Research Article

Microclonal propagation of plant process modeling and optimization of its parameters based on neural network

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ABSTRACT

Objective: This article describes the development and study of the models of neural networks for the evaluation and prediction of the results of the sterilization stage. These results allow to optimize its parameters and to conduct a required amount of simulation experiment with simultaneous changes of several (including all) parameters. **Methods:** For modeling study, researchers used two paradigms of artificial neural network, i.e., multilayer perceptron and radial basis function network. Preliminary laboratory experiments confirmed the adequacy of the developed models, while the smallest approximation error corresponds to the model in the form of a radial basis function network. Seeds of rare, disappearing, and medicinal plants growing on the territory of the Belgorod region were considered as explants. **Results:** This article carried out an imitation experiment based on the developed model. This experiment made it possible to identify the optimal parameters of the sterilization stage (the type of sterilant, its concentration, and the time of processing of plant explants) for plants seeds that were not used to construct the model, for which no laboratory experiments were used. **Conclusion:** The highest predicted percentage of sterile explants and aseptic viable seedlings was determined for these plant species.

KEY WORDS: Artificial neural networks, Microclonal propagation, Modeling, Optimization, Simulation experiment, Sterilization of plant explants

INTRODUCTION

At the present time, there is a rapid reduction of ranges and complete disappearance of many plant species due to human economic activities. There are about 1400–1500 species of plants raised in such economically developed region of Russia as the Belgorod region. Today among them, >30 plant species are included in the list of the Red Data Book of the Russian Federation and >200 species are rare and endangered, so they require effective protection at the regional level.[1] Conservation of biodiversity of plants is necessary to maintain ecological conditions of existing and economic development of human society, while the conservation of genetic resources is the main source of important selection features.[2,3] Thus, the problem of conservation and reproduction of rare and endangered plant species is becoming extremely urgent today. Its effective solution is possible when using the technology of microclonal reproduction, based on the method of cell and tissue culture. This approach has several advantages in comparison with traditional methods of maintaining plant collection which are high reproduction rates; miniaturization of process, resulting in space savings; improvement of planting material; and the possibility of a long deposition of samples with less storage costs. Under "*in vitro*" conditions, it is possible not only to propagate and root those plants that are difficult to reproduce in the traditional way but also to obtain enough material for the reproduction of rare and endangered plants.[4-7]

However, it should be noted that the process of optimization of the parameters of microclonal propagation of plants to obtain high-quality planting material is long, time-consuming, and expensive. It requires the establishment and repetition of a significant number of laboratory experiments. At the same time, there is a heavy expenditure of expensive components contained in the nutrient medium, as well as valuable time and human resources. These are collecting

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materials for experiments, providing with sterile instruments before each series of experiments, dishes, nutrient mediums, necessary indoor conditions, etc. In addition, analyzing the results of such experiments, it is necessary to work with a large amount of mixed, different, sometimes weakly structured information.

The foregoing determines the perspective of using modern information technology tools and modeling methods for solving optimization problems of microclonal propagation of plants, including data mining techniques that are successfully used in forecasting and managing process and objects in various spheres, including when solving various problems in biotechnology.[8-13]

Specialized mathematical and situational models will allow to identify cause-effect relationships both between parameters of processes inside individual stages of microclonal propagation and between parameters that are outputs and inputs of various stages of this technology.

MATERIALS AND METHODS

During scientific research, the authors of the article used the methods of system analysis, theory of sets, data mining (artificial neural networks of various paradigms), mathematical statistics, and methods of experimental studies of biotechnological processes.

The process of microclonal reproduction is multistage and includes:

- The selection and preparation of plant explants, which can be different organs, tissues, cells, and seeds of plants;
- The process of sterilization of plant explants and introducing them into culture *in vitro*;
- Production and cultivation of aseptic plants on a synthetic nutrient medium;
- Microclonal reproduction of regenerative plants;
- Adaptation of microclonal plants to soil conditions,[5-7] while sterility of received culture plays one of the defining roles; therefore, optimization of parameters of the sterilization stage is of particular importance.[5,7]

In this investigation, there were carried out laboratory experiments, modeling process, and simulation experiments for this stage.

The scientific researches were carried out according to the schedule of the technical task on the project of the state task "Research of the methods and modeling of the processes in biotechnology and plant systematics."

