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The usage of cell selection *in vitro* in the creation of tomato and eggplants breeding lines with resistance to fusarium (*Fuzarium oxysporum*)

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Aim. The development of a biotechnological system of assessing and selecting the forms of egg-plant and tomato which are tolerant to fusarium wilt in the culture of the tissues, isolated *in vitro*. **Methods**. The studies were carried out according to the standard biotechnological methods and using the standard equipment. The high-grade and hybrid seeds of egg-plant and tomato genotypes of F_1–F_4 breeds with different tolerance to fusarium wilt were used in the studies. The cell selection was carried out in the media with different content of the selective agent (20, 40 and 60 % of the total medium volume). **Results**. It has been determined that the samples can be reliably differentiated in the selective media with liquid culture filtrate on the stages of induction and proliferation of callusogenesis according to their resistance in the field. The effective concentrations of the liquid culture filtrate of *F. oxysporum* in the selective media necessary for the selection of resistant callus clones are 20−30 % for acceptable genotypes and 40–50 % and higher for moderately resistant and resistant samples. According to the selective assessment (2012−2013) of initial breeding lines of egg-plant and tomato and the ones, selected via the cell selection, there were 4 highlighted promising lines of egg-plant and 5 lines of tomato that exceeded the control samples and the initial genotypes in their resistance to fusarium and in fertility. **Conclusions**. The possibility and efficiency of the biotechnology of accelerated creation of and express-tests on the breeding lines of egg-plant and tomato resistant to *F. oxysporum* were substantiated and experimentally proved in order to cut the time necessary for the attainment of resistant initial material.

Keywords: *in vitro*, *Fusarium*, selective factor, fungal culture filtrate, resistance source, selection.

INTRODUCTION

The report of the Seed Production Association, entitled "Climate Changes and Seed Production" highlighted the expectable rise of the temperature by 11 degrees till 2050. Such changes will have a negative impact on agricultural production throughout the world, along with more frequent and prolonged droughts and floods, amid the ever-increasing negative impact of pests and diseases. In order to prevent significant losses in agricultural production the world's leading breeding and seed centers face the need to revise generally acceptable approaches of scientific research. The adaptation of plants to stress conditions is a complex set of processes occurring at the molecular, cellular, physiological and biochemical level, while most signs of stress tolerance are determined by the set of genes, linked into complicated systems. For this reason, the efficiency enhancement of stress tolerance by means of traditional breeding methods is much more complicated.

Some ways of creating breeds that would be resistant to disease-producing factors include the methods of cell selection, based on the *in vitro* selection of tolerant biotypes, which develop adaptively in the course of microevolutional processes in the populations. This approach makes it possible to cut the time of selection. The selection of regenerant plants which are resistant/tolerant to stresses *in vitro* has a number of advantages compared to the selection in the field: quick and more precise assessment of quantitative attributes of polygenic resistance; a big quantity of genotypes analyzed within a comparatively short period of time [1, 2]. The fungal culture filtrates of the disease-producing agents are commonly used as selective media for the selection of material that would be resistant to necrotrophic

phytopathogens [3]. *In vitro* culture selection with the usage of culture filtrates of fungus as selective agents is even more effective than the usage of pure pathogenic culture [4]. The fungal culture filtrate was used in the selection of genotypes of chickpea, flax, cotton [4], gladiolus, wheat [2, 5, 6], and cucumber [7].

We used this approach in our study while selecting the sources of resistance to wilting, the main cause of which in the forest-steppe zone is fungi of *Fusarium* Link *(Fusarium solani* and *Fusarium oxysporum),* the spreading of which has been 37−69 % in the zones of solanaceous vegetables in recent years. This results in significant losses of crops (30−40 %) [8].

The aim of this study was to develop a biotechnological system of assessing and selecting the forms of egg-plant and tomato, tolerant to fusarium in the culture of the *in vitro* isolated tissues.

