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Optimization of the number of evaluations for early blight disease in tomato accessions using artificial neural networks

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SUMMARY

The efficacy of artificial neural networks (ANN) to solve complex problems can optimize evaluation processes for early blight disease on tomato plants, reducing required time and resources. The objective of the study was to verify the efficiency of ANN to predict the area under the disease progress curve (AUDPC) to reduce the number of assessments and establish the best time to evaluate early blight disease in tomato accessions. The severity of this disease was evaluated in one hundred and thirty-five tomato accessions from the Germplasm Vegetable Bank of the Federal University of Viçosa (BGH-UFV) in three experiments. The area under the disease progress curve (AUDPC) was calculated with data from six evaluations of the disease's severity. Several ANN MLP types (Multi-Layer-Perceptron) were trained, taking into account AUDPC values for desired output. Different numbers and assessment combinations for early blight disease severity were used as input. ANN's were efficient at predicting AUDPC and reduced the number of evaluations from six to two. The twelfth and eighteenth days after pathogen inoculation are the best to evaluate the severity of early blight disease. Genotype by environment affects the efficiency in predicting the AUDPC. ANNs were efficient at predicting the area under the early blight disease progress curve (AUDPC) with fewer evaluations, and as such optimized assessment of this disease in tomato accessions.

1. Introduction

The tomato (*Solanum lycopersicum* L.), originally from South America, is one of the world's most cultivated crops (Ranjan et al., 2012). It has a high yield, but is economically risky, mainly due to disease. Early Blight Disease (EBD), caused by the fungus *Alternaria tomatophila* Simmons, causes tomato crop losses (Brun et al., 2013).

A wide range of hosts, high variability, and a prolonged active stage all hamper the control of EBD (Singh et al., 2014). Relative humidity higher than 80% and moderate temperatures, of around 27 °C, favor the disease (Foolad et al., 2000). EBD is controlled primarily with fungicides. A few resistant tomato cultivars are also available (Ashrafi and Foolad, 2015). Chemical control is expensive and causes problems for humans and the environment (Hariprasad and Niranjana, 2009), however resistant cultivars can reduce this problem. This makes identification of sources of resistance in tomato accessions of germplasm banks highly valuable.

Data from six to eight evaluations of EBD severity processed at regular intervals, of- the area under the disease's progress curve (AUDPC) (Mukherjee et al., 2010) can identify superior tomato genotypes for breeding programs (Grigolli et al., 2011; Foolad and Ashrafi, 2015; Rani et al., 2015). These assessments are labor-intensive and are usually carried out in the field using a large number of genotypes (Foolad et al., 2008; Kumar and Srivastava, 2013).

Artificial neural network (ANN) models were used to predict average regional wheat yields and production (Alvarez, 2009), environmental impact on strawberry production, (Khoshnevisan et al., 2013) and biological and environmental factors influencing single pea seed mass (Dacko et al., 2016). The efficiency of ANNs to model complex problems can predict AUDPC using fewer evaluations. This reduces labor and costs during selection of tomato accessions resistant to EBD. ANNs were also applied to predict genetic resource characteristics in plant breeding (Pandolfi et al., 2009; Bari et al., 2012; Emamgholizadeh et al., 2015). ANN computational models, which mimic the human brain in recognizing data patterns and regularities, represent an alternative as a universal substitute for complex functions (Gianola et al., 2011). They can have superior performance compared to conventional statistical models by being

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non-parametric, not requiring detailed information on the physical processes of the system studied, as well as tolerating data loss.

The objective of this study was to verify the efficiency of ANNs to predict the area under the progress disease curve (AUDPC) of Early Blight Disease (EBD) using fewer evaluations to establish the best time for its evaluation in tomato accessions.

