf{b}_i), indexed by i . Additionally, the distribution of random effects vector is $\mathbf{b}_i \sim N(\mathbf{0}, \sigma^2 \mathbf{D})$, where \mathbf{D} is its scaled variance-covariance matrix and the distribution of the residuals can be denoted as $\boldsymbol{\varepsilon}_i \sim N(\mathbf{0}, \sigma^2 \mathbf{A}_i)$, where $\mathbf{A}_i = \mathbf{I}$ is the residual covariance matrix for all i accessions. The table 2 shows the equations used to represent $\boldsymbol{\eta}_i(\boldsymbol{\phi}_i)$, can be represented by the sigmoid format. All nonlinear equations can be found in ARCHONTOULIS & MIGUEZ (2015) and Fernandes et al. (2020) for von Bertalanffy's model.

For the nonlinear models in table 2 (from Gompertz to von Bertalanffy), ϕ_{1i} represents the asymptotic weight (g); ϕ_{2i} is when the curve reaches its inflection point, where growth is maximized; ϕ_{3i} is the scale parameter, which controls the steepness; and ϕ_{4i} is a constant associated with asymmetric growth, present only in Richards' model. Parameter estimation was done according to the method proposed by LINDSTROM & BATES (1990), which is divided into two steps: the first consists of minimizing the nonlinear sum of squares, known as the Penalized Nonlinear Least Squares (PNLS) Step, which makes use of the Gauss-Newton algorithm. The second resembles estimating the variance components of linear mixed models; and is therefore, called the Linear Mixed Effects (LME) Step by maximizing the log-likelihood function.

Table 1 - Mean, standard deviation (SD), maximum and minimum values of total dry matter per plant (TDMP, in grams) for 60, 90, 120, and 150 days after planting (DAP).

DAP	Average ± SD	Median	Minimum	Maximum
60	0.97 ± 0.30	0.93	0.45	1.60
90	4.44 ± 0.96	4.34	2.86	6.72
120	17.70 ± 3.59	17.40	11.80	28.80
150	22.60 ± 4.70	22.10	13.00	36.10

The quality of fit of the models was evaluated using likelihood-based measures (AIC and BIC), based on the fitted values (Mean Squared Error-MSE and Mean Absolute Error-MAE) and the adjusted coefficient of determination (R_{adj}^2).

The AKAIKE Criterion (1974), known as AIC (Akaike Information Criterion), can be obtained by: $AIC = -2 \cdot \ln(L) + 2 \cdot p$, where SCHWARZ'S (1978) criterion, also named BIC (Bayesian Information Criterion), is described as follows: $BIC = -2 \cdot L + p \cdot \log(N)$. For both measures, L is the logarithm of the likelihood ratio obtained by estimation and p represents the number of parameters in the model and N is the sample size.

Measures based on the comparison between observed and estimated values are calculated as follows:

$$MSE = \sum_{i=1}^{30} \sum_{j=1}^4 (y_{ij} - \hat{y}_{ij})^2 / N$$

and $MAE = \sum_{i=1}^{30} \sum_{j=1}^4 |y_{ij} - \hat{y}_{ij}| / N$, in which y_{ij} and \hat{y}_{ij} are, respectively the observed and

estimated values of total dry matter per plant (Y) for the i -th access ($i = 1, \dots, 30$) at the j -th time ($j = 1, \dots, 4$) and N represents the sample size ($N = 120$). The best models are those with the lowest AIC, BIC, MSE and MAE values.

The R_{adj}^2 is used to check the proportion of the total variability explained by the regression model and is calculated as follows (HOJJATI & HOSSEIN-ZADEH, 2018): $R_{adj}^2 = 1 - [N - 1 / N - p](1 - R^2)$, where R^2 is the coefficient of determination ($R^2 = 1 - RSS/TSS$), TSS being the Total Sum of Squares and RSS the residual sum of squares, the value of N represents the same sample size as described above. This measure varies in the range between 0 and 1, and the closer to 1, the better the model.