MATERIALS AND METHODS

The studies were carried out according to the standard biotechnological methods and using the standard equipment [9]. The quantity of plants in one group was 40. The high-grade and hybrid seeds of egg-plant and tomato genotypes of F_1–F_4 breeds with different tolerance to fusarium were used in the study. The seed-lobes of seven-day seedlings were used as donor explants for cell selection. They were bedded out into our specifically designed MS media, modified by growth regulators (6-BAP – 6-benzylaminopurine, IAA – 3-indolylacetic acid). The cell selection was carried out in the media with different content of the selective agent – fungal culture filtrate (FCF) of the pathogenic fungus *F. oxysporum* (20, 40 and 60 % of the total medium volume). The control samples were culture media without FCF. The selection of resistant breeding lines was carried out according to the two-stage scheme. The cultivation went on at 22−24 °C, 16-h photoperiod and the lighting level of 2,000 lux.

The level of FCF influence on the development of explants in the *in vitro* culture was measured concurrently with the first differentiation of the samples according to the occurrence of calluses on day 16 of the cultivation. The influence of the complex of FCF toxins on the growth and development of the calluses was assessed visually according to the following five-score grading scheme: 0 – highly resistant, the development of the tissues is the same as in the control samples; 1 – resistant, the amount of chlorotic tissue is up to 25 %, the growth of calluses is intensive; 2 – moderately resistant, the amount of chlorotic tissue is up to 50 %, the growth of calluses is moderate; 3 – susceptible, the amount of chlorotic tissue is up to 75 %, the growth of calluses is inhibited; 4 – highly susceptible, the amount of chlorotic tissue is more than 75 %, calluses do not grow.

The regenerant plants acquired after the two-stage selection were reproduced, grown, implanted and adapted to unsterile conditions according to the commonly used methods. Further assessments of the indications were carried out according to the Methodology for assessing distinctiveness, homogeneity, and stability of breeds [11].

RESULTS AND DISCUSSION

The assessment of the susceptibility of egg-plants of various origins to FCF of *Fusarium* fungi in the culture of isolated tissues revealed the influence of the selective medium on the viability and the parameters of callusogenesis of cotyledonary explants. The investigated samples of egg-plant were divided into groups of resistance towards FCF of pathogens by the reaction of viability to the effects of the selective medium: 1 (resistant) – interspecific hybrid b. c. 13 (b. c. − biotechnological catalogue) genotypes of West-Asian type b. c. 47, b. c. 40; 2 – moderately resistant genotypes of West-Asian type b. c. 50, b. c. 5, b. c. 37, b. c. 50, and genotypes of East-Asian type b. c. 35, b. c. 30, b. c. 8; 3 – susceptible − genotypes of East-Asian type b. c. 10, b. c. 29.

It was determined that at the 20 % FCF concentration of *Fusarium* oxysporum, the viability of calluses decreased to 78.7 % for the sample b. c. 47 of West-Asian origin and to 24.2 % for the sample b. c. 29 of East-Asian origin (Fig. 1).

The viability of egg-plant calluses in the medium with FCF 40 % was in the range from 33.3 % (b. c. 47) to 20.4 % (b. c. 29). The egg-plant calluses of West-Asian subspecies (b. c. 40), cultivated at the high concentration of FCF, had the lowest result of viability 13.2 %, and the calluses of East-Asian subspecies were totally inhibited and did not develop.

Thus, at the stage of induction and proliferation of callusogenesis in primary explants, the samples could be reliably differentiated according to the level of resistance in the field. The optimal conditions for selection by resistance are defined as the following sublethal concentrations: 20 % FCF for the samples of East-Asian origin and 40 % FCF for the samples of West-Asian origin. 11 lines of egg-plant were acquired from

