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INTRODUCTION INTO CULTURE IN VITRO OF RARE AND ENDANGERED PLANT SPECIES OF BELGOROD REGION FLORA (BY THE EXAMPLE OF THE FAMILY FABACEAE) USING NEURAL NETWORK HARDWARE

Elena V. Maslova*, Natalia I. Gulya, Kristina A. Degtyareva, Irina V. Batlutskaya, Olga A. Ivashchuk, Vjacheslav I. Fedorov

Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia

Abstract:

In this article mathematical modeling of methods of neural networks of one of the stages of microclonal reproduction of plants – sterilization of plant explants of rare and endangered plants of the Legume family (Fabaceae) of flora of the Belgorod region is carried out. The authors selected the most effective sterilizing chemicals, their concentration and exposure time for both Astragalus albicaulis and Astragalus dasyanthus. Evaluation of the influence of sterilization regimes on the preparation of aseptic explants A. albicaulis and A. dasyanthus was performed according to the viability of plant explants of the studied species.

Keywords: *micropropagation, preservation under the conditions in vitro, plant explants sterilization, cell and tissue culture, math modelling, forecasting effort, neural networks.*

Corresponding author:

Elena V. Maslova,

Belgorod State University, 308015,
Belgorod, Pobeda Street, 85,
Russia
E-mail: maslova@bsu.edu.ru

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INTRODUCTION:

The problem of conservation of rare and endangered plant species is currently important both for a particular region and for the whole country. At present, a rationale is using modern biotechnological methods in vitro to renew and conserve valuable endangered plant species [1-7]. The basis of the cell and tissue culture technique is ability of the plant to vegetative propagation using somatic cells. To preserve the plants as the explants it's better to choose seed material. It gives a chance to reserve genetic diversity of species, with using a small amount of scarce material [8].

This process is multistage, and parameter optimization at each stage is a lengthy, labor-intensive and cost-based process, that requires a good number of experimental set up and replication. At the same time, there is a heavy expenditure of expensive elements, that are part of nutrient medium, as well as expenditures of valuable time and human labour. The identifying the optimal parameter of introduction into culture in vitro situation is complicated by necessity to work with a vast amount of the dissimilar, sometimes weakly structured information. Therefore, the authors suggest the process simulation with using the artificial neural network (ANN).

The authors have already successfully tested this method for some representatives of Legume family, which are rare and medicinal species and grow on the Belgorod Region territory [9,10].

The project goal was to optimize the most important stage of introduction into culture in vitro, that is sterilization, of some representatives of the family Fabaceae (Leguminosae) on the basis of smart modeling with using of the artificial neural network (ANN).

The studies were pursued according to the technical requirement of academic research work "Research of methods and modeling of processes in biotechnology and plant systematics".

MATERIALS AND METHODS:

Materials

The objects of research were plants of family Fabaceae, growing in the Belgorod Region – Astragalus white-stemmed Astragalus albicaulis DC. And Astragalus woolly-flowered Astragalus dasyanthus Pall. Both of them are rare and endangered species and are protected at the regional and federal levels [11,12].

Astragalus albicaulis DC. belongs to the genus Astragalus, subfamily Faboideae, family Fabaceae, order Fabales. It has Eastern European steppe species category and the 4th rarity status. Therefore, it is a very valuable species. Species distribution area covers European part of Russian Federation, south-west regions of West Siberia, Ukraine and the Republic of Moldova. A. albicaulis is a subshrub with lignescent at the bottom, grey biennial boughs. Annotinous beanstalks have a lenth of 5-10 cm and could be rising or straight, thin, rounded and thickly white-pressed-villose. Stipules are white- or black-villose, lower ones are ovoid, upper ones are lanceolate. Leaves have a length of 2-6 cm, with very short and thin leafstalks. Folioles 3-4 are coupled, 10-20 mm long, oblong or ovate, pointed, rarer pointless, pressed-villose on both sides, greyish. Pedicels are 10-15 cm long, greyish. Racemes are bulbous, ovoid, rather porous, 2-10 cm long; the bracts are lance-linear, 3-5 mm long, white- or black-white-villose; a calyx is about 15 mm long; half-pressed white- and usually striped-black-villose; the teeth are fibriform. Corolla is white or flexen. The beans are stalkless, protruding, oblong, leathery, thickly and squarrously white- and downy, two-chambered. The plant flourishes from May to July, fructifies from June to August [13].

