Mathematical modeling of the process of sterilizing potato explants and obtaining viable potato microclones

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Abstract. The aim of the studies is obtaining optimal regime parameters of the process of decontamination of plants’ explants by investigating integral action of sterilizing agent and by modifying nutrient medium. The studies of integral influence of technological factors, which have an impact on the process of explants’ sterilization and output of viable plants, have been carried out by the method of experimental design. As a result of the analysis of literature data and searching experiments three main controllable factors, which influence on a percentage of viable potato sprouts, have been highlighted: a concentration of the antibiotic «Cefotaxime», a concentration of the cytokinin of the 6-benzylaminopurine (6-BAP) and a concentration of the active substance of the nutrient medium. The method of experimental design for three-factor experiment has been realized to study optimum region. The optimal values of regime parameters of the process of decontamination of explants of plants’ material have been determined on the basis of regression equations and two-dimensional sections of yield surfaces: the concentration of the antibiotic «Cefotaxime» in the nutrient medium – 335…365 mg/l, the concentration of the phytihormone 6-BAP – 0.95…1.05 mg/l, the concentration of the commercial drug «Belizna» (the active substance is sodium hypochlorite) – 2.5…2.6 %. At that the percentage of viable plants will amount to 86.0 %.

1. Introduction

One of the main tasks of agricultural production consists in finding effective ways to increase crop production and reduce its cost through implementation of promising production methods, namely, micropropagation of healthy crop plants with their planting in an artificial nutrient medium, following adaptation and completion of growing in the real growing conditions [1-3]. Rapid cultivation of healthy plants with high bio-productivity can be ensured under optimal conditions at all stages of microclonal propagation: selection of the source material of plants; sterilization and introduction of explants in the invitro culture; micropropagation and replication of plants [4-6].

Today, the use of mathematical modeling for the purpose of optimizing regime parameters in agricultural production through a multifactor experiment allows for effective choice of microclimate indicators for biotechnological methods of cultivating plants in a short time. In addition, tools of mathematical modeling make it possible to study the integral influence of technological factors, which
have an impact on the process of explants’ sterilization and output of viable plants, with obtaining adequate mathematical model, which ensures effective optimization of the technological process.

Such an approach involves conducting repeatable experiments on several selected factors with three levels of variation, which corresponds to the techniques of Rechtschafner design, Box-Behnken design and others.

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2. Study techniques

The potato varieties Impala and Red Scarlett have been chosen for studying. These released and recommended for cultivation in the Volgograd region varieties are of high food value owing to low content of reducing sugars in pulp, what is necessary for producing chips and fried products.

Potato tubers were flushed in the water flow elaborately to obtain etiolated plantlings removing surface contaminations by soap solution. Then tubers were processing by diluted solution of potassium permanganate for 20 minutes, placed into carton boxes with perforation, whose bottom was paved with wet filter paper, and germinating at 25 °C for 7-14 days. Sprouts 2…4 cm long were broken off from sprouted potato, flushing in the water flow with the use of common soap for 10 minutes, releasing the material from coarsened external tissues and contaminations. Then meristem tissue was identified and extracted from potato sprouts for its following introduction in the invitro culture.

All experiments on determining efficiency of the method of explants’ sterilization were carried out repeatedly. Statistical analysis was performed according to the established procedures.

A multifactor experiment was carried out with the use of the matrix of Rechtschafner design. As a result of the analysis of literature data and searching experiments three main controllable factors, which influence on a percentage of viable potato sprouts, have been highlighted. They are a concentration of the antibiotic «Cefotaxime» [7], a concentration of the phytohormone 6-BAP and a concentration of the «Belizna» (the active substance is sodium hypochlorite). Levels and intervals of variation were assigned for these factors (Table 1).

| Factors | Levels of factor | Interval of variation ε |
|---------|-----------------|------------------------|
| Concentration $X_1$ of the antibiotic «Cefotaxime» in the nutrient medium, mg/l | 0 350 | 500 150 |
| Concentration $X_2$ of the phytohormone 6-BAP, mg/l | 1 0.5 1.5 0.5 |
| Concentration $X_3$ of the commercial drug «Belizna» (the active substance is sodium hypochlorite), % | 3.0 2.5 3.5 0.5 |

Estimation of condition of plants under study was carrying out for two weeks since the day of beginning of the experiment by visual inspection (figure 1).
A percentage of viable plants $Y$, %, was accepted as an output index at the stage of laboratory and field studies.

Sterile viable explants differed in absence of the signs of fungal and bacterial infection and strong growth as well.

