ARTICLE
BIOINFORMATICS
DOI: 10.5504/BBEQ.2012.0073

A BIOMATHEMATICAL MODEL FOR *PHOMA TRACHEIPHILA* CITRUS RESISTANCE SCREENING

Khaled Khanchouch[1, 2], Mohamed Rabeh Hajlaoui[2], Hakan Kutucu[3]
[1]University of Tunis, ISAJC Department of Techniques, Tunis, Tunisia
[2]National Institute of Agronomic Research, Laboratory of Biotechnology, Tunis, Tunisia
[3]Izmir Institute of Technology, Department of Mathematics, Izmir, Turkey
Correspondence to: Khaled Khanchouch
E-mail: khanchouchkhaled@yahoo.fr

ABSTRACT

The causal agent of Mal Secco, Phoma tracheiphila, is responsible for many important losses in the Citrus crop worldwide. The resistance enhancement of Citrus susceptible to the pathogen infection depends on the availability of a valid test for disease assessment. However, the resistance analysis tests used give controversial results. In this paper, we propose a new mathematical model to conduct a rapid and efficient resistance screening test. This model has the advantage to give a strict evaluation of the resistance and not a relative estimation as in the usual tests. The results obtained by this model are in concordance with those observed in the orchards.

Biotechnol. & Biotechnol. Eq. 2012, **26**(5), 3282-3285

Keywords: fungi, citrus, biomathematics, modelling

Introduction

Among the various threats which inflict a critical phytosanitary state in the citrus plantation, Mal Secco disease is the most serious problem in the Mediterranean and the Black Sea region (3). The causal tracheomycosis agent, *Phoma tracheiphila*, is very difficult to be eradicated from the infected areas. Trials conducted in different natural orchards using fungicide treatments showed unsatisfactory results (6). The usual breeding methods used to improve the resistance level of the sensitive cultivars were not successful in reducing the impact of the attacks observed in the natural citrus fields (9).

Limitation of the conventional control methods employed to protect susceptible host plants oriented researchers towards the screening of new varieties tolerant to the pathogen (5). The reaction of many citrus species and cultivars towards the toxin of the parasite and the radiation treatments was investigated to develop new approaches of selection of Citrus lemon resistant cultivars (3, 8).

Regenerated somaclone variants induced by toxin and seedless ones obtained using radiation treatments of buds, need to be evaluated for their promised enhanced resistance level to the pathogen infection. The conventional assessment analysis tests used based on the relative statistical comparing methods do not allow to have a real evaluation which reflects the inner reaction of the host plant. As a consequence, many controversial results for the same tested genotype can be observed between the different laboratories using the common relative comparative analysis methods (10, 12).

In order to improve the genetic researches for the resistance screening towards the causal agent of Mal Secco, a valid and simple technique of the disease evaluation is required (5). The new analysis method should be able to discriminate between the tested plants based on their proper resistance level and give a real statement of the new selected genotype towards the pathogen infection. The development of this new tool of analysis is based on a mathematical model.

The most known models developed in the phyotopathological studies are: Monomolecular, Exponential, Gompertz and Logistic models. The logistic model which was proposed firstly to represent human population growth was later developed by Van der Plank as being more appropriate for most polycyclic plant diseases (1,13). This growth model is the most widely used one for describing natural epidemics of plant diseases (4, 7).

In the case of Mal Secco citrus disease, the natural infection occurs twice during the year. The first infection, in autumn, is produced by the conidia of the pathogen which penetrate via the injured wood and then the parasite is further developed in the xylem vessels. The development of the mycelium inside the plant produces fructification bodies called pycnidia on the surface of the destroyed tissues. Pycnidia are responsible for the second foliar infection in the spring. However, the artificial infection in the laboratory rapid tests allows to observe only a monocyclic development of the disease after infection by one type of the asexual propagules of the fungi.

Since the upper canopy of the susceptible host plants is the main target of the air born infection, a monocycle foliar laboratory rapid test is used for the artificial infection of the tested plants. As the evaluation of the resistance to Mal Secco encounters several technical difficulties for many years a new methodology of screening has been urgently needed (10). In the present work a new data analysis of the symptomatological severity index of the disease is proposed to describe more precisely the specific reaction of each tested cultivar. Results

obtained after the incubation period are processed by means of both polynomial interpolation method and least-square approximations using adequate types of regression curves of the disease. The provided fitted curves of the infected cultivar are compared and are used to determine the differential resistance reaction of the tested genotype.