Astragalus dasyanthus Pall. Belongs to the genus Astragalus, subfamily Faboideae, family Fabaceae, order Fabales. It has Eastern European steppe rare species category and the 3th rarity status. Species distribution area covers Balkan Peninsula, Hungary, Ukraine, the Republic of Moldova and in the south of European part of Russian Federation [14]. It is perennial herbaceous plant with procumbent and erect villous ramuses. Leaves have petiolate form and 12-14 pairs of folioles. Folioles are stalkless, oblong and ovate, have 25-20 mm long and about 6 mm at widths. Stipules his acuminate. Inflorescences are lush bulbaceous racemes consisting of 10-20 yellow flowers, 3-6 cm long. Flowers are located on the pedicels, 15 cm long. The length of the flowers is about 15-20 mm. Flowers have flexen papilionaceous corolla and lush, downy campanulate calyx. The fruit of plant is villose bean. All parts of the plant have a yellowish-white cotton. The plant has slight smell and sweetish taste. A. dasyanthus flourishes from June to July, fructifies from July to September [14].

Plant seeds of the species Astragalus albicaulis and Astragalus dasyanthus were used to introduce into culture in vitro as plants explants. The seeds were harvested in the summer period on the territory of Belgorod Region and the botanical garden of Belgorod State University in 2017. The processes of

collection, drying, plant material keeping were carried out according to established procedures [15, 16].

Experiments on introduction into in vitro culture

Manipulations on introduction into culture in vitro were carried out in the laboratory of 'Innovative methods of research of plant objects' of the department of biotechnology and microbiology of the NRU BelSU with observance of the standard aseptic conditions rules in laminar boxes of microbiological safety Lamsystems class II type A2.

Sterilization of nutrient media, materials, instruments and equipment was carried out according to the techniques adopted in the work on cell and tissue culture [17-19].

At the stage of sterilization, plant explants were treated with five different sterilizing agents: lysoformin 3000, biocide, liquid bleach (5-15%), chloramine B, and silver nitrate. The following values of the parameters varied: the concentration of the sterilizer (c,%) and the treatment time (t, min).

After sterilizing, plant explants were washed with sterile distilled water three times for 15 minutes.

To evaluate the effect of aseptic solutions, plant explants were placed in the Murashige-Skoog nutrient medium without hormones [20]. Next, the seeds were cultivated in a thermostat at a temperature of 22-24 °C. The experiment was carried out three times. The effect of the sterilization mode was evaluated by the number of sterile and viable explants.

Modeling and optimization of the sterilization stage

To develop an adequate model of optimization and evaluation of the sterilization process, reflecting the cause-effect relationship between the parameters and the result of the sterilization stage, the artificial neural network (ANN) device was chosen. Basic data for modeling were obtained by carrying out a series of experiments on the sterilization of plant seeds. As a result, 195 experiments were carried out for each plant species: 45 experiments with each of the sterilizing agents. The results were divided into a training sample (150 experiments) and validation sample (45 experiments).

To build the specific models, the ANN of the following topologies is most often used: multilayer perceptron and networks with radial-basis function (RBFN). The processes of constructing and researching models, as well as simulation experiments, were carried out using a package of applied programs and functions of the Neural Network Toolbox of the MATLAB computer system.

RESULTS AND DISCUSSION:

As a result of laboratory experiments, preliminary data on the most effective sterilizing agent, its concentration and time were obtained. The average results of the sterilization mode impact on the preparation of sterile explants of *A. albicaulis*, and *A. dasyanthus* with an assessment of their viability are presented in Table 1.

Table 1 - Modes of sterilization and evaluation of their effect by the number of sterile and viable plant explants of *A. albicaulis* and *A. dasyanthus.*

Sterilizing agent	Time, min	Concentration, %	*A. albicaulis*	*A. dasyanthus*
Lysoformin 3000	10	5	57.5±1.8/ 8.5±1.2	77±1.9/ 16.1±2.5
		10	59.4±1.5/ 11.4±0.9	80.3±2.1 / 15.8 ±0.9
		15	64.0±2.8/ 10.2±1.3	78.9±2.7/ 15.5±1.3
	20	5	70.2±1.3/ 13.5±1.2	78.9±2.3/ 12.7 ±0.8
		10	73.39±5.83/ 12.48±1.67	82.6±4.87/ 11.2 ±0.72
		15	76.7±3.1/ 8.7±1.0	85.3±3.1/ 8.5±0.3
	30	5	77.9±2.5/ 1.2 ±0.2	87.2±1.5/ 5.5 ±0.7
		10	81.3±2.0/ 0 ±0	89.2±0.9/ 2.1±0.8
		15	87.0±1.9/ 0 ±0	97.8±0.5/ 0 ±0
Biocide	10	1	10±1.8/ 0 ±0	30.1±2.1/ 8.1±1.2