3. Mathematical modeling

As a result of calculations the regression equations were obtained in coded form:

$$Y_i = 79.6 - 0.1x_1 - 0.1x_2 - 14.6x_3 + 0.3x_1x_2 + 0.6x_1x_3 - 0.5x_2x_3 - 6.9x_1^2 - 5.5x_2^2 - 8.0x_3^2. \quad (1)$$

Adequacy of the obtained mathematical models was verified by Fisher’s ratio test:

$$F = \frac{S_{ad}^2}{S^2(y)} \quad (2)$$

where $S^2(y) = \left( \frac{\sum_{i=1}^{N} \sum_{q=1}^{k} \left[ y_{iq} - \bar{y}_i \right]^2}{N(n-1)} \right)$ is the error experiment variance, $S_{ad}^2(y) = n \sum_{i}^{N} (\bar{y}_i - \bar{y})^2 / (N - [k + 1])$ is the dispersion of the model inadequacy, here: $y_i$ is the random quantity, which was calculated according to the mathematical relationship; $\bar{y}_i$ is the arithmetic mean value of the random quantity; $y_{iq}$ is the value of the $i$-th quantity in the $q$-th experiment; $n$ is the number of the experiment replications; $N$ is the number of the rows of the design matrix; $k$ is the number of the factors.

The following value of cutting force was obtained: $F = 1.381$, i. e. $F_{0.05} > F$ (here $F_{0.05} = 2.599$ is the table value of Fisher’s ratio test at confidence level 5 %). Thus, the mathematical models are adequate to the experiment results. Then the optimal values of the factors were determined (table 2).

| Factor | Optimal values$^a$ |
|--------|---------------------|
| Concentration $X_1$ of the antibiotic «Cefotaxime» in the nutrient medium, mg/l | 0.05 |
| Concentration $X_2$ of the phytohormone 6-BAP, mg/l | 0.03 |
| Concentration $X_3$ of the commercial drug «Belizna» (the active substance is sodium hypochlorite), % | 0.92 |

$^a$ In coded form in the numerator, in decoded form in the denominator.
The obtained second-order mathematical model was reduced to typical canonical form for analysis and systematization. As a result of calculations, which were performed on computer, the regression coefficients $B_{11}, B_{22}, B_{33}$ in canonical form and the values of optimization criterion in the optimal point $Y_*$ were obtained.

Regression equation (1), which is expressed in canonical form, is given by:

$$Y_* - 86.3 = -6.8x_1^2 - 5.5x_2^2 - 8.1x_3^2.$$  \hspace{1cm} (3)

As all coefficients at squared terms have negative signs, then yield surfaces, which are described by equation (1), are three-dimensional paraboloids with coordinates of the surfaces’ centers in the optimal values of the factors.

When two-dimensional section of yield surfaces on regression equation (2) is considered relatively to «concentration of the antibiotic «Cefotaxime» in the nutrient medium» ($X_1$) and «concentration of the phytohormone 6-BAP» ($X_2$), the factor «concentration of the commercial drug «Belizna» (the active substance is sodium hypochlorite») equals to the optimal value $X_3 = -0.92$ (figure 2).

![Figure 2. Two-dimensional section for studying influence of the factors $X_1$ and $X_2$ when $X_3 = -0.92$ on the percentage of viable plants $Y$.](image)

The following optimal values of the factors can be recommended: $X_1 = -0.1...0.1$ and $X_2 = -0.1...0.1$.

When two-dimensional section of yield surfaces on regression equation (1) is considered relatively to «concentration of the antibiotic «Cefotaxime» in the nutrient medium» ($X_1$) and «concentration of the commercial drug «Belizna» (the active substance is sodium hypochlorite») ($X_3$), the factor «concentration of the phytohormone 6-BAP» equals to the optimal value $X_2 = 0.03$ (figure 3).
The following optimal values of the factors can be recommended: $X_1 = -0.1...0.1$ and $X_3 = -1.0...-0.8$.

When two-dimensional section of yield surfaces on regression equation (1) is considered relatively to «concentration of the phytohormone 6-BAP» ($X_2$) and «concentration of the commercial drug «Belizna» (the active substance is sodium hypochlorite)» ($X_3$), the factor «concentration of the antibiotic «Cefotaxime» in the nutrient medium» is at the optimal value $X_1 = -0.05$ (figure 4).

The following optimal values of the factors can be recommended: $X_2 = -0.1...0.1$ и $X_3 = -1.0...-0.8$.

Analysis of represented two-dimensional sections has shown that the following optimal values of the factors can be recommended for ensuring the maximal percentage of viable plants: $X_1 = -0.1...0.1$ (335...365 mg/l), $X_2 = -0.1...0.1$ (0.95...1.05 mg/l), $X_3 = -1.0...-0.8$ (2.5...2.6 %). At that the percentage of viable plants $Y$ will amount to 86.0 %.

4. Conclusion
As a result of experimental studies and following processing of the results of multifactor experiment the optimal values of the parameters, which influence on viability of potato microclones, have been...
obtained. Three main controllable factors, which influence on a percentage of viable potato sprouts, have been highlighted: \( X_1 \) is a concentration of the antibiotic «Cefotaxime», \( X_2 \) is a concentration of the phytohormone 6-BAP and \( X_3 \) is a concentration of the commercial drug «Belizna».

Analysis of represented two-dimensional sections has shown that the following optimal values of the factors can be recommended for ensuring the maximal percentage of viable plants: \( X_1 = -0.1\ldots0.1 \) (335…365 mg/l), \( X_2 = -0.1\ldots0.1 \) (0.95…1.05 mg/l), \( X_3 = -1.0\ldots-0.8 \) (2.5…2.6 %). At that the percentage of viable plants \( Y \) will amount to 86.0 %.

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