Materials and Methods

Inoculum preparation

A highly pathogenic single-spore Tunisian isolate of *Phoma tracheiphila* was cultured on a modified potato dextrose agar with a concentration of 1 % glucose. After two weeks of incubation pycnidia were harvested and pycnidiospores were suspended in sterile water with the aid of a fine painter's brush. The pycniospore suspension solution adjusted to a concentration of spores of 10^6/ml was used to inoculate the tested plants.

Inoculation method

Three cultivars of *Citrus limon* BURM. F.: Eureka, Monachello and Interdonato, were inoculated by deposing 15 µl of the adjusted pycnidiospores suspension solution on the inoculation sites prepared by pricking each leave in four different places using three entomological pins mounted on a cork. A completely randomized design with three repetitions was adapted for all the experiments. One hundred and twenty inoculation points were assessed per each cultivar. To determine the development of the disease, a scale of six classes was used to evaluate the reaction of the tested plants. The disease classes are defined according to the progress of the disease using the empirical scale of Luisi et al. (5) slightly modified as follows:

Degree 0: No sign of infection;

Degree 1: Chlorotic halo around the inoculation point;

Degree 2: Chlorosis of the vein close to the inoculation point;

Degree 3: Vein Chlorosis extended as far as the leaf edge;

Degree 4: Generalized chlorosis;

Degree 5: Vein browning.

We associated for each degree its respective class of sensibility and thus determined six disease classes of sensitivity from class zero to class five. After an incubation period of 30 days, the tested plants were rated using the above described empirical scale and the severity of the disease was determined by calculating the mean of the rated score for each cultivar.

The experimental results from the artificially inoculated plants were analyzed by one-way ANOVA analysis. Mean separation was done with Newman–Keuls test at $P = 0.05$.

Data processing

Data analysis of the experimental results obtained for each inoculated plant allowed calculating the cumulative percentage frequency for each class. Calculation of the cumulative frequency was done as follows:

$$yi = \left[\frac{\sum_0^i (frequency\ of\ xi)}{N}\right] 100$$

where i is the respective class, varying from "0" to "5"; yi is the cumulative frequency for the respective class, xi; xi is the class i varying from "0" to "5"; N is the total number of the inoculation points for each cultivar.

Fitting model

Least-square approximations for linear, exponential and polynomial regressions were used to fit the set data points. To assess the precision of fitting, the coefficient of determination (R^2) was calculated for each regression curve.

Polynomial interpolation

The experimentally obtained data (xi,yi), i = 0,…, 5, of the different tested cultivars were interpolated with a polynomial of degree five. The coefficients of the polynomial function were determined by solving the corresponding linear system of six equations (2).

Results and Discussion

Resistance assessment of the inoculated plants was attempted on the basis of the severity of the disease symptoms following foliar inoculation. After the incubation period all the tested plants developed the pathological symptoms caused by the parasite. The cultivars Eureka and Monachello expressed, respectively, the highest and the lowest degree of the disease index. However, Interdonato cutivar, showed an intermediate index of the disease severity. The statistical analysis indicated significant differences between the three inoculated cultivars at $P = 0.05$. According to this analysis, the tested plants were ranked in three different groups of resistance (**Table 1**).

TABLE 1

Infection severity rating of lemon cultivars

Cultivars	Severity of the disease (means)	Ranked group*
Eureka	4.216	I
Interdonato	2.225	II
Monachello	0.8	III

* I: sensitive; II: tolerant; III: resistant

TABLE 2

Coefficients of determination* of the three regression models

Cultivars	Linear regression	Exponential regression	Polynomial regression
Eureka	0.7152	0.9568	1
Interdonato	0.7887	0.8650	1
Monachello	0.6250	0.5690	1

* The coefficient of determination of the polynomial regression given in this table corresponds to the polynomial function of degree 5.

The coefficients of determination for the processed data were calculated using the linear, the exponential and the polynomial regression method for the three resistance groups.