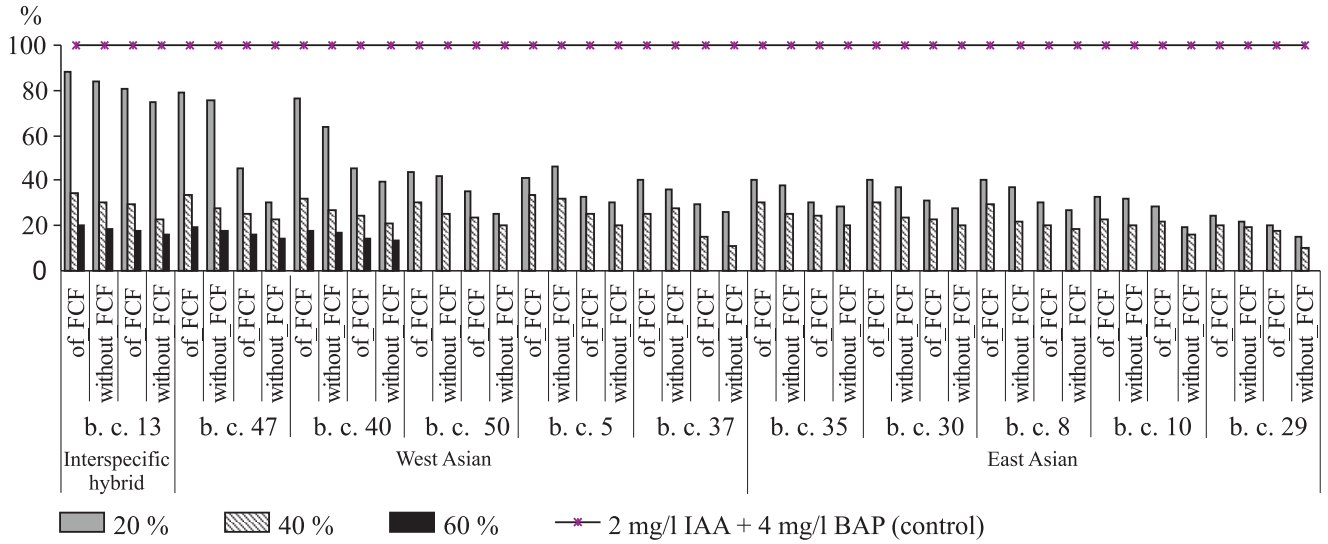

Fig. 1. The influence of various concentrations of FCF of *Fusarium* oxysporum on the viability of egg-plant calluses in the selective medium, average for 2011−2013

