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Research Article

Approaches to the Construction of Simulation Model of the Process Optimization of Rare Plants Microclonal Propagation

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ABSTRACT

The present time is characterized by the active use of information technologies and promising modelling methods for the development of various fields of science, providing opportunities for obtaining new knowledge about processes and facts, based on simulation experiments, high-precision model assessments and forecasts. In this aspect, the creation and research of methods and models of processes in biotechnology, aimed at contributing to the effective solution of the problems of biodiversity conservation and food security, are extremely urgent. The authors present approaches to constructing an imitation model for the optimization of the process of rare plants microclonal reproduction (on the example of the Belgorod region), which allow to identify the most effective ways of explants sterilization, to define the optimal composition of nutrient media for regenerants growth (tube plants) and growth regulators (phytohormones) for propagation of mini-plants. Such adequate models will be the main elements of creation an economically efficient technology for obtaining isolated plant cultures, using modern biotechnological methods.

Keywords: biodiversity of rare and endangered plant species, microclonal propagation of plants, mathematical and simulation modelling, process optimization, intelligent modelling methods, situational approach.

INTRODUCTION

In modern socio-economic and environmental conditions, the problem of biodiversity conservation of rare and endangered plants is extremely urgent, because of the rapid decrease of habitats of many species, due to the human economic activity. At the same time, many endangered plant species are considered worldwide as the main source of agricultural crop improvement for the next few decades. More than 30 out of 1400-1500 plant species, widespread in the Belgorod region, are included in the Red Book of Russia. More than 200 plant species require effective protection as rare and endangered at the regional level [1]. If now there is no solution to this problem, then environmental problems will inevitably turn into socio-economic ones.

The technology of clonal micropropagation of plants, based on the method of cell and tissue culture, is actively developing at the present time and has a wide range of practical applications. Isolated plant cells and callus tissues, cultivated *in vitro*, are capable of producing valuable biologically active substances for medicine, perfumes, cosmetics and other industries: alkaloids, steroids, glycosides, hormones, essential oils, flavonoids, etc. The advantage of cellular technologies is the ability to use rare and endangered from the territory of the region plants, as well as growing in different natural conditions plants, for the synthesis of secondary metabolites and for receiving environmentally safe products all the year round. Herewith, the productivity of cultured cells, as a result of cell selection, can significantly exceed the productivity of whole plants and bring a greater yield of the biologically active substance [2-4].

The use of isolated tissues culture allows to obtain healthy, virus-free planting material; to propagate plants, which are difficult to breed in a traditional way; to receive hundreds of thousands of plants per year from one meristem; to create collections of rare and endangered plant species and store them in *in vitro* conditions [5, 6].

At present time, the search for the most optimal composition of nutrient media and the use of growth regulators (phytohormones) on regenerants (test tubes), as well as targeted selection of test tubes materials and mini-plants for propagation, are long-term, labor-intensive and expensive processes, that require a significant number of experiments. The tasks are complicated by the need to work with large volumes of different data, including weakly structured and formalized. To effectively solve this problem, it is possible to use modern information technologies and modelling methods, including intelligent modelling methods [7-10].

In the aspect of the investigated problem, the issues of obtaining plant material by the microclonal propagation method, are described in the works of N.M. Abramenko, A.A. Borisov, R.G. Butenko, N.A. Grin, A.V. Milekhin, Y.N. Prihodko, T.B. Reshetnikova, S.L. Rubcov, R.V. Stakanova, N.I. Starichkova, A.V. Soloviev, I.K. Sorokina, O.Y. Surkov, M.T. Upadyshev, A.M. Chernec, S.N. Shevchenko et al. Various approaches to the realization of microclonal propagation of plants are presented in the works of B. Bhagwat, W.I. David Lane, E.H. Kokking, Z.H. Morel, L. Guarino et al., L.A. Withers, P.K. Bajburina, K.Z. Gamburg, N.I. Rekoslavskaya, S.G. Shvecov and others. The analysis of scientific publications has shown, that the world leaders in the field of microclonal propagation of plants *in vitro* are the Netherlands, the USA, India, Israel, Italy, Poland and other countries. In the United States of America, microclonal reproduction involves about 100 laboratories, 5 of which have a capacity of 15-20 million plants per year, 8-10 laboratories - from 2-10 million, the rest less than 1 million of plants. Only 37 of the 248 commercial laboratories in Western Europe, with a total annual output of 212 million plants, produce more than 1 million of plants.

However, the analysis of scientific publications of Russian and foreign scientists shows, that at present there are no scientific papers, related to the use of modelling methods (including intellectual data processing) in biotechnology.