2. Material and methods

The resistance of one hundred and thirty-five tomato accessions from the Germplasm Vegetable Bank of the Federal University of Viçosa (BGH-UFV) for Early Blight Disease (EBD) was evaluated in three experiments: the first from October 2009 to January 2010 with 33 accessions; the second from July to October 2010 with 51 accessions; and the third from September to December 2010 with 51 accessions (Laurindo et al., 2015). These accessions were randomly selected from approximately 840 accessions of tomato stored in the BGH-UFV (Silva et al., 2001). Information on passport data, morphoagronomic characteristics, pest and disease resistance assessments are available on the World Wide Web and can be accessed at www.ufv.br/bgh. EBD susceptible tomato cultivars Débora and Santa Clara (Grigolli et al., 2011) were used as the control in the three experiments. Pattern resistant as the control to early blight was not used, because resistance to this disease in the cultivated species of tomato is rare (Foolad et al., 2008).

The experiments were conducted at the experimental field of the Vegetable Crop Sector of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais, Brazil (20°45′14″S, 42°52′53″W and altitude of 648,74 m) with a randomized complete block design with three replications. Each parcel had five plants and the three central ones were evaluated.

The tomato seeds were sown in 128 cell trays with commercial vegetable cultivation substrate Plants with four definitive leaves were transplanted into the field in a 1.0×0.5 m spacing. Plants were cultivated with a single stem tied vertically with narrow ribbon. Pesticide application stopped two weeks before inoculation and during assessment for EBD severity.

Forty-five days after transplanting, tomato plants were inoculated with a pathogenic mixture for tomatoes of conidia VBH2, 129's, 149's and 151 of *Alternaria* spp. isolates from the Brazilian states of Minas Gerais, Santa Catarina, Paraná, and Rio Grande do Sul, respectively. We obtained these isolates from the collection of cultures at the Phytopathogen Population Biology laboratory of the Department of Phytopathology of the UFV. The isolates were cultivated on V8 CaCO3 agar (175 ml V8 juice, 3 g CaCO3, 20 g agar, 1 l of water) at 25 ± 2 °C under a 12 h photoperiod to produce the inoculum.

Distilled water was added to the plates on the fifth day of incubation with the conidia and mycelium removed using a brush. The plates were maintained uncovered at 22 ± 2 °C under six 40 W black light lamps (Sylvania® —1.20 m long with 3 cm between them and positioned at 30 cm above the plates) with controlled 16 h:8 h dark and light photoperiods. After this period, the conidia was removed with 10 ml of distilled water per plate followed by swabbing the surface of the colony with soft bristle brush. The suspension was filtered through sterile double layer gauze in a beaker and the concentration adjusted to 2×10^3 conidia ml^{-1}. A conidia suspension with equal volume per isolate was prepared.

Inoculations in the three experiments were carried out on January 10, October 06, and December 6, 2010 at around 04:00 P.M. with 10 ml of suspension applied per plant with a knapsack sprayer.

The plants were irrigated by sprinkling after inoculation to ensure high ambient humidity. Assessment for EBD severity was initiated three days after inoculation, from the 13th to the 28th of January; 09th to the 24th of October, and the 09th to the 24th of December 2010 at three day intervals, totaling six evaluations per experiment.

Scores were assigned in the field for the leaves of each plant by estimating the severity of the disease. The average score for each leaf represented the overall score per plant, which we used to estimate the area under the disease progress curve (AUDPC) (Simko and Piepho, 2012), with the expression: $AUDPC = \{\sum_{i+1}^{n-1}[(y_i + y_{i+1})/2] * (t_{i+1} - t_i)\}$. A common approach to determine AUDPC is through a simple midpoint (trapezoidal) rule that breaks up a disease progress curve into a series of trapezoids, calculating the area of each, and then adding up the areas. Where: y_i and y_{i+1} = percentage of damaged leaf area in the evaluation i and the next i + 1; t_i and t_{i+1} = time interval between evaluations; and n = total number of evaluations. This formula shows that AUDPC is calculated by multiplying each assessment value by its respective weight. For each assessment, the weight is the duration between the midpoint of the previous time interval to the midpoint of the subsequent time interval.

ANN MLP type (Multi-Layer-Perceptron) with back propagation algorithm and Levemberg-Marquardt optimization were developed with Neural Network Toolbox Matlab software (The MathWorks, 2012). In this methodology the sample input (EBD severity) is presented to the network, which will produce an output response, which is compared to the desired one (AUDPC). By the deviations between the predicted and observed value (AUDPC) an error is generated, which is used to readjust the connection weights (synaptic weights). In this way, after several iterations, the synaptic weights are adjusted innumerable times allowing the network to predict values from input data.