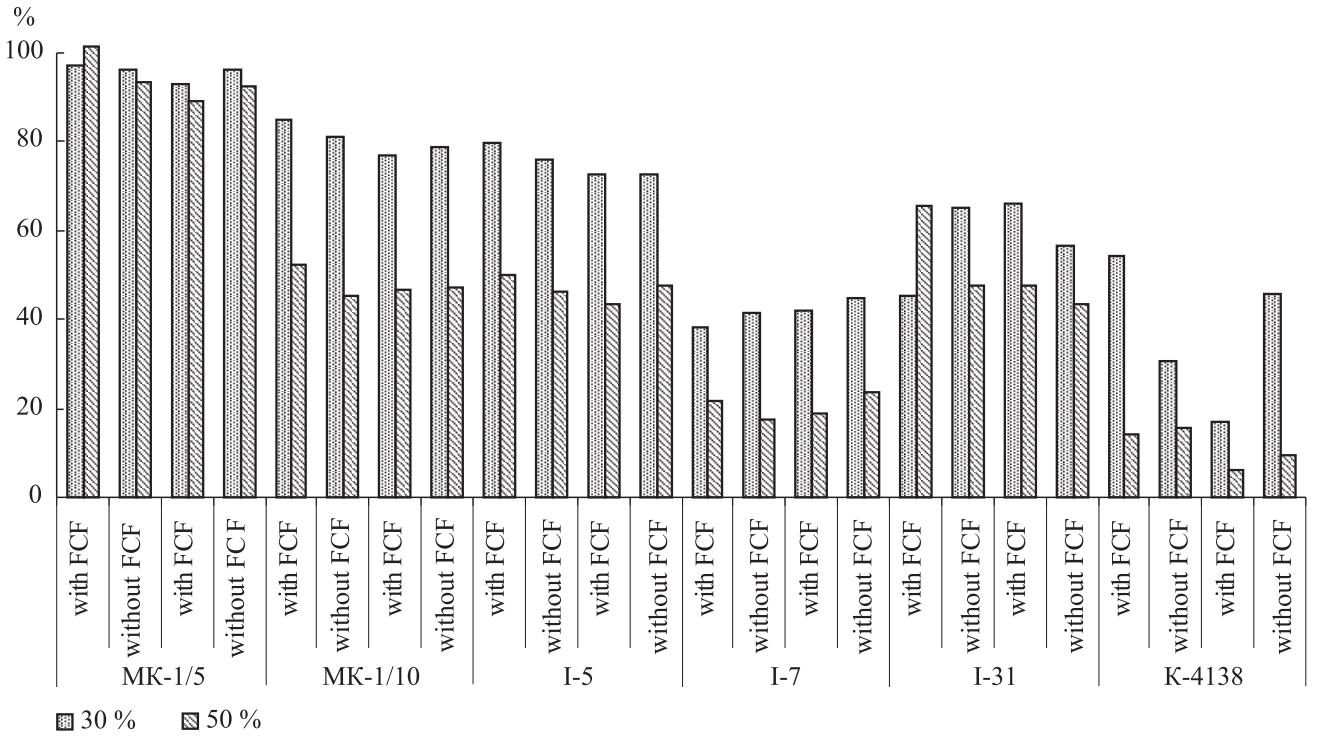

Fig. 2. The influence of various concentrations of FCF of *Fusarium* oxysporum on the viability of tomato calluses in the selective medium (2011−2013)

the egg-plant calluses, selected in the medium with 40 % FCF in the following subcultures.

The investigated tomato samples were divided by the level of tolerance in the *in vitro* culture: 1 – resistant – MK-1/5, Eleanora; 2 – moderately resistant – K-4138, I7, I31; 3 – susceptible – I5; 4 – highly susceptible – Ukrainskyy teplychnyy 285, MK-1/10.

The process of cell selection in the tomato culture revealed an inversely proportional dependence between the quantity of viable explants and the FCF concentration in the culture medium (Fig. 2). The influence of the pathogen was especially high on the explants of I7 genotype – the rate of explants that survived after 30 % FCF had been added to the medium decreased to 32 %, 50 % FCF − to 17.9 compared to 83.3 % for the con-

trol sample. In genotypes MK-1/5 and K-4138 which were the most resistant of all samples, the rate of viable calluses in both 30 % and 50 % FCF media did not differ from that for the control sample. The standard for indeterminant samples – Ukrainskyy teplychnyy 285 – died entirely in the selective media which indicates its susceptibility to fusarium.

FCF contributed to the decrease of average cubic capacity of callus in all the investigated genotypes of tomato. For instance, for MK-1/5 the cubic capacity in the control sample was 804.8 cmm, in the medium with 30 % FCF – 276.0 cmm, and in the medium with 50 % FCF – only 66.5 cmm. For MK-1/10 genotype the cubic capacity was 476.7, 128.3, and 3 cmm respectively. The most inhibiting influence of FCF on this parameter was observed for MK-1/5, I5, MK-1/10 and I7 genotypes.

The efficient FCF concentration of *F. oxysporum* for the selection of tolerant callus clones from susceptible genotypes (4 and 3 according to the scale) is 30 %. The FCF concentration of *F. oxysporum* equal and above 50 % can be used for resistant and moderately resistant (1 and 2 according to the scale) samples. The tolerant callus clones of 5 genotypes were selected from the samples in the selective media with 30 and 50 % FCF.