METHODS

In the course of scientific research, the following methods will be used: the methods of experimental research; methods of system analysis; mathematical and computer modelling; theory of sets and mathematical logic; situational approach and methods of expert assessment; artificial intelligence technologies; author's methods for implementing complex situational assessment, developed on the basis of the above methods synthesis.

On the basis of the development stages assessment of methods for process modelling in biotechnology, the engineering approach will allow to perform the analysis of the costs for implementing the above methods and schemes. An innovative approach involves the use of cost-effective methods for process modelling in biotechnology.

MAIN PART

The purpose of the scientific research is to obtain new knowledge, which allows to optimize the processes of microclonal propagation of plants, to build an effective technology for obtaining an isolated culture of rare and endangered plant species, on the basis of creating and exploring adequate mathematical, simulation and situational models of the studied processes.

To achieve this goal, the following tasks were set:

- To conduct the analysis of existing methods and approaches to the studied process modeling.
- To carry out the series of laboratory experiments, allowing to justify the choice of modeling parameters and to form necessary database.
- To perform a systematic description of the technology for obtaining an isolated culture of rare and endangered plant species (on the example of the Belgorod region) by modern

biotechnological methods; to select the effective methods for sterilization of plant explants; to select and optimize the nutrient media for cultivation into plant cultures for each species *in vitro*.

- To develop the simulation model for the implementation of microclonal propagation.
- To develop the methods, mathematical and situational models, with the help of devices for intelligent modeling.
- To provide software implementation of created methods and models, with convenient user interface; to carry out simulation and real experiments, with processing and interpretation of the results; to investigate the sensitivity to the assumptions, made in the process of models construction.
- To evaluate the effectiveness of the obtained results, in comparison with the current scientific and technological level.

Sequential scheme of construction the simulation model for optimization the process of microclonal propagation of rare plants, proposed by the authors, is presented in Figure 1.