The maximum number of training evaluations was refereed as 10,000; the MSE (mean square error) minimum to stop training was set at 1.0×10^{-7} and the largest number of successive failures for cross-validation (early stopping) was six. To ensure the same weight for the input parameter during training, input and output data was standardized for the range between −1 and 1 by the equation: $V_n = 1 + 2(V_{obs} - V_{max})/(V_{max} - V_{min})$ in which V_n is the normalized value, V_{obs} observed, V_{min} the minimum sample, and V_{max} the maximum sample.

The AUDPC was used as the desired. Different numbers and combinations of evaluations were used as an input file per layer constitutive of network configurations. Networks trained with data from the 2nd, 3rd, 4th, 5th, and 6th evaluations and those from all evaluation combinations two by two, three by three and four by four with data for EBD severity were tested to verify the possibility of predicting the AUDPC with one, two, three, or four evaluations.

The first evaluation (evaluation 1), on the third day after inoculation, was not included in the data input layer of the MLP because most of the plants had 0% leaf area affected by EBD and therefore it proved to be uninformative.

The MLP networks were trained individually and simultaneously using data from the three experiments to evaluate the influence of genotype by environment. The networks individually trained with data from experiments 1, 2, and 3, were named "Network 1", "Network 2" and "Network 3", respectively. The networks trained with simultaneous data were named "Network 4". 70% of plant information was selected at random to train and update the networks, 15% for cross-validation (early stopping), and 15% as a test sample.

Two hidden layers with six neurons each and an output layer with one neuron were used for all MLP. We used the hyperbolic tangent activation function for the hidden layers, and the linear function for output. Each MLP configuration was trained 1000 times, because free parameters randomly generated at the beginning of training may influence the final result (Soares et al., 2014). A total of 120,000 MLP networks (4×30 network combination evalu-

Table 1
Pearson's correlations between observed and estimated area under Early Blight Disease progress curve (AUDPC) for the best trained network for evaluation combinations (Eval.) of diseased leaf area by Early Blight in tomato plants in experiments 1 (E1), 2 (E2) and 3 (E3).