As plant explants, there were considering seeds of rare, endangered, and medical plants growing in the Belgorod region. All of them belong to the family Labiatae Juss (Lamiaceae): *Bellevalia sarmatica* (Georgi) Woronow, *Nigella damascena* (L.), *Echinacea purpurea* (L), *Hyssopus cretaceus* Dubjan, *Prunella grandiflora* (L.) *Sholl*., and *Salvia sclarea* L.[14-16]

There was carried out the development of models for estimating and predicting the results of the sterilization stage using neural network.

The specific topology and parameters of artificial neural networks were determined by constructing and following test for adequacy of the two types of artificial neural networks, i.e., radial basis function network and multilayer perceptron. There were considered various data structures of artificial neural networks, in which there were varied the number of hidden layers (1 or 2), the number of neurons in the hidden layers, and the type of activation function.

To evaluate the adequacy of the constructed models, the following criteria were used:

The mean square error (MSE) that is minimized in the process of training artificial neural networks is as follows:

$$MSE = \frac{1}{N_{tr}} \sum_{q}^{N_{tr}} = 1 (y_q - d_q)^2 \quad (1)$$

Here, d_q is the values of the output parameter obtained empirically; y_q is the values obtained theoretically with the help of the model; N_{tr} is the volume of training sample.

The coefficient of determination R2, which characterizes the part of the dispersion in relation to the average value of the required output $\bar{d}$, was obtained from the training sample This is explained by the constructed model:

$$R^2 = \frac{\sum_{q=1}^{N_{tr}} (\bar{d}_q - yq)^2}{\sum_{q=1}^{N_{tr}} (d_q - \bar{d}q)^2} \quad (2)$$

The average approximation errors on the training and test samples $\bar{A}_{tr}$, and $\bar{A}_{prog}$ that give a general idea of the quality of the constructed mathematical model:

$$\bar{A} = \frac{1}{N_{tr}} \sum_{q=1}^{N_{tr}} \left| \frac{d_q - y_a}{dq} \right| \times 100\% \quad (3)$$

It is most important to evaluate the predictive capabilities of the model on the set of data that constitute the test sample N_{test} and are not used in the training process of the model. The values of the output parameter are calculated for the input patterns from

the test sample using the constructed artificial neural network and then the average approximation error $\bar{A}_{prog}$, for the test sample, is calculated according to a formula (3). If $\bar{A}_{prog}$, is <10%, then the constructed artificial neural network has a good predictive feature.

To develop and evaluate the adequacy of the models, there were generated training and test samples based on the results of laboratory experiments on the sterilization of seeds of three species of plants of the family Labiatae, that is *B. sarmatica, N. damascena,* and *E. purpurea*.

Based on the developed model, there were conducted simulation experiments to determine the optimal parameters for sterilization stage of the seeds of three other plant species (*H. cretaceus, P. grandiflora,* and *S. sclarea*).

To construct artificial neural network and carry out simulation experiments, there was used an application program package Neural Network Toolbox of the MATLAB system, which makes it possible to implement artificial neural networks with different paradigms.

Laboratory experiments were carried out using various sterilizing agents: Lysoformin 3000, biocide, liquid bleach (5–15%), chloramine B, and silver nitrate. The concentration (%) and the time of seeds treatment were varied for each type of sterilizing agents. Sterilization of nutrient medium, materials, instruments, and equipment was carried out according to the methods adopted in the work about cell and tissue culture. To evaluate the effect of aseptic solutions, plant was placed on the Murashige and Skoog medium without hormones. Next, the seeds were cultivated in a thermostat at a temperature of 22–24°C. There were used 10 seeds of each species for each mode taking into account the time and concentration of the sterilizing agent. The experiment was carried out thrice. The effect of the sterilization regime was evaluated by the number of sterile and viable explants (%).

There were conducted 585 experiments to build, train, and test models for adequacy. There were conducted 195 experiments with each plant species. The results were divided into training (450 experiments) and test (135 experiments) samples.

Laboratory and simulation experiments were carried out according to the technical task of the study "Research of methods and modeling of processes in biotechnology and plant systematics."

The authors suggest the modernization of the traditional process of microclonal propagation by optimizing its parameters on the basis of specially developed models of estimates and forecasts.

There was made a system description of the studied biotechnological process, and there was constructed its functional model with the use of the graphical notation IDEF0, as demonstrates in Figures 1 and 2.