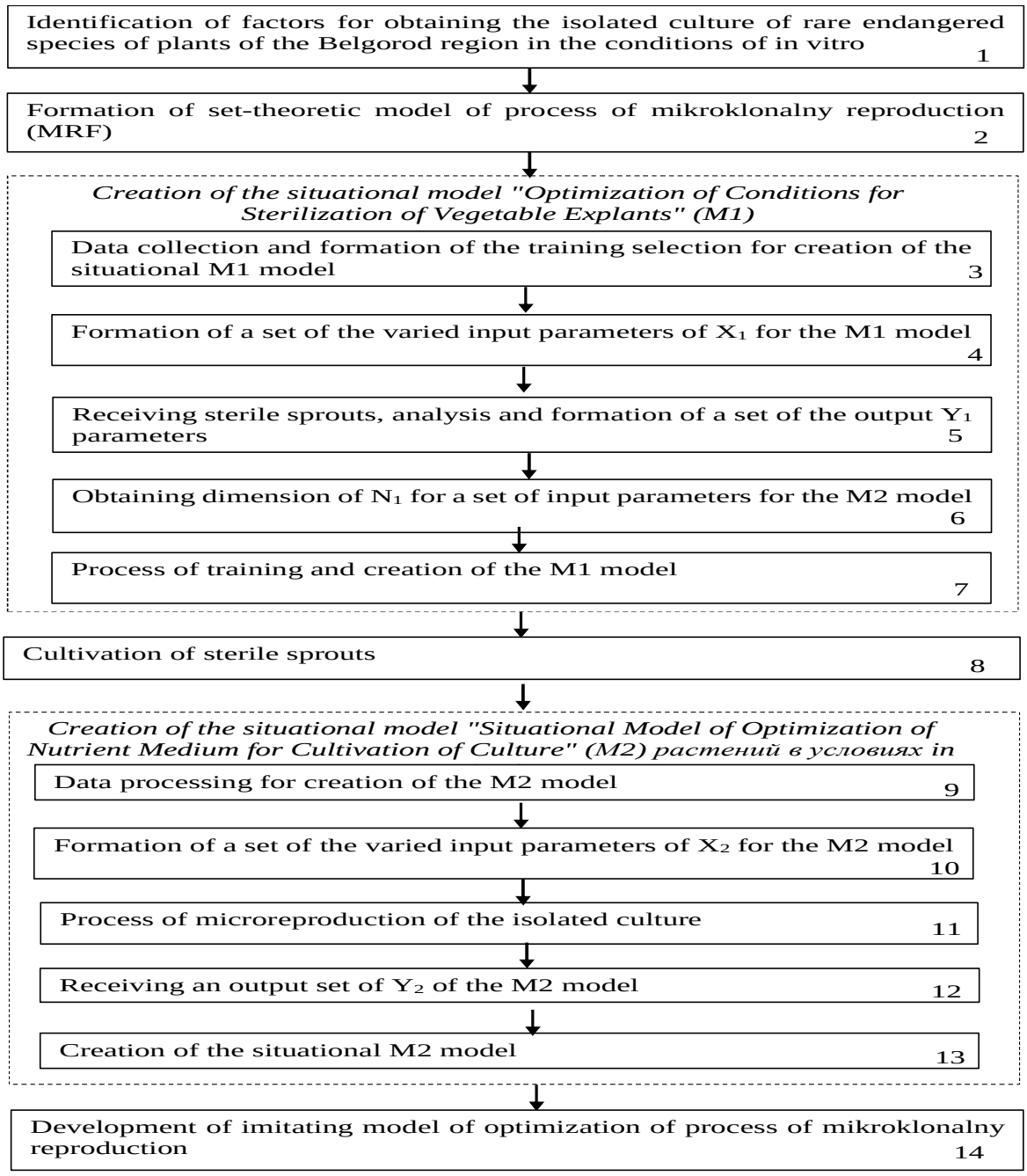

Figure 1. Sequential scheme of construction the simulation model for optimization the process of microclonal propagation.

At the initial stage 1, theoretical and empirical studies are conducted to identify factors, that

have a fundamental influence on the result of the microclonal propagation of rare plants. The following factors were identified:

- Aseptic conditions: sterilization of rooms, laboratory tools and dishes, nutrient medium and plant explants.
- Physical factors: photoperiod, temperature, acidity of the nutrient medium.
- Composition of the nutrient medium: macroelements, microelements, organic substances, vitamins, chelated iron solution, calcium chloride, growth-regulating chemicals.

At the second stage, based on the system description of the process, the corresponding set-theoretic model was formed:

$$MRF=<\Omega, X_1, X_2, Y_1, Y_2, F_1, F_2, F, O_1, O_2, O>, \quad (1)$$

Where Ω is a set of unregulated influence factors: external factors, as well as specified parameters of the environment and equipment; X_1 – is a set of variable input parameters for the situational model M1, determining the conditions for the sterilization of plant explants and the result of seedlings germination; X_2 - is the set of variable input parameters of the situational model M2 for optimization of the nutrient medium, for growing the plant culture *in vitro*, Y_1, Y_2 - are the output variables of the models M1 and M2, respectively: Y_1 - is the number of viable seedlings (%), Y_2 - is the growth index (the length of growth of plant explant, mm); F_1 - is the set of mappings on X_1, Y_1, F_1: $(X_1) \rightarrow Y_1$; O_1– ratio, O_1: (X_1^i, Y_1^k); F_2 – the set of mappings on X_2, Y_2, F_2: $(X_2) \rightarrow Y_2$; O_2– ratio, O_2: (X_2^j, Y_2^m); F: $(Y_1) \rightarrow Y_2$ – the set of mappings on Y_1, Y_2; O: (Y_1^l, Y_2^m).

At the third stage, the data is collected and the training sample is formed, for optimization the conditions of plant explants sterilization (situational model M1).

At the 4th stage, the set of input variables of the model M1 $X_1=\{X_{11}, X_{12}\}$ is formed, where X_{11} - chemical reagents for the sterilization of plant explants, X_{12} - time for sterilization.

At the 5th stage, sterile seedlings are obtained, set Y_1 is formed and analyzed.

At the 6th stage, the dimension N_1 (the number of germinated sterile sprouts) of the input set X_2 of the model M2, is defined.

At the 7th stage, there is the process of training and creation of the model M1.

After implementing the process of cultivation of sterile sprouts (stage 8), the situational model for optimization the nutrient medium for growing the plant culture *in vitro* is developed. For this, at the 9th stage, the analysis of germinated sprouts is carried out and the parameters of their qualitative state are evaluated.

At the 10th stage, the set of varied input parameters of the model M2 is formed: M2: $X_2=\{X_{21}, X_{22}, X_{23}\}$, where X_{21} - are the growth regulators (auxins), X_{22} - are the growth regulators (cytokinins), and X_{23} - are the growth regulators (gibberellins). After the process of microclonal propagation (stage 11), we obtain the output set Y_2 (stage 12).

At the 13th stage, there is the creation of the model M2. Based on the results of all the stages, described above, a simulation model for the microclonal propagation process is developed at the 14th stage.