The studies on the correlated connections between the parameters of *in vitro* and *in vivo* culture have indicated the efficiency of cell selection in selective medium with 40 % FCF. There has been a significant average positive correlated dependence found between the following parameters: "cubic capacity of callus – height of plants" (r = 0.76); "cubic capacity of callus – plant productivity – general crop productivity" (r = = 0.78); close correlation was defined between the parameters "viability of callus clones – damage level" (r = = –0.70). The rest of the correlation coefficient values were within the study error values. Thus, there is a reliable possibility of selecting regenerant plants, resistant to fusarium wilt, among callus clones which are resistant to stress factors.

According to the selective assessment (2012−2013) of initial breeding lines of egg-plant and tomato and the news ones, selected via the cell selection, there have been 4 highlighted promising lines of egg-plant and 5 lines of tomato, exceeding the control samples and the initial genotypes in their resistance to fusarium wilt and in all the basic parameters: height of plants, quantity and mass of fruit, productivity, market value of fruit (Table).

By the crop productivity parameter, most of the egg-plant genotypes exceeded the Almaz breed which is resistant to fusarium wilt. It was determined that cultivation of new breeds created as a result of selection in 40 % FCF provides big increase in crop productivity – S. m. 62 − by 44.21 %, S. m. 63 − by 30.9 %, S. m. 79 − by 29.61 %, L-21 − by 26.18 %. The parameters of market value of egg-plant fruit were also high – 98−99 %.

According to the phytopathologic assessment of initials material of egg-plant b. c. 47, b. c. 40, b. c. 36, resistance to fusarium wilt was about 5 points according to the scale.

Genotypes S. m. 62, S. m. 63, S. m. 79, L-21, acquired as a result of the selection in the media with FCF, were characterized by the resistance to the disease with 7 points that corresponds to 'resistant'. Only the line L-21, created from the genotype sample b. c. 11 that was susceptible to the disease, got 5 points. The reason is that egg-plant genotypes of East-Asian origin lack genetic resistance to the phytopathogen.

The level of resistance to fusarium wilt of tomato plants, screened in the selection media with different content of *F. oxysporum* FCF, exceeded the values of the control samples induced without the addition of the selective agent by this characteristic. The resistance of determinant samples T. m. 42 and T. m. 52 was at the level of the standard breed Eleanora (7 points – resistant). In the indeterminant genotypes – T. m. 11 and T. m. 46 – the level of resistance was also 7 points, which is higher than that for the standard breed Ukrainskyy teplychnyy 285 (5 points). The samples, singled out in the selection media, exceeded the control by the main agricultural features such as fruit weight, plant productivity, and general crop productivity. Moreover, the crop productivity of samples T. m. 42, T. m. 52 (determinant), T. m. 11, T. m. 46 (indeterminant) exceeded the standard breeds. The exception is genotype T. m. 15 whose crop productivity was twice lower than that for the standard breed Eleanora, but exceeded the control sample T. m. 27 two-fold by this characteristic. The biggest increase in crop productivity compared to the standard breed was registered in indeterminant samples T. m. 11 and T. m. 46–24.24 %, and in determinant sample T. m. 52–14.14 %. The market value of the selected samples was not lower than 90 %. The acquired data indicate high efficiency of selection in the *in vitro* culture.

The characteristics of morphobiological features of egg-plant and tomato breeding samples, average for 2011–2013