This model allows to reveal the interrelation of all stages and parameters of the process of microclonal reproduction.

Modernization of the process is reflected in the presented functional model due to the introduction of new information stream and subprocess. Thus, the stream "Information support for optimization of process control" was introduced as a mechanism in Figures 1 and 2. When the process is detailed, there is introduced the subprocess "Simulation experiments," the output of which is determined by the information control stream for the sterilization subprocesses and the introduction

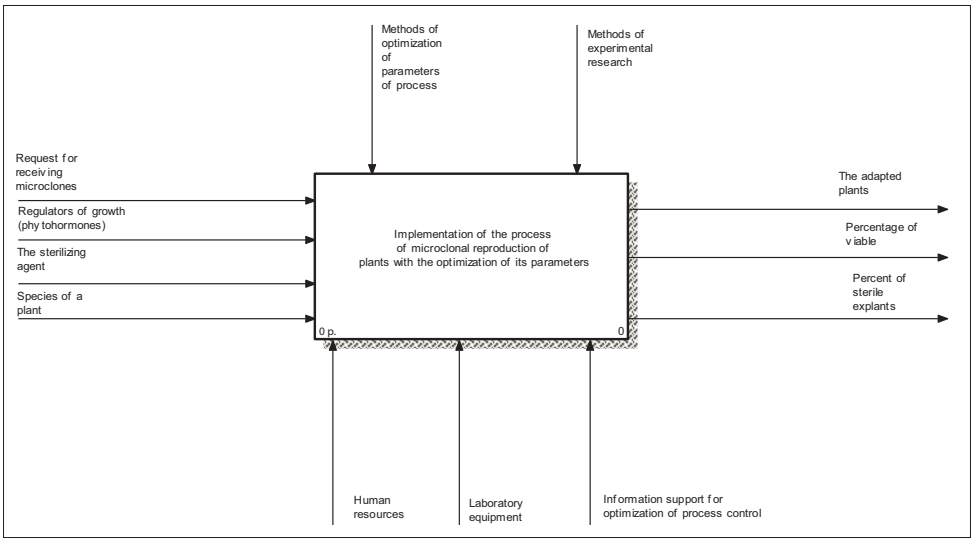

Figure 1: Context diagram of the process of microclonal reproduction of plants with optimization of its parameters

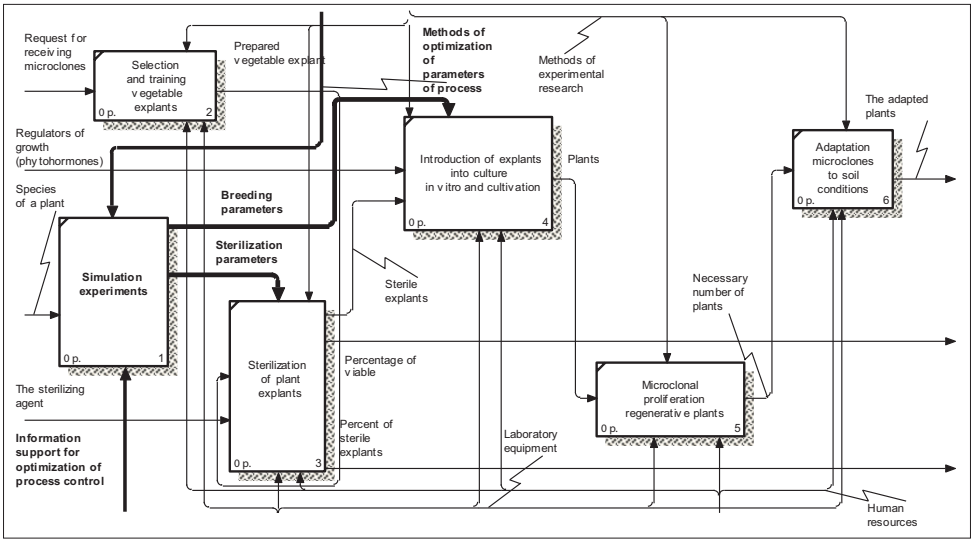

Figure 2: Decomposition of the process of microclonal reproduction of plants with the optimization of its parameters

of explants into the culture *in vitro*. It is represented in Figure 2, where introduced new control loops with the participation of these subprocesses.