Scientific researches were carried out according to the scheduled plan of the technical task on the project "Research of methods and modelling of processes in biotechnology and plant systematics". The seeds of angiospermous plants, growing on the territory of the Belgorod region, were chosen as initial plant explants for introduction into the culture of *in vitro*. Seed collection was made after the flowering phase, since May 2017, taking into account the fruiting time of each species. The collection of plant material was carried out in dry sunny weather, in the period of the full seeds ripening. The crowns of the stems were cut off with fruiting inflorescences, were placed in bags and labeled. Cameral processing included: drying the collected material in the laboratory, separating the seeds from the remains of the dry parts of the inflorescences (cups, coronets, trichomes), placing them in separate paper bags, indicating the date and place of collection of the plant on the label. In parallel, the collection and herbarization of the whole plant is carried out, according to the generally accepted methods, for

the documentation of the material [11-13]. Species identification is carried out using the plant determinant [14].

CONCLUSION

The main result of scientific researches is an increase of the efficiency of solving the problems of plant biodiversity conservation, and contributing to the country's food security, including the support of import substitution of agricultural products.

The implementation of the research results in the form of developed methods and models, software implementation and knowledge base will accelerate the process of obtaining an isolated culture of rare and endangered plant species, by modern biotechnological methods: the selection of effective methods for sterilization plant explants; the selection and optimization of nutrient media for cultivation into plant cultures for each species *in vitro*.

Methodological recommendations for obtaining an isolated culture of rare and endangered plant species, with modern biotechnological methods, were developed for the Belgorod region. Application of these recommendations will ensure the selection of effective methods for sterilization of plant explants; selection and optimization of nutrient media for cultivation into plant cultures for each species *in vitro*, using modelling methods.

DEDUCTIONS

The results of the research will allow to contribute to the implementation of the program on import substitution of Federal Agency for Scientific Organizations, and will also allow for selection organizations and agro-industrial complexes to use economically expedient modern methods of process modelling in biotechnology.

REFERENCES

1. The Red Data Book of the Belgorod Region [Electronic resource]. Available at: https://cicon.ru/krasnaya-kniga-btlgorodskoiobl.html

2. Butenko R.G., 1999. Biology of cells of higher plants in vitro, and biotechnologies on their basis. Moscow: FBK-PRESS, 160 p.

3. Ditchenko T.I., 2007. Culture of cells, tissues and organs of plants. Minsk: BSU, 46 p.

4. Timofeeva O.A., Nevmerzhitskaya Y.Y., 2012. Clonal micropropagation of plants. Kazan: Kazan University, 56 p.

5. Zholobova O.O., 2012. Conservation of rare and endangered plant species in the culture invitro and assessment of the level of their intraspecific polymorphism: the author's abstract of the dissertation of PhD Biol. Sciences. NRU "BelSU": Belgorod, 24 p.

6. Maslova E.V., Gulya N.I., 2015. Determination of the most effective regime for sterilization of plant explants of rare specie Astragalusalbicaulis DS (Fabaceae) in the flora of the Belgorod Region, for its obtaining in the culture in vitro. Materials of the collection of scientific works of the II International Youth Contest "Youth in Science: New Arguments": 48-50.

7. Ivashuk O.A., Konstantinov I. S., Shcherbinina N. V., Kvanin D.A., Gakhov R.P., 2015. Automated management of biotechnosphere of local urban areas. International Business Management, Volume: 9, Issue: 7: 1598-1603.

8. Ivashuk O.A., Shcherbinina N. V., Lazarev S. A., Ivashchuk O. D., 2015. Increasing efficiency of territory technospheric safety management, based on improving of Earth's surface satellite images. International Journal of Soft Computing 10 (6): 420-4265.

9. Ivashchuk O.A., Zemlyakova A.V., Shcherbinina N.V., Petrov S.P., Buryak J.A., Fedorov V.I., Bogat D.V., 2016. Electronic model of the waste management schedule. Scientific bulletins of BelSU. Series Economics, Computer Science, No. 2 (223), Issue 37: 162-167.

10. Ivaschuk O.A., Ivashchuk O.D., 2013. Models of data mining in information systems of ecological safety. Scientific Bulletin of Belgorod State University. Series Economics, Computer Science, No. 15 (158), Issue 27/1: 163-168.

11. Skvortsov A.K., 1977. Herbarium: methodological and technical manual. Moscow: Nauka, 200 p.

12. Herbarium art. Reference manual under the editorship of D. Bridson, L. Foreman. Kew: The Royal Bot. Garden, 1995, 341 p.
13. Gulya N.I., Maslova E.V., Petrova I.V., 2016. The main stages of clonal micropropagation in vitro, for the conservation of rare and endangered plant species. Collection of theses of the 20th International Pushchino School-Conference of Young Scientists "Biology – is the science of the XXI century": 221.
14. Maevskiy P.F., 2006. Flora of the midland of the European part of Russia. Moscow: Association of Scientific Publications KMK, 600 p.