Initial sample	Culture medium	New genotype	Plant height, cm	Fruit parameters		Productivity, kg/plant	Resistance by CMEA scale, point	General crop productivity, t/ha	Crop productivity increase, %	Saleable condition, %
				Number per 1 plant	Average weight, g					
Egg-plant										
Almaz st.	MS without FCF		60.69 ± 1.4	9.33 ± 0.11	170.3 ± 0.4	1.58 ± 3.1	7	23.3	0.0	97.9
b. c. 47	MS without FCF	S. m. 47	62.41 ± 2.5	9.07 ± 0.21	186.31 ± 1.2	2.03 ± 6.8	5	27.5	18.02	96.7
	MS+40 % FCF	S. m. 62	62.42 ± 4.4	8.40 ± 0.13	214.58 ± 3.81	1.73 ± 8.4	7	33.6	44.21	99.7
	MS without FCF	S. m. 40	58.34 ± 2.2	9.13 ± 0.23	189.43 ± 1.4	1.72 ± 5.9	7	23.7	1.72	94.5
	MS+40 % FCF	S. m. 63	62.91 ± 4.2	8.40 ± 0.16	217.93 ± 0.3	1.82 ± 7.6	7	30.5	30.90	99.3
b. c. 36	MS without FCF	S. m. 36	62.93 ± 1.2	9.13 ± 0.13	219.60 ± 5.3	1.99 ± 6.7	5	28.1	20.4	96.9
	MS+40 % FCF	S. m. 79	64.12 ± 4.4	8.61 ± 0.15	213.33 ± 5.3	1.83 ± 6.3	5	30.0	29.61	99.7
b. c. 11	MS without FCF	S. m. 11	58.24 ± 1.2	8.62 ± 0.16	215.32 ± 8.3	1.83 ± 6.3	5	25.3	8.6	95.2
	MS+40 % FCF	L. 21	51.91 ± 2.1	8.50 ± 0.36	210.20 ± 6.3	1.78 ± 6.5	5	26.4	26.18	98.4
Tomato determinant										
Eleanora st	MS without FCF		54.72 ± 3.4	15.73 ± 2.0	80.1 ± 2.3	1.26 ± 0.3	7	50.2	0.0	98.3
MK-1/10	MS without FCF	T. m.27	47.81 ± 3.6	5.91 ± 1.0	44.0 ± 1.9	0.32 ± 0.1	3	12.0	–76.10	60.8
	MS+30 % FCF	T. m.15	49.73 ± 2.6	10.92 ± 3.3	52.6 ± 3.0	0.61 ± 0.2	5	24.2	–51.79	90.1
I31	MS without FCF	T. m.34	51.72 ± 3.2	17.51 ± 1.7	53.2 ± 3.3	1.03 ± 0.2	5	48.2	–3.98	94.5
	MS+50 % FCF	T. m.42	58.44 ± 1.7	19.43 ± 2.2	60.6 ± 4.2	1.23 ± 0.4	7	52.3	4.18	98.6
I7	MS without FCF	T. m.48	47.22 ± 2.5	15.73 ± 2.3	69.5 ± 2.7	1.30 ± 0.1	5	56.1	11.75	92.2
	MS+30 % FCF	T. m.52	50.01 ± 2.0	19.14 ± 3.0	73.4 ± 5.0	1.4 ± 0.2	7	57.3	14.14	99.3
Tomato indeterminant										
Ukrain. teplychnyy 285, st	MS without FCF		155.7 ± 3.3	20.82 ± 2.8	158.5 ± 4.3	3.30 ± 0.5	5	13.2 kg/m^2	0.0	98.5
MK-1/5	MS without FCF	T. m.5	159.3 ± 4.2	23.5 ± 2.6	142.7 ± 2.2	3.35 ± 0.2	5	13.4 kg/m^2	1.52	97.1
	MS+50 % FCF	T. m.11	167.7 ± 3.5	27.3 ± 2.1	146.4 ± 1.5	4.10 ± 0.3	7	16.4 kg/m^2	24.24	98.7
K-4138	MS without FCF	T. m.43	165.3 ± 3.8	21.3 ± 2.0	169.1 ± 2.7	3.60 ± 0.3	5	14.4 kg/m^2	9.09	95.2
	MS+30 % FCF	T. m.46	170.8 ± 4.4	24.0 ± 2.0	170.8 ± 3.4	4.10 ± 0.4	7	16.4 kg/m^2	24.24	98.3

New breeds are recommended as the source of resistance towards wilt and heat resistance in the process of creating breeds and hybrids of egg-plant and tomato.