Eval.	Network 1				Network 2				Network 3			
	E1	E2	E3	Mean	E1	E2	E3	Mean	E1	E2	E3	Mean
2	0.20	0.00	0.38	0.19	−0.37	0.10	−0.37	−0.21	0.60	0.00	0.36	0.32
3	0.75	0.71	0.61	0.69	0.66	0.88	0.67	0.74	0.60	0.79	0.71	0.70
4	0.80	0.88	0.79	0.82	0.84	0.97	0.82	0.88	0.89	0.91	0.84	0.88
5	**0.92**	**0.95**	**0.82**	**0.90**	**0.93**	**0.97**	**0.84**	**0.91**	**0.86**	**0.93**	**0.92**	**0.91**
6	0.78	0.89	0.79	0.82	0.85	0.92	0.82	0.86	0.76	0.82	0.86	0.81
2 e 3	0.67	0.73	0.64	0.68	0.52	0.88	0.60	0.66	0.68	0.75	0.70	0.71
2 e 4	0.79	0.46	0.75	0.67	0.11	0.97	0.02	0.36	0.89	0.94	0.85	0.89
2 e 5	0.95	0.93	0.84	0.91	0.82	0.97	0.77	0.85	0.90	0.88	0.92	0.90
2 e 6	0.78	0.60	0.79	0.72	0.65	0.91	0.69	0.75	0.82	0.46	0.85	0.71
3 e 4	0.86	0.89	0.80	0.85	0.86	0.97	0.83	0.89	0.90	0.89	0.84	0.88
3 e 5	0.96	0.86	0.90	0.91	0.93	0.99	0.89	0.94	0.92	0.95	0.96	0.95
3 e 6	0.87	0.83	0.81	0.83	0.80	0.97	0.89	0.89	0.90	0.92	0.92	0.91
4 e 5	0.96	0.91	0.86	0.91	0.97	1.00	0.91	0.96	0.94	0.91	0.95	0.93
4 e 6	**0.96**	**0.94**	**0.89**	**0.93**	**0.95**	**0.99**	**0.95**	**0.96**	**0.97**	**0.97**	**0.98**	**0.97**
5 e 6	0.93	0.89	0.86	0.90	0.93	0.97	0.87	0.92	0.91	0.94	0.95	0.93
2, 3 e 4	0.82	0.85	0.79	0.82	0.56	0.96	0.57	0.70	0.90	0.92	0.86	0.89
2, 3 e 5	0.97	0.93	0.86	0.92	0.89	0.98	0.87	0.91	0.94	0.98	0.96	0.96
2, 3 e 6	0.86	0.73	0.85	0.81	0.66	0.97	0.46	0.69	0.92	0.84	0.93	0.90
2, 4 e 5	0.97	0.92	0.83	0.91	0.47	1.00	0.49	0.65	0.98	0.89	0.98	0.95
2, 4 e 6	0.95	0.92	0.92	0.93	0.47	0.99	0.32	0.59	0.94	0.25	0.92	0.70
2, 5 e 6	0.96	0.95	0.92	0.94	0.49	0.97	0.75	0.74	0.93	0.96	0.96	0.95
3, 4 e 5	0.98	0.96	0.92	0.95	0.97	1.00	0.91	0.96	0.98	0.92	0.97	0.96
3, 4 e 6	0.97	0.95	0.88	0.93	0.96	0.99	0.96	0.97	0.95	0.91	0.96	0.94
3, 5 e 6	0.99	0.71	0.93	0.88	0.98	0.99	0.95	0.97	0.97	0.98	0.98	0.98
4, 5 e 6	**0.97**	**0.95**	**0.90**	**0.94**	**0.98**	**1.00**	**0.97**	**0.98**	**0.99**	**0.99**	**0.99**	**0.99**
2, 3, 4 e 5	0.98	0.94	0.92	0.95	0.37	1.00	0.37	0.58	0.98	0.99	0.98	0.98
2, 3, 4 e 6	0.96	0.92	0.93	0.94	0.77	0.99	0.75	0.84	0.96	0.97	0.97	0.96
2, 4, 5 e 6	1.00	0.98	0.94	0.97	0.40	1.00	0.49	0.63	0.99	0.81	1.00	0.93
3, 4, 5 e 6	**1.00**	**0.94**	**0.95**	**0.96**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
2, 3, 5 e 6	0.98	0.96	0.90	0.94	0.92	0.99	0.91	0.94	0.98	0.97	0.98	0.98
Means	0.88	0.84	0.83		0.70	0.94	0.70		0.90	0.85	0.90	
	0.85				0.78				0.88			

Network 1; Network 2; Network 3: MLP trained with data from experiments 1, 2, and 3, respectively. Values in bold refer to measurements with improved AUDPC prediction with 1, 2, 3, and 4 evaluations.

ations × 1000 trainings) were trained. The network with the lowest mean square error was selected from the 1000 trained by combining the ratings tested in the four networks, resulting in 120 selected networks (4 networks × 30 combinations).

3. Results

The results from data with one evaluation showed less satisfactory results than that with two evaluations (Table 1). Average correlation of 0.19, −0.21, and 0.32 AUDPC between values calculated and predicted by the "Network 1", "Network 2" and "Network 3", respectively, was obtained. The best results were those from the fifth evaluation, with an average correlation between calculated and predicted AUDPC of 0.90, 0.91, and 0.91 for "Network 1", "Network 2" and "Network 3", respectively (Table 1).

The ANNs trained with data from two evaluations provided better results for evaluations 4 and 6 (Table 1), when carried out on the twelfth and eighteenth days after inoculation, with an average correlation of 0.93, 0.96, and 0.97 for "Network 1", "Network 2", and "Network 3", respectively. The three evaluations showed better results with four, five, and six evaluations with estimates of 0.94, 0.98, and 0.99. Correlation estimates close to 1.00 were observed with four evaluations. Evaluations 3, 4, 5, and 6 provided better results with average correlation estimates of 0.96, 1.00, and 1.00 for "Network 1", Ñetwork 2" and "Network 3", respectively.