The results are shown in **Table 2**. Polynomial regression functions of different degrees were tested for the disease curves and their respective values of the coefficient of determination R^2 were determined (**Table 3**).

TABLE 3

Coefficients of determination computed for the polynomial regressions

Cultivars	Degree 2	Degree 3	Degree 4	Degree 5
Eureka	0.9299	0.9951	0.9997	1
Interdonato	0.9456	0.9732	0.9980	1
Monachello	0.9150	0.9891	0.9990	1

Elevated values of the coefficient of determination R^2, calculated for the tested groups of resistance indicate the strength of fitting using the polynomial regression method of degree 5. The linear and the exponential regression methods revealed to be less accurate in describing the different reactions of the infected plants. Their respective coefficients of determination decreased respectively starting from group I to group III of resistance. However, for the polynomial fitted curves, the values of the coefficients of determination remained highly significant to describe the different phytopathological reactions of the different tested groups of resistance.

The performance of the polynomial model was validated by the phytopathological experimental test, the statistical analysis by the software Statistica 5.1, and the calculation of the parameters of the polynomial curves using the software *Mathematica* 6.0 (**Table 4**).

The response of the studied cultivars as recorded and analyzed by the mathematical model reflects the natural behavior of the tested plant in the natural fields. The determined polynomial coefficients were in concordance with the ones obtained by *Mathematica* using the Hermite interpolation technique to find fitting curves to given sets of data (11).

On the other hand, the coefficient parameters of the interpolated polynomial were comparable to those of the regression polynomial function. This can be explained with the higher coefficient of determination R^2 of the polynomial curve of degree five obtained for all the tested cultivars.

According to the above results, the polynomial mathematical model was retained to describe the phytopathological reaction of the infected plants. Using the interpolation polynomial function it is now possible to estimate the development of the disease with precision. The polynomial model allows evaluating the reaction of the tested plants at the data points within the range of the determined classes. Drawing the representative polynomial fitted curve for the three groups of resistance, it was found that the polynomial with an upper concave curve is correlated with the statistical group I of resistance, while group II of resistance was characterized with a mixed convection regression curve. Group III of resistant plants showed a lower concave curve (**Fig. 1**).

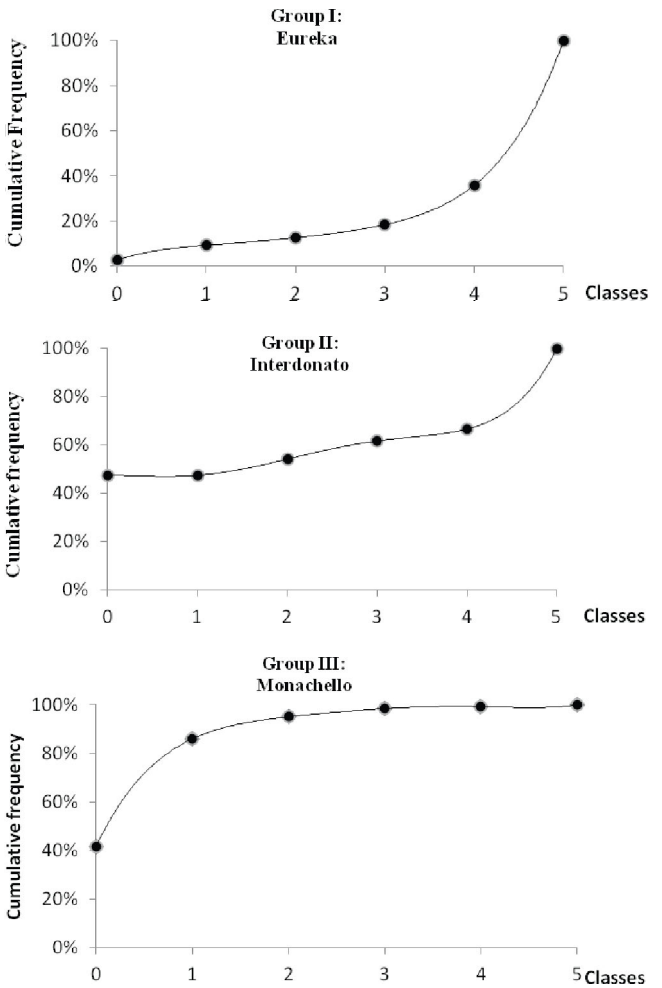

Fig. 1. Polynomial regression fitted curves for the cumulative disease classes frequency of the infected *Citrus limon* cultivars.