		3	12.7±1.8/ 0 ±0	33.5±2.1/ 7.9±2.0
		5	15.4±3.2/ 0 ±0	36.7±2.1/ 7.7±1.2
	20	1	20.1 ±2.1/ 3.0 ±0.4	40.1±3.4/ 6.1±1.2
		3	45.1±2.8/ 0 ±0	47.1±1.8/ 5.9±3.1
		5	56.1±3.2/ 0 ±0	50.7±2.1/ 5.1±0.4
	30	1	61.2±1.8/ 0 ±0	61.7±3.2/ 4.8±0.7
		3	74.4±3.8/ 0 ±0	69.9±0.9/ 0 ±0
		5	77.3±3.4/ 0 ±0	84.4±2.1/ 0 ±0
Liquid bleach (5-15%)	10	50	59.1±1.1/ 57.1±0.1	70.1±1.5/ 30.7±1.7
		100	60.4±1.7/ 57.1±1.2	82.7±1.1/ 29.3±2.4
	20	50	61.2±1.7/ 55.98±1.9	90.1±1.1/ 28.8±2.1
		100	64.67±5.25/ 55.97±5.09	93.3±6.8/ 23.3±5.08
	30	50	65.8±0.8/ 49.1±0.9	90.4±1.6/ 17.8±2.9
		100	69.1±0.9/ 41.2±0.8	95.4±1.8/ 7.8±2.3
Chloramine B	10	1	48.1±1.4/ 31.1 ±0.9	97.1±2.1 3.7±1.1
		5	35.5±1.5/ 28.4 ±0.9	93.1±2.1/ 3.1±0.5
		10	30.4±1.9/ 27.2±1.6	92.1±1.3/ 3.1±0.2
	20	1	26.8±1.7/ 26.8±1.6	91.1±1.2/ 0 ±0
		5	31.37±5.25/ 25.49±1.96	88.89±11.11/ 0 ±0
		10	39.1±1.7/ 21.1±1.4	80±2.3/ 10.7±3.2
	30	1	41.1±1.5/ 12.1 ±0.7	57±2.3/ 9.7±3.2
		5	51.1±2.6/ 0±0	87.3±2.3/ 7.7±3.2
		10	61.2±2.6/ 0 ±0	89.3±2.3/ 5.7±3.2
Silver nitrate	10	0.05	93.1±2.1/ 45.0 ±0.7	81.1±2.1/ 5.0 ±0.1
		0.1	90.2±2.1/ 41.1 ±0.7	87±2.1/ 2.3 ±0.1
	20	0.05	91.1±2.1/ 36.1±2.6	90.8±2.8/ 0±0
		0.1	95.1±1.3/ 34.85±6.28	96.70±3.33/ 0±0
	30	0.05	96.5±2.1/ 29.7±2.1	97.8±2.1/ 0±0
		0.1	98±2.1/ 21.4±2.1	98.1±2.5/ 0±0

Figures 1 and 2 show the results of a comparative analysis of the sterilizing agents effect on the number of sterile of sterile and viable plant explants of *A. albicaulis* and *A. dasyanthus.* (treatment time t=20 min, the concentration readings (c,%) are presented in Figures.