The RNAs "Network 1" showed the highest correlation estimates with, a 0.88, 0.84, and 0.83 average for experiments 1, 2, and 3, respectively (Table 1). Similar results were found for "Network 2" and "Network 3". "Network 2" had the highest average correlation for experiment 2 (0.94) and the lowest for 1 and 3 (0.71 and 0.70, respectively). "Network 3" showed a higher correlation estimate for experiment 3 (0.90), and lower for 1 (0.89) and 2 (0.85).

"Network 4", trained using data from the three experiments simultaneously, showed an overall average of 0.90 for the correlation estimate (Table 2). This estimate was higher than those for "Network 1", "Network 2", and "Network 3"(0.85, 0.78 and 0.88, respectively) (Table 1). "Network 4", trained with data from one evaluation, performed best with evaluation 5 (0.91 correlation) (Table 2). Two evaluations showed better results with 4 and 6, (0.96 correlation). With three evaluations, the best results were obtained from 4, 5, and 6, (0.99 correlation). With four evaluations, the best results were obtained with 3, 4, 5, and 6, (1.00 correlation). These evaluations were the most important to calibrate "Network 4", as with "Network 1", "Network 2", and "Network 3".

Evaluations 4 and 6 predicted the AUDPC with considerable precision (Fig. 1). In this situation, the predicted and observed AUDPC ranking showed few changes, with correlations of 0.96, 0.98, and 0.95 for experiments 1, 2, and 3, respectively (Table 2). Three evaluations, 4, 5, and 6, allowed a more accurate selection of the best genotypes (Fig. 1), with similar observed and predicted AUDPC values, with correlations of 0.99, 1.00, and 0.99 for the experiments 1, 2, and 3, respectively (Table 2). Evaluations 3, 4, 5, and 6 showed the same estimated and predicted RNAs values as those measured (Fig. 1) with a correlation coefficient of 1.00 in the three experiments (Table 2).

4. Discussion

Artificial neural networks (ANN) simulate the biological neural system, mimicking the human brain's learning processes to solve