Conclusions. The possibility and efficiency of a biotechnology of faster creation and express assessment of cell lines of egg-plant and tomato, resistant to *F. oxysporum*, in order to cut the time necessary for the acquisition of resistant initial material, have been grounded and experimentally proven. The selective *in vitro* system for egg-plant and tomato was developed, which makes the selection of valuable genotypes 2−3 times faster.

Використання клітинної селекції *in vitro* для створення стійких до фузаріозного в'янення (*Fusarium oxysporum*) селекційних ліній баклажана і томата

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Мета. Розробити біотехнологічну систему оцінки та добору в культурі ізольованих тканин *in vitro* форм баклажана і томата, толерантних до фузаріозного в'янення. **Методи.** Досліди виконували загальноприйнятими біотехнологічними методами із застосуванням стандартного обладнання. Використано різний за рівнем толерантності до фузаріозного в'янення сортовий і гібридний насіннєвий матеріал генотипів баклажана і томата поколінь F_1–F_4. Клітинну селекцію здійснювали на середовищах з різним вмістом селективного агента (20, 40 і 60 % від об'єму середовища). **Результати.** Встановлено, що на селективних середовищах з фільтратом культуральної рідини (ФКР) на етапі індукції та проліферації калюсу можна достовірно диференціювати зразки відповідно до рівня їхньої стійкості за польових умов. При доборі стійких калюсних клонів ефективними концентраціями ФКР *F. oxysporum* у селективних середовищах для сприйнятливих генотипів є 20–30 %, а для середньостійких і стійких зразків − 40–50 % і вище. За результатами проведеної у 2012−2013 рр. селекційної оцінки вихідних і нових відібраних клітинних ліній баклажана і томата виділено чотири перспективні лінії баклажана і п'ять ліній томата, що перевищують контрольні варіанти і вихідні генотипи за стійкістю до фузаріозного в'янення та за продуктивністю. **Висновки.** Для скорочення терміну отримання стійкого вихідного матеріалу обґрунтовано та експериментально доведено можливість і ефективність біотехнології прискореного створення і експрес-оцінювання стійких до *Fusarium oxysporum* клітинних ліній баклажана і томата.

Ключові слова: експерименти *in vitro*, селективний фактор, фільтрат культуральної рідини, стійкий матеріал, селекція.

Использование клеточной селекции *in vitro* для создания устойчивых к фузариозному увяданию (*Fusarium oxysporum*) селекционных линий баклажана и томата

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Цель. Разработка биотехнологической системы оценки и отбора в культуре изолированных тканей *in vitro* форм баклажана и томата, толерантных к фузариозному увяданию. **Методы.** Опыты проводили по общепринятым биотехнологическим методам с применением стандартного оборудования. Использовали различный по уровню толерантности к фузариозному увяданию сортовой и гибридный семенной материал генотипов баклажана и томата поколений F_1–F_4. Клеточную селекцию осуществляли на средах с разным содержанием селективного агента (20, 40 и 60 % от объема среды). **Результаты**. Установлено, что на селективных средах с фильтратом культуральной жидкости (ФКЖ) на этапе индукции и пролиферации каллуса можно достоверно дифференцировать образцы в соответствии с уровнем их устойчивости в полевых условиях. При отборе устойчивых каллусных клонов эффективными концентрациями ФКЖ *F. oxysporum* в селективных средах для восприимчивых генотипов является 20–30 %, для среднеустойчивых и устойчивых образцов − 40–50 % и выше. По результатам проведенной селекционной оценки среди отобранных клеточных линий баклажана и томата выделены четыре перспективные линии баклажана и пять линий томата, превышающие контрольные варианты и исходные генотипы по устойчивости к фузариозному увяданию и по продуктивности. **Выводы.** Для сокращения периода получения устойчивого исходного материала обоснована и экспериментально доказана возможность и эффективность биотехнологии ускоренного создания и экспресс-оценки устойчивых к *Fusarium oxysporum* клеточных линий баклажана и томата.

Ключевые слова: *in vitro*, *Fusarium*, селективный фактор, фильтрат культуральной жидкости, устойчивый материал, селекция.

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