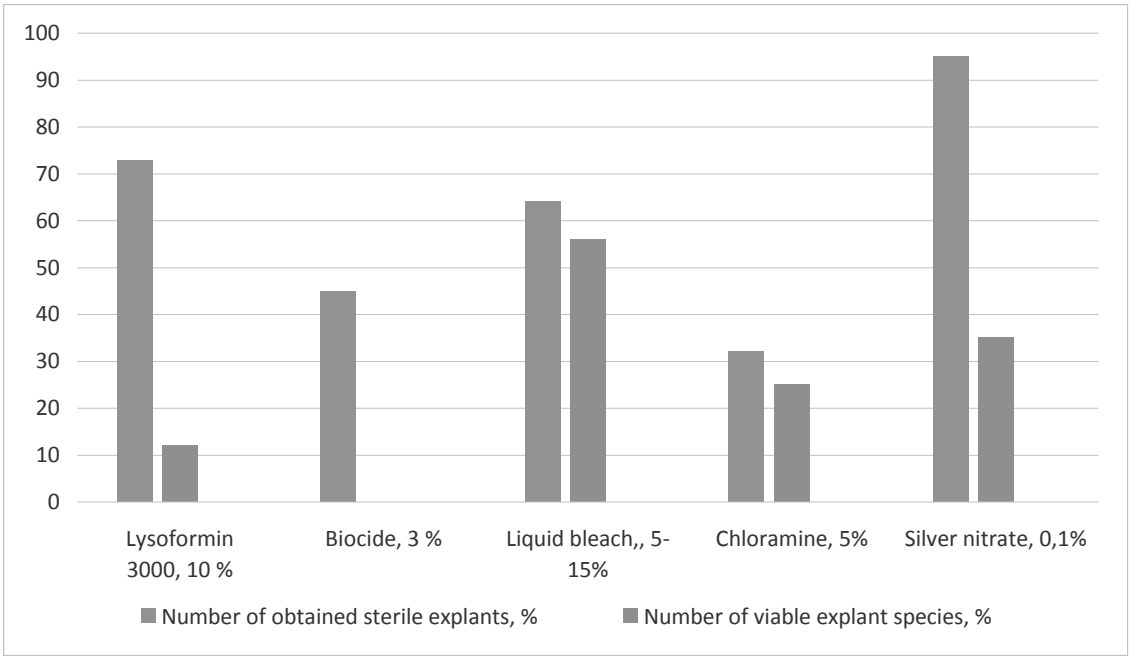

Fig. 1. - Modes of sterilization and evaluation of their effect by the number of sterile and viable plant explants of *A. Albicaulis*

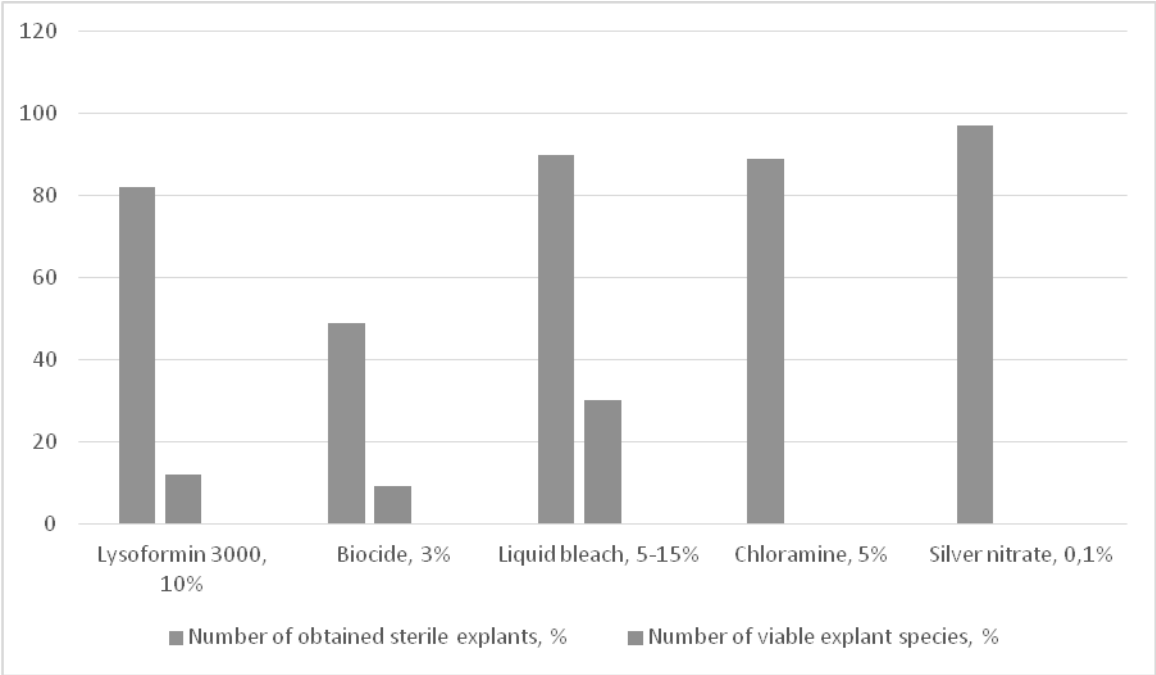

Fig. 2. - Modes of sterilization and evaluation of their effect by the number of sterile and viable plant explants of *A. dasyanthus*

As can be seen from the Figures 1 and 2, the optimal sterilizing agents for *A. Albicalius* are liquid bleach (5-15%) and silver nitrate (0.1%) and for *A. Dasyanthus* it is liquid bleach (5-15%).