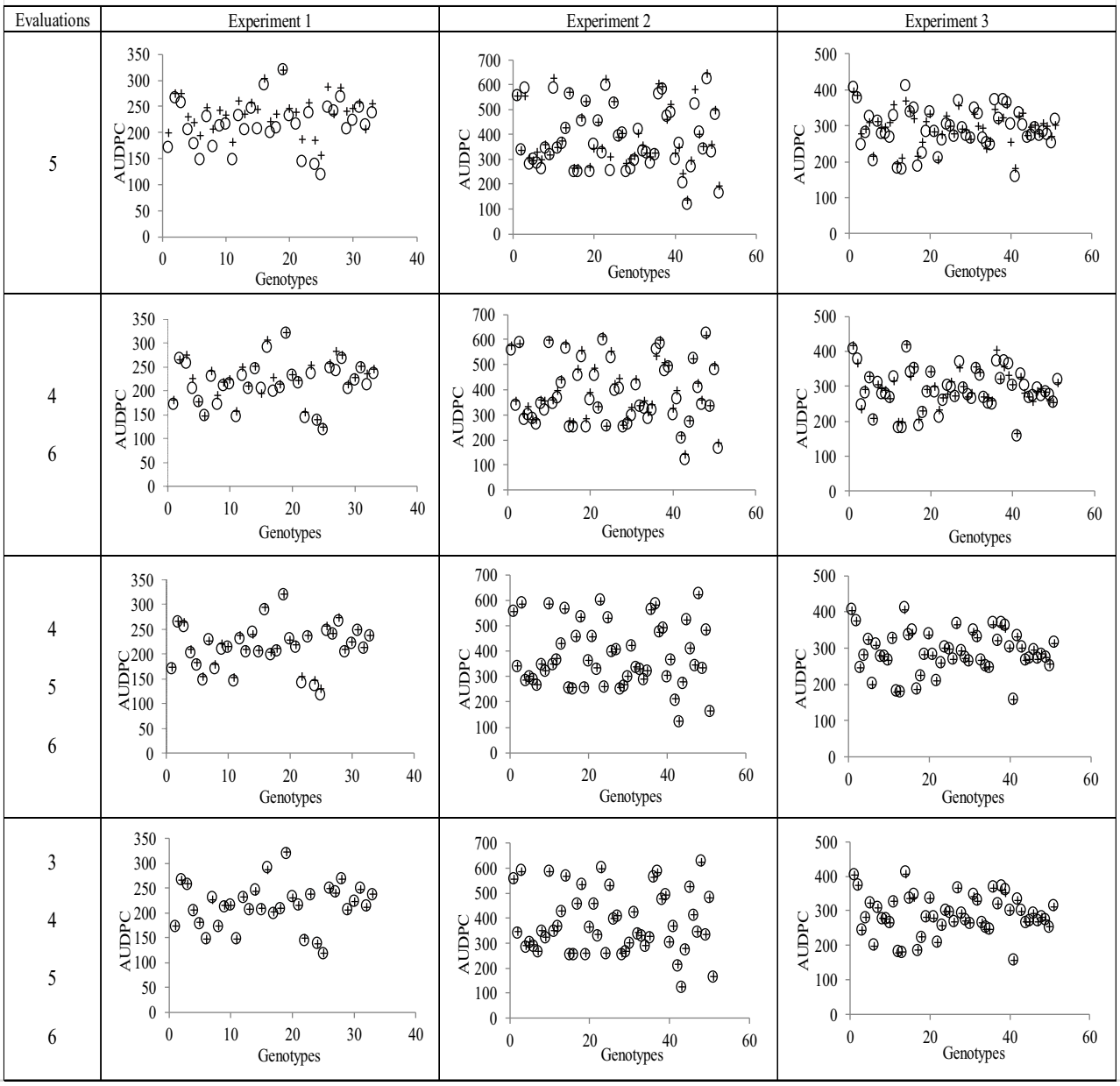

Fig. 1. Area under Early Blight Disease progress curve (AUDPC) observed (o) for mean tomato accessions in three experiments and estimated (+) the best settings of 'Network 4' with 1, 2, 3, and 4 evaluations.

complex problems (Odabas et al., 2013). This technique has advantages of being non-parametric, tolerates data losses, and does not require detailed information on the system to be modeled (Silva et al., 2014) to solve complex problems of a linear or non-linear nature (Azevedo et al., 2015). Therefore, it may be useful to predict AUDPC with fewer evaluations.

The low correlation between evaluation 2 and observed AUDPC values explains the less satisfactory results, considering the input layer of RNAs. The development cycle of Early Blight Disease (EBD) has two stages, a slower initial one, followed by one with a logistical pattern and rapid progress (Chelal et al., 2015). Evaluation 2 was uninformative therefore, due to its taking place during the disease's initial stage. On the other hand, the most satisfactory results for a single evaluation in the input layer of the neural networks with evaluation 5 data, performed 15 days after inoculation, is due to its higher association with the AUDPC.

The best results for networks trained with data from evaluations 4 and 6 indicate a greater AUDPC association. The lowest average correlation for "Network 1" (0.93) indicates that 86.49% (R^2) of the variation in the AUDPC is explained by its predicted value. The determination coefficient higher than 0.92 for "Network 2" and "Network 3" indicates that the AUDPC can be predicted with considerable accuracy with two evaluations at 12 and 18 days after the tomato plant inoculation with Early Blight Disease.

Lower evaluation numbers reduces labor per individual evaluated, financial costs for evaluation experiments, and facilitates more treatments. This demonstrates the potential of ANNs since a large number of genotypes requires a slow and laborious process to evaluate EBD resistance at early stages in breeding programs (Chaerani and Voorrips, 2006).

Correlations higher than 0.98 with data from the three evaluations (4, 5 and 6) in the input layer "Network 2" and "Network 3" show that values predicted by ANNs can explain 96.04% (R^2)

Table 2
Pearson's correlations between observed and estimated area under the Early Blight Disease progress curve (AUDPC) of best network trained to combinations of leaf area evaluations for Early Blight Disease in tomato plants in the test sample in experiments 1 (E1), 2 (E2), and 3 (E3).