All analyses were performed in R software (R CORE TEAM, 2020). The databases were adjusted, the descriptive measures were calculated, and the graphs were generated with the aid of the packages *dplyr* (WICKHAM et al., 2022) and *ggplot2* (WICKHAM, 2016), respectively. The models were fitted considering all 30 accessions previously described with the inclusion of mean (general) fixed

Table 2 - Equations used to represent $\eta_i(\phi_i)$.

Reference	Equation*
Gompertz	$y_{ij} = \phi_{1i} \exp\{-\exp[-\phi_{3i}(x_{ij} - \phi_{2i})]\} + \varepsilon_{ij}$
Logistic	$y_{ij} = \phi_{1i} \{1 + \exp[-\phi_{3i}(x_{ij} - \phi_{2i})]\}^{-1} + \varepsilon_{ij}$
Richards	$y_{ij} = \phi_{1i} \{1 + \phi_{4i} \exp[-\phi_{3i}(x_{ij} - \phi_{2i})]\}^{-\frac{1}{\phi_{4i}}} + \varepsilon_{ij}$
von Bertalanffy	$y_{ij} = \phi_{1i} [1 - \phi_{2i} \exp(-\phi_{3i} x_{ij})]^3 + \varepsilon_{ij}$
Linear Mixed Effect (LME) Model	$y_{ij} = \phi_{1i} + \phi_{2i} x_{ij} + \varepsilon_{ij}$

*The parameters can be decomposed as: $\phi_{1i} = \beta_1 + \phi_{1i}$, $\phi_{2i} = \beta_2 + \phi_{2i}$, $\phi_{3i} = \beta_3 + \phi_{3i}$ and $\phi_{4i} = \beta_4 + \phi_{4i}$.

effects and random effects (individual per access) with the aid of the *nlme* package (PINHEIRO & BATES, 2022), with the *nlme* function.

RESULTS AND DISCUSSION

There was a convergence for all models used, so parameter estimation for all accessions was possible. LINDSTROM & BATES (1990) emphasize that model selection procedures must be developed and studied using real data when proposing their algorithm. Although, the methodology has never been applied before for the growth of total dry matter accumulation in garlic accessions, the convergence for all models and the proximity between actual and estimated values (Table 3) confirmed the efficiency of this method for this application. This provided support for its application in different areas.

Initially observing the estimates of the overall mean fixed effects, the asymptotic weight ($\hat{\beta}_1$) estimated by the equations yielded similar results, averaging around 24g and ranging from 22.57g by the Richards model estimation to 26.60g by the von Bertalanffy equation (Table 3). The time to inflection point ($\hat{\beta}_2$) of the growth curve for the interpretable parameters showed results close to 100 days, with approximately 98 by the Gompertz model and 106 by the Logistic model 115 according to Richards. The result for this parameter was close to 10 days for the von Bertalanffy model. The scale parameter ($\hat{\beta}_3$) also showed divergent results among equations, ranging between 0.04 and 0.21. The result for fixed effects estimates of asymptotic weight ($\hat{\beta}_1$) and scale parameter ($\hat{\beta}_3$) for the Gompertz, Logistic, and von Bertalanffy models were like those found by PUIATTI et al. (2018), who used data like that used in this study. Compared to the same authors, there was a divergence in the estimation of time to the