The constructed functional model shows that the state of the sterilization stage (as one of the components of the internal control loop of the introduced modernized process) determines not only the quality of the aseptic material but also the number of viable sterile seedlings. This generally determines the result of the microclonal propagation process. Thus, the identification of a cause-effect relationship between inputs and outputs of the sterilization stage and the optimization of its parameters will increase the effectiveness of the process management of microclonal reproduction.

According to the set-theoretical approach, the stage of sterilization of plant explants can be formally represented using the following model:

$$ST=<W, \Omega, X, Y, F, O> \quad (4)$$

Here, W is the set of subprocesses of the sterilization stage;

Ω is the set of external influences on the W elements, as well as the specified parameters of the environment and equipment;

X is the set of variable input parameters that determine the conditions and result of the sterilization stage of plant explants. Preliminary empirical studies have shown[5,7] that the result of obtaining a sterile culture that will be characterized by good growth directly depends on the correct choice of parameters such as the type of sterilizing agent (X_1), its concentration (X_2,%) and time treatment of plant explants with a sterilizing agent (X_3, min);

Y is an output variables models, that is the number of sterile explants (Y_1, %) and aseptic viable seedlings (Y_2, %);

F is the set of mappings carried out on W; Ω, X, Y, F: (W, Ω, X, Y) → Y;

O is the set of relations over the elements W; Ω, X, Y, O: (W^k; X^i, Ω^l Y^j), and the arities of k, i, l, j depend on the laboratory conditions and the type of plant explants.

The most important components of the set of mapping F are the functional dependencies that reflect the cause-effect relationships between input and output parameters of the sterilization stage: $Y_1 = F_1(X_1, X_2, X_3, \Omega)$, $Y_2 = F_2 (X_1, X_2, X_3, \Omega)$, and $Y_2 = F_3 (Y_1, \Omega)$. The authors used the artificial neural network to simulate these dependencies. The important property of such is the possibility of parallel processing of information simultaneously by all neurons.[17,18] On the basis of such models, it is possible to carry out simulation experiments with the modification of several (including all) parameters simultaneously.

There were implemented artificial neural networks of two paradigms to obtain a model that provides the possibility of estimating and predicting the results of the sterilization stages of plant explants with the choice of optimal parameters, that is a multilayer perceptron and radial basis function network. As indicated in clause 2.1, training and test samples are formed on the basis of experimental data obtained during sterilization of seeds of rare and endangered plant species of the family Labiatae, that is *B. sarmatica, N. damascena,* and *E. purpurea*.

There were obtained results of the research and evaluation of the adequacy of neural network models with different structures. The results have showed that artificial neural network has the best prognostic

capabilities in the form of radial basis function network with 143 neurons in the hidden layer and radial basis activation functions. For this model, the MSE will be MSE = 10^{-6}; the determination coefficient will be R^2 = 99.89; average errors of approximation of the training and test samples will be $\bar{A}_{tr}$ = 0.86%, $\bar{A}_{prog}$ = 0.98%. Table 2 shows the results of an assessment of the adequacy of artificial neural network with other structures, which also showed good approximation results.

The artificial neural network inputs are the parameters X_1, X_2, $X_3 \in X$, and the outputs parameters are Y_1, $Y_2 \in Y$. The interlayers reflect the biochemical processes occurring in the processed explants.

The developed model can be used to evaluate, predict, and determine the optimal sterilization parameters when introducing into the culture *in vitro* other representatives of plants of the family Labiatae, which are rare and endangered and grow on a similar territory.

The results obtained during the simulation experiments are presented in Table 3.

The developed model was used to conduct simulation experiments on the selection of optimal parameters for the seed sterilization stage. There were injected plants *H. cretaceus, P. grandiflora,* and *S. sclarea* into the culture *in vitro*. The plants belong to the family Labiatae and grow in the Belgorod region. Laboratory experiments were not conducted with these plant species. Various combinations of input parameters X_1, X_2, and X_3 are shown as an input of the model. They had a wider range of variation limits and with a smaller variation step that it was possible to implement in a laboratory experiment. Thus, it was varied from 1% to 100% in increments of 0.01. The sterilization time was from 1 to 30 min in increments 1.

CONCLUSION

The following results and conclusions were obtained based on the study.