As can be seen form the Figures 1 and 2, the sterilization parameters determine the results of this stage in different way: active seed germination is not sufficient criterion to ensure a high percentage of viable explants.

To construct a model that provides the possibility of estimating and predicting the plant explants sterilization results with the choice of optimal parameters, a network RBF with 132 neurons in a hidden layer was realized. Figure 3 shows the structure of the network, constructed in the MATLAB system.

For this model, the standard error of estimate *mse* = 10^{-6}; determination coefficient R^2 = 99.73; the average error of approximation for the plant species studied: $\overline{A}_{об.} = 0.89$ %, $\overline{A}_{прог.} = 0.96$ %.

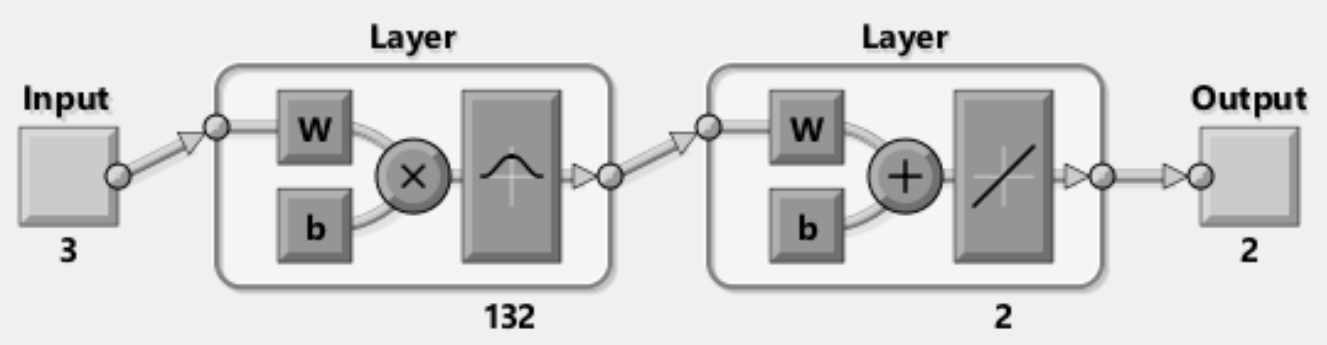

Fig. 3. Structure of the RBF for simulating plant explants sterilization, constructed in the MATLAB system.

Thus, with the help of the constructed models, simulation experiments were performed to identify the optimal conditions for plant explants sterilization: the type of sterilizing agent, its concentration and the time of sterilization.

In the framework of the experiments, all the possible combinations of these parameters were submitted to the models input with a change in the concentration of the sterili Optimization of sterilization process of plant explants when introduced into culture in vitro based on artificial neural networks Olga A. Ivashchuk, et al. 1704 agent from 0 to 100% (by 0.01) and the sterilization time from 0 to 30 minutes (by 1), which cannot be achieved in the laboratory conditions.

Table 2 - Optimal sterilization parameters obtained by the computer experiment

Plant type	Sterilizing agent	Time, min	Concentration %	Number of viable explants, %
A. albicaulis	Liquid bleach (5- 15%)	19	48.4	56.4%
A. dasyanthus	Liquid bleach (5- 15%)	11	96.5	29.3%

CONCLUSION:

Laboratory experiments on the introduction of plants *astragalus albicaulis* и a*stragalus dasyanthus* in vitro in the process of their sterilization stage were carried out.

Assessment of the impact of sterilization stages on obtaining sterile and viable plant explants of *A. albicaulis* и *A. dasyanthus.* It has been found that active seeds germination is not sufficient criterion to ensure a high percentage of viable explants.

The RBF network with 132 neurons in a hidden layer is implemented. The network reflects the dependence of the number of viable germinated explants on the parameters of the sterilization stage (the type and concentration of sterilizing agent, time of its influence on the seeds under the study). For this model, the standard error of estimate *mse* = 10^{-6}; determination coefficient R^2 = 99.73; the average error of approximation for the plant species studied: $\overline{A}_{об.} = 0.89$ %, $\overline{A}_{прог.} = 0.96$ %. This goes to prove that there is an adequacy and good predictive capabilities of the model.

Simulation experiments were carried out and optional conditions for sterilization of the plant explants were

identified. The conditions provide an opportunity to obtain X % of viable germinated explants for *A. albicaulis* и Y % for *A. dasyanthus*.

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