Evaluations	Network 4			Means
	E1	E2	E3	
2	0.04	−0.01	0.33	0.12
3	0.72	0.77	0.67	0.72
4	0.83	0.95	0.82	0.87
5	**0.93**	**0.96**	**0.84**	**0.91**
6	0.88	0.89	0.83	0.87
2 e 3	0.60	0.73	0.64	0.65
2 e 4	0.84	0.96	0.82	0.87
2 e 5	0.95	0.96	0.85	0.92
2 e 6	0.89	0.89	0.85	0.88
3 e 4	0.86	0.96	0.84	0.89
3 e 5	0.96	0.98	0.90	0.95
3 e 6	0.91	0.93	0.92	0.92
4 e 5	0.97	0.99	0.91	0.96
4 e 6	**0.96**	**0.98**	**0.95**	**0.96**
5 e 6	0.96	0.97	0.93	0.95
2, 3 e 4	0.86	0.97	0.84	0.89
2, 3 e 5	0.97	0.98	0.91	0.95
2, 3 e 6	0.93	0.94	0.93	0.93
2, 4 e 5	0.96	0.99	0.90	0.95
2, 4 e 6	0.96	0.98	0.96	0.97
2, 5 e 6	0.97	0.97	0.94	0.96
3, 4 e 5	0.98	1.00	0.93	0.97
3, 4 e 6	0.97	0.99	0.97	0.97
3, 5 e 6	0.98	0.98	0.98	0.98
4, 5 e 6	**0.99**	**1.00**	**0.99**	**0.99**
2, 3, 4 e 5	0.98	1.00	0.93	0.97
2, 3, 4 e 6	0.97	0.99	0.97	0.98
2, 4, 5 e 6	1.00	1.00	0.99	0.99
3, 4, 5 e 6	**1.00**	**1.00**	**1.00**	**1.00**
2, 3, 5 e 6	0.99	0.98	0.98	0.98
Means	0.89	0.92	0.88	
	0.90			

Network 4: MLP trained with data from experiments 1, 2, and 3 simultaneously. Values in bold refer to measurements with improved AUDPC prediction with 1, 2, 3, and 4 evaluations.

of AUDPC variation. Estimates of correlations close to 1, for networks trained with information from evaluation 4, show that a large number of evaluations may not be necessary. Network training with data from evaluations 3, 4, 5, and 6 indicate that those of 1 and 2 (at three and six days after disease inoculation) are not necessary, due to a correlation equal to 1 between the AUDPC calculated and predicted for networks 2, 3, and 4. The potential for using the RNAs is confirmed by studies involving pathosystems such as *Phytophthora infestans* in tomato, *Magnaporthe grisea* in rice, and *Fusicladium oleagineum* in olives (Wang et al., 2008; Zhang et al., 2011; Roubal et al., 2013).

The best performances for "Network 1", "Network 2", and "Network 3" in experiments 1, 2, and 3, respectively, indicate interactions of genotype per environment, as seen for tomato crops at different times and in different environments (Roselló et al., 2011; Leiva-Brondo et al., 2012; Panthee et al., 2013). The use of data from multiple experiments in network training processes can reduce the effect of genotype per environment in the ANN's (Azevedo et al., 2015). Increased AUDPC prediction efficiency using data from three experiments in training "Network 4" compared with 1, 2 and 3, is due to data collection from different scenarios improving ANN efficiency, which becomes more general (Azevedo et al., 2015).

The best results for the evaluation of "Network 4", the same as that for "Network 1", "Network 2", and "Network 3", indicate higher AUDPC correlation. The average correlation of 0.96 (R^2 = 92.16%), with data from evaluations 4 and 6 to predict AUDPC for "Network 4" indicates high accuracy for distinguishing the best genotypes. Evaluations 4, 5, and 6 in training "Networks 4" allowed an average correlation of 0.99, indicating that observed and predicted AUDPC values were almost the same. Satisfactory results provided by the ANNs are justified by their improved efficiency in conventionally predicting regression techniques (Silva et al., 2014).

5. Conclusions

ANNs were effective at predicting the area under Early Blight Disease progress curve (AUDPC) in tomatoes, optimizing the assessment efficiency for disease severity and reducing from six to two the number of evaluations to be carried out on the twelfth and eighteenth days after inoculation of this disease.

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