There is presented modernized process of microclonal propagation of plants with the optimization of its parameters. That is achieved by introducing a new subprocess, that is carrying out a simulation experiment to optimize parameters.

There was carried out a systematic description of the process under the investigation. It has been developed its functional model that demonstrates the emergence of internal control loops through the introduction of new information stream and subprocess. There is one of the components of the internal control loop. The stage of sterilization of plant explants determines not only the quality of aseptic material but also the number of viable sterile seedlings. It generally determines the result of the process of microclonal reproduction.

There was developed a set-theoretical model of the sterilization stage of plant explants, presented by the authors of the modernized process of microclonal propagation of plants with the optimization of parameters. There were found the cause-effect relationships necessary for the optimization of the parameters of this stage: $Y_1 = F_1 (X_1, X_2, X_3, \Omega)$, $Y_2 = F_2 (X_1, X_2, X_3, \Omega)$, and $Y_2 = F_3 (Y_1, \Omega)$, here X_1 is a type of sterilizing agent; X_2 is its concentration; X_3 is processing time of sterilizing agent of plant explants; Ω is set of external influences; Y_1 is a number of sterile explants; and Y_2 is a number of aseptic viable seedlings.

Table 2: Results of experiments for the selection of artificial neural network characters

Network topology	Number of neurons in the hidden layer	Function activation of hidden layer neurons	MSE, 10^{-5}	R^2, %	$\bar{A}_{tr}$ %	$\bar{A}_{prog}$ %
RBF	143	Radial-basic	0.1	99.89	0.86	0.98
Perceptron	9	Linear	0.18	99.72	0.92	2.36
Perceptron	11	Linear	0.14	99.87	0.77	4.64
Perceptron	15	Linear	0.15	99.82	0.,82	3.12
Perceptron	17	Linear	0.19	99.75	0.88	4.18
Perceptron	19	Linear	0.2	99.58	0.97	3.48
Perceptron	8	Sigmoid	0.16	99.88	0.33	1.35
Perceptron	12	Sigmoid	0.12	99.86	0.61	2.44
Perceptron	13	Sigmoid	0.14	99.88	0.42	1.39
Perceptron	15	Sigmoid	0.17	99.85	0.55	1.6

Table 3: Optimal parameters of the sterilization stage obtained on the basis of simulation experiments

Species of a plant	Sterilizing agent X_1	Sterilization time X_3, minutes	Concentration of the sterilizing agent X_2, %	Number of sterile explants Y_1, %	Number of viable explants Y_2, %
H. cretaceus	Lysoformin 3000	9	7.12	74.2	15.9
P. grandiflora	Belizna (5–15%)	16	77.1	79.3	32.1
S. sclarea	Silver nitrate	18	0.12	94.3	55.3

H. cretaceous: *Hyssopus cretaceous, P. grandiflora*: *Prunella grandiflora, S. sclarea: Salvia sclarea*

To model the dependences, F_1, F_2, and F_3 artificial neural networks were used. Various structures of artificial neural networks were constructed and investigated for two paradigms: Multilayer perceptron and radial basis function network. To form training and test samples, there were used laboratory experiments to sterilize seeds of rare, endangered, and medicinal plants growing in the Belgorod region: *B. sarmatica, N. damascena,* and *E. purpurea*.

Analysis of simulation results allowed to choose an adequate neural network model for carrying out simulation experiments to evaluate and predict the results of the sterilization process with the choice of optimal parameters: Radial basis function network with 143 neurons in the hidden layer and radial basis function (MSE = 10^{-6}; determination coefficient R^2 = 99.89; average apprXoximation errors on the training and test samples $\bar{A}_{tr}$ = 0.86%, $\bar{A}_{prog}$ = 0.98%).

With the help of the developed model, simulation experiments were carried out and optimal sterilization parameters were selected for three plant species belonging to the family Labiatae, for which no laboratory experiment was performed. *H. cretaceus* - a sterilizing agent is lysoformin 3000 with a concentration of 7.12% and a sterilization time of 9 min. *P. grandiflora,* sterilizing agent, is liquid bleach (5–15%) with a concentration of 77.1% and a sterilization time of 16 min. *S. sclarea,* sterilizing agent, is silver nitrate with a concentration of 0.12% and a sterilization time of 18 min.

Determined by the simulation experiments, the number of viable sterile seedlings for these plants are 15.9, 32.1, and 55.3% respectively.

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