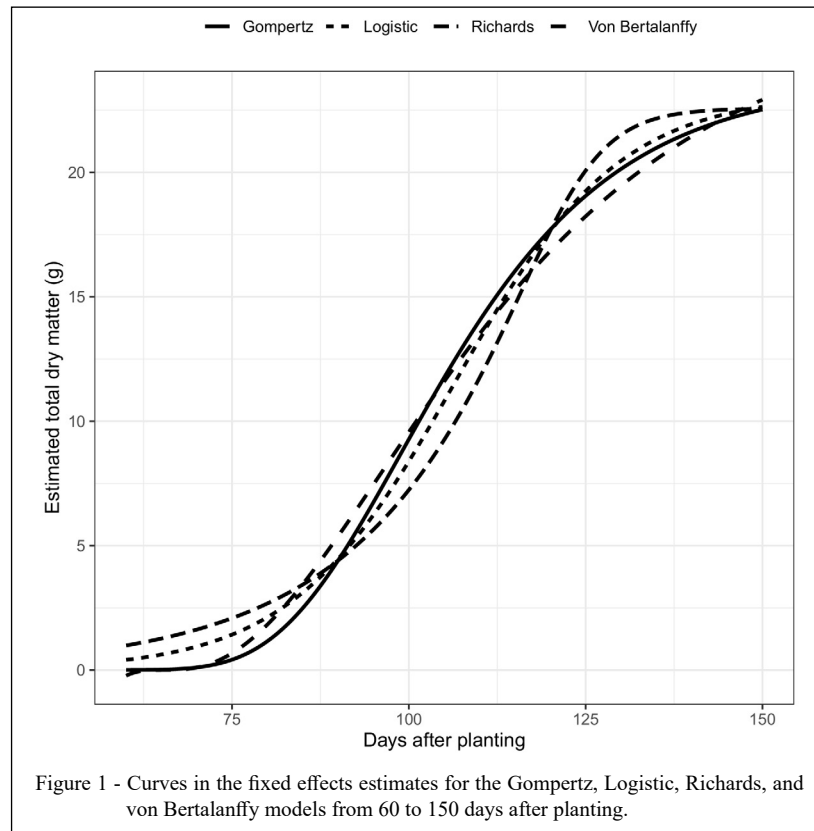
time to inflection point ($\hat{\beta}_2$). This difference may be caused by differences in model specifications. REIS et al. (2014) also reported analogous results for $\hat{\beta}_1$ and $\hat{\beta}_3$ or the Gompertz, Logistic, and von Bertalanffy models.

The shape of the curves for the populational mean, observed in figure 1, confirms what ARCHONTOULIS & MIGUEZ (2015) stated about the sigmoid shape of the Gompertz, Logistic, and Richards models. This behavior of the growth curves also corroborated with the results reported by PUIATTI et al. (2018) and REIS et al. (2014), both for garlic dry matter accumulation data, MAIA et al. (2009) applying the methodologies to the growth of banana trees and SILVA et al. (2017), who worked with micronutrients of cassava intercropped with banana trees, confirming that this behavior of growth curves is a common feature in the study of plant growth.

All the models used showed reasonable fit quality measures, indicating they were suitable methodologies for describing total dry matter accumulation in garlic accessions. This is confirmed by noting that the values of for R_{adj}^2 nonlinear methods were higher or equal to 0,9805, minimizing the residuals almost completely. The LME showed the lowest value for this metric ($R_{adj}^2 = 0,9272$), confirming that nonlinear models are more suitable for fitting the relationships with sigmoidal format. We can also observe that the von Bertalanffy model, which had lower results than the others, presented MAE and MSE of, respectively, 1.10 and 1.70 (Table 3), which indicates considerable proximity between observed and expected values even for the least prominent model. Applying the NLM method with quantile regression, PUIATTI et al. (2018) and REIS et al. (2014) also obtained satisfactory results in terms of fit by using the NLM methodology.

Table 3 - Mean (fixed) effect estimates (parameters $\hat{\beta}_1$, $\hat{\beta}_2$, $\hat{\beta}_3$ and $\hat{\beta}_4$) for the four equations used to adjust total dry matter per plant, with standard errors shown in parentheses.

Model	Parameters			
	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_4$
Gompertz	23.69 (0.9069)	98.90 (0.5913)	0.06 (0.0027)	-
Logistic	23.17 (0.8632)	106.56 (0.6931)	0.09 (0.0025)	-
Richards	22.57 (0.8238)	115.90 (5.6231)	0.21 (0.1598)	4.16 (3.7557)
von Bertalanffy	26.60 (1.1627)	10.28 (1.6764)	0.04 (0.0019)	-