Based on the geometrical analysis of the respective polynomial function curve of each infected plant, it is possible to determine the resistance degree of the tested host plant. Three types of polynomial curves can be used to describe three resistance groups:

TABLE 4

Parameters of the polynomial functions of degree 5

Cultivars	Regression curve
Eureka	$y = 0.187x^5 - 1.736x^4 + 6.701x^3 - 12.43x^2 + 13.944x + 2.5$
Interdonato	$y = 0.263x^5 - 2.534x^4 + 7.638x^3 - 5.798x^2 + 0.430x + 47.5$
Monachello	$y = 0.208x^5 - 3.159x^4 + 18.611x^3 - 54.340x^2 + 82.847x + 41.666$

Type A: with an upper concave convection characterizes group I of resistance;

Type B: with a mixed convection curve characterizes group II of resistance;

Type C: with a lower concave convection characterizes group III of resistance.

Analysis of the inoculation data using both the polynomial model and usual statistical tools revealed concordance between the results obtained by the two methods regarding the resistance classification of the infected plants. In contrast, the polynomial model was able to evaluate the resistance level of the infected plants without using comparative methods. Based on the inner response of the tested plants, the polynomial model describes the repartition of the different classes of the infected plant to determine precisely its level of resistance.

Using this new methodology of analysis the proper reaction of the susceptible host plant can be revealed regardless the phytopathological response of the other cultivars used in the same test. This new approach of data analysis allows to perform the resistance tests in different laboratories all over the world and to obtain the same results for the same tested genotype.

Conclusions

Taking into account the obtained results, the model proved to be a promising new method for the resistance screening towards the Mal Secco citrus disease. The polynomial model as described was revealed to be an accurate method to discriminate between the three main levels of resistance observed in the natural orchards of the *Citrus limon* cultivars. Our analysis, using the polynomial model, will be performed to other cultivars to test the intermediate resistance levels recorded between the three main groups of resistance.

Acknowledgements

The authors would like to thank TA for their financial support.

REFERENCES

1. **Bacaër N.** (2011) In: A Short History of Mathematical Population Dynamics, Springer-Verlag, London, 35-39.
2. **Gareth W.** (2009) Linear Algebra with Applications, 7th Ed., Jones & Bartlett Publishers, p. 55.
3. **Gulsen O., Uzun A., Pala H., Canihos E., Kafa G.** (2007) Sci. Hortic.-Amsterdam, **112** (2), 184-190.
4. **Jeger M.J.** (2004) Annu. Rev. Phytopatol., **42**, 61-82.
5. **Luisi N., De Cicco V., Cutuli G. , Salerno M.** (1978) Proc. Int. Soc. Citriculture, **1**, 197-200.
6. **Pionnat J.C.** (1982) Fruits et Agrumes, **37**(4), 237-248.
7. **Segarra J., Jeger M.J., Van den Bosch F.** (2001) Phytopathology, **91**, 1001-1010.
8. **Sesto F., Grimaldi V., Pennisi A.M.** (1990) Advances in Horticultural Science, **4**, 97-102.
9. **Solel Z., Salerno M.** (1988) In: Compendium of Citrus Diseases (J.O. Whiteside, S.M. Garnsey, L.W. Timmer, Eds.), American Phytopathological Society Press, St. Paul, MN, 18-20.
10. **Solel Z., Spiegel-Roy P.** (1978) Phytoparasitica, **6**(3), 129-134.
11. **Wolfram S.** (1999) The Mathematica Book, Version 4, Cambridge University Press, p. 1469.
12. **Tuzcu O., Cinar A., Kaplankiran M., Erkilic A., Yesiloglu T.** (1989) Fruits, **44**(3), 139-148.
13. **Van der Plank J.E.** (1963) Plant Disease: Epidemics and Control, Academic Press, New York &. London, p. 349.