The measures based on the likelihood function (AIC and BIC) point to the best fit, in that order, for the Richards and Logistic models, with values of 472.74 and 492.45, respectively (Table 4). The metrics that consider the difference between fitted and estimated values (MAE and MSE) showed superiority when estimated by the Logistic model in relation to the others, presenting results of 0.61 and 0.59, in that order. Therefore, the Logistic equation was the one that presented the best results according to most measures of goodness of fit. The choice of the Logistic model as the best fit was also reported by REIS et al. (2014) and PUIATTI et al. (2018), which was expected since the datasets for both researches are like this one. However, MAIA et al. (2009), fitting banana tree growth, and CARINI et al. (2020), applying nonlinear models for lettuce growth, also found a higher quality of fit for the Logistic model, showing that this equation is a good alternative for plant growth modeling in general. Observing these same metrics for the Logistic model (MSE = 0.61 and MAE = 0.59), such results are certified by looking at figure 2, where the proximity between these lines and the observed points is almost zero, just like the previously presented results.

Observing figure 3, we note that the accessions 4826 and 4505 stand out in asymptotic weight (\hat{b}_1) since values greater than zero show that the respective access presents effects higher than average. This means that these accesses tend to distance themselves from the average of the others, accumulating a greater amount of TDMP. As for the time to the time to inflection point and scale parameter (\hat{b}_2 and \hat{b}_3) the highest estimated values are respectively from accessions 4826 (which presented an estimated value of \hat{b}_1) and 4567. This means that these two accessions reach the inflection point of the curve at a higher time (\hat{b}_2) and present the scale parameter (\hat{b}_3) higher than the average of the other accessions.

CONCLUSION

The use of Nonlinear Mixed Effect Models provided advantages in the adjustment in relation to traditional nonlinear models, presenting excellent results in adjustment quality and obtaining estimates close to the actual values from the estimation of individual effects per access in a single adjustment per equation for all accessions.

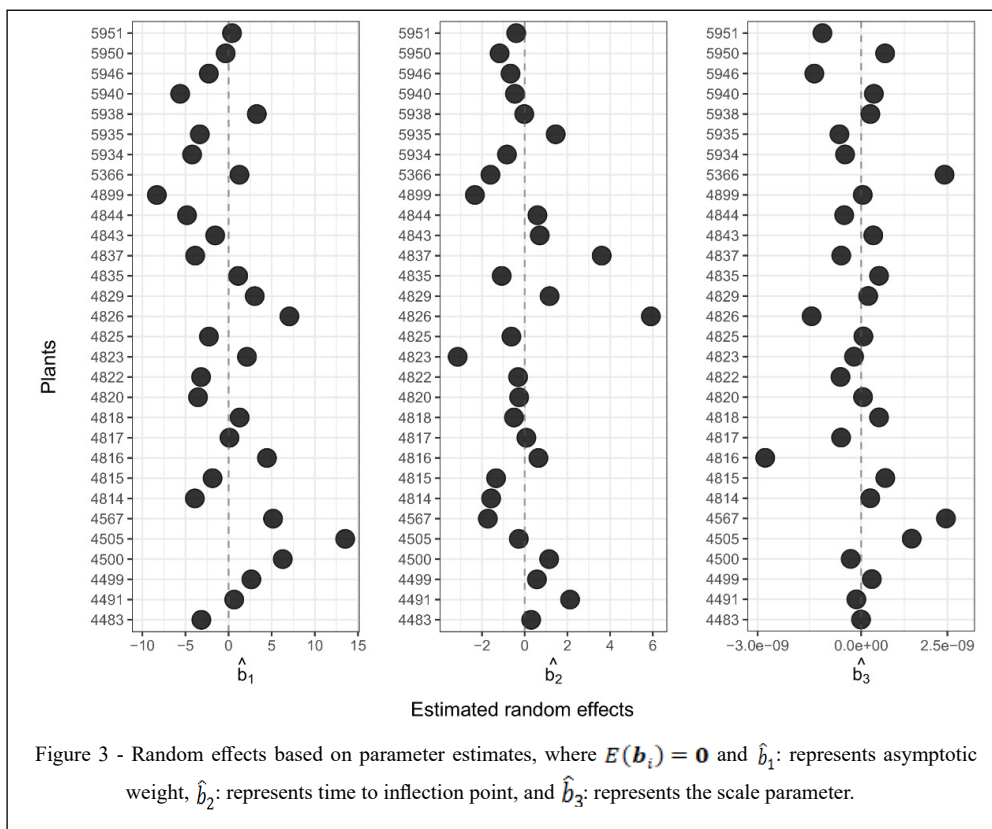
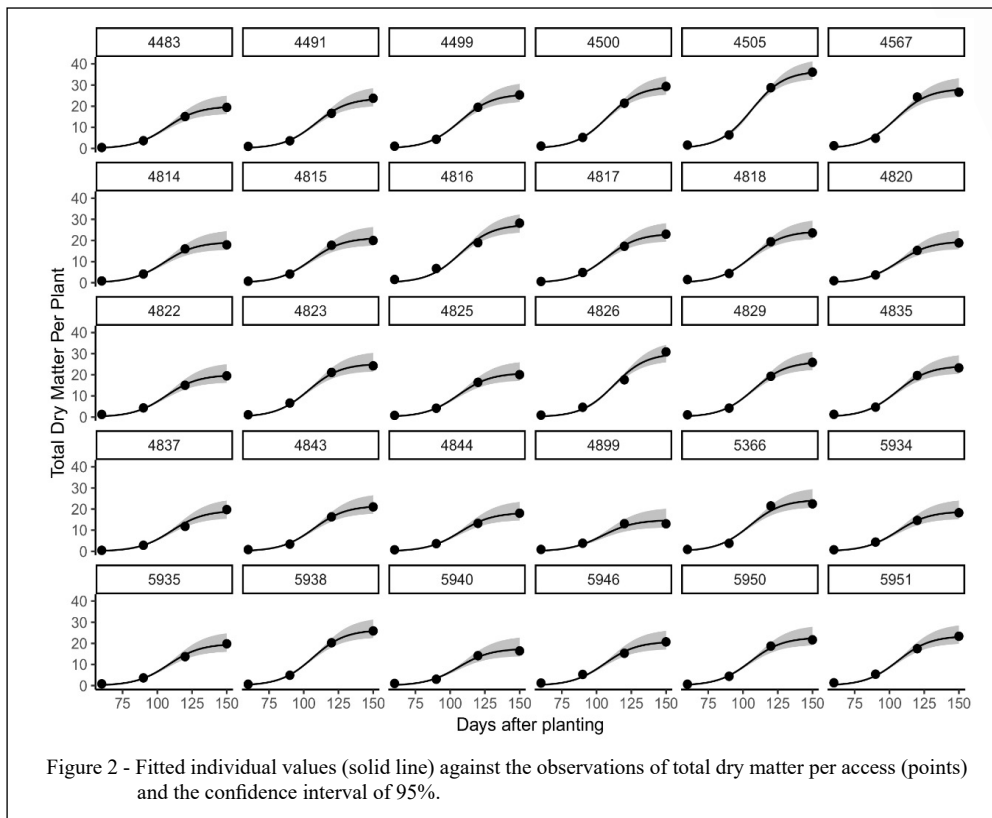


Table 4 - Quality of fit measures for the Gompertz, Logistic, Richards and von Bertalanffy equations.

Model	AIC	BIC	R_{adj}^2	MAE	MSE
Gompertz	491.53	511.05	0.9873	0.83	1.11
Logistic	472.94	492.45	0.9931	0.61	0.59
Richards	472.74	497.83	0.9902	0.64	0.86
von Bertalanffy	529.39	548.91	0.9805	1.10	1.70

The Logistic model, as in other studies, presented a better fit than the others, with results close to the Richards model. The approach allowed us to identify the best garlic accessions according to each parameter of the growth model through mean deviations quantified by random effects through a precise estimation, confirmed by the goodness-of-fit measures.

ACKNOWLEDGEMENTS

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Brazil (grant number 301004/2009-9). And was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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