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ORIGINAL ARTICLE

Producing of potato varieties resistant to fusarial wilt by cell selection

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The experiment has determined the characteristic features of callusogenesis, regeneration as well as of receiving resistant to fusariosis of potatoes cell lines in simulated conditions of biotic stress. The technologic elements of potato cell selection towards its resistance to *Fusarium oxysporum* and *Fusarium sabucinum* were presented. We succeeded in separating the lines which during a 3-year period of research were characterised by a higher resistance point as compared to the original varieties Gurman and Tyras. The plants-regenerants received on the selective medium were characterised by higher resistance (by 10–35 %) to the pathogens action as compared to the regenerants of a control variant. The received lines were estimated on their yield capacity and starch content. The lines deviations on economically valuable indices either increased or decreased as to characteristic value. Herewith, the deviation upward is 25–75 % depending on characteristic. The combination of maximal characteristic values was found in lines 12 and 28 of Gurman variety. The received lines were given to the selection-breeding plot for further testing.
Key words: potatoes, culture in vitro, cell selection, selective medium, phytotoxic metabolites, plants-regenerants.

Introduction

Potato is one of the most important food and feed crop in the world. It is grown on all continents practically in every country. In Ukraine potato is grown on the area of more than 1.5 ha 10^6. The variety has become a determinant of modern potatoe growing efficiency in the last few years.The potatoe yield is determined by 50 % by the potato variety. The variety value can not be overestimated. Being different in biological and economic characteristics, the varieties make the basis of any technology (Podgayetskiy & Gorbas, 2013).

Under current conditions the selection capabilities on augmenting the obtainable genetic variability have greatly increased (Polozhenets, Tymoschuk & Zhuravska, 2006). They have new methods of mutations inducing, chromosome and gene engineering, protoplast fusion and others, though the main sources of hereditary variation, which are used in selection practice are hybridization and mutagenesis. That is why, together with using the recombinant sources of genetic variabilities it is expedient and perspective to find and to work through the effective methods of genetic variabilities expansion by means of technologies in vitro using different selective agents. Being based on the induced or spontaneous somaclonal variability, it is possible to choose in the cell and fiber culture and in selection conditions some genotypes resistant to unfavorable factors of the environment including the ones resistant to the agents of the diseases. A number of researchers deal with the problem of cell-less selection agents (culture filtrates, toxins). Such approach was used when using the potato plants resistant to *Phytophthora infestans* (Behnke, 1980), *Rhizoctonia solani* (Kalashnikova, 2003), *Fusarium oxysporum* (Bolic, Foroughi-Wehr & Kohler, 1986).

The task of the given research is to work through the criteria of receiving the regenerants on the selective medium and to estimate the selected lines on the level of test-tube plants and in the field conditions.

Material and methods

The potato varieties of the Institute of Potato Growing selection with different resistance to *Fusarium oxysporum, Fusarium sambucinum:* Gurman (enduring) and Tyras (sensitive) were used in the research.

Murashige-Scoog (MC) (Murashige & Scoog, 1962) with different concentrations of hormones and aminoacids served as the growing medium. The resistance on the level of regenerating callus was determined by calculating the amount of regenerants, which have

survived on the selective medium as well as by the amount of callus offsets, which were formed. There were 250 new callus in the experimental samples and 100 ones – in a control one. Later only the amount of established regenerants was taken into account.
The resistance analysis on the plant level was made in laboratory conditions by spraying conidium suspension. The plant resistance to diseases as well as qualitative and quantitative indices were determined in field conditions by using the methods of selection.

Results

As follows from the results of the research, on the fiber level somaclonal lines resistant to phytotoxic metabolites (FTM) *Fusarium oxysporum, Fusarium sambucinum* have been received. But it should be mentioned that not all lines were able to regenerate. Throughout the duration of selection process some of them lost the morphogen competence. From literary sources no cases of receiving regenerants in all cell- and callus clones have been found. From among the resistant callus lines of Gurman variety only 36 % were able to regenerate, and of Tyras variety – only 2.8 %. The problem of receiving regenerants from resistant cell- and callus lines is one of the most important and difficult in cell selection. The offsets regeneration from such cells is very complicated and the frequency of plants formation is low.
While developing selective medium, it was supposed that cell screening with a higher resistance to phytotoxic metabolites of fungus *Fusarium oxysporum, Fusarium sambucinum* takes place in a more hostile selective environment. Cell proliferation resulted in callus formation and later in regenerants formation, which were characterised by a higher resistance. The results of the experiments on plants regeneration conducted in selective conditions showed that higher concentration of toxic metabolites in the environment lowered the amount of the received regenerants (Table 1).

Table 1. Yield of plants-regenerants from suspensive culture of potato on a selective medium with different concentrations of FTM

Variant	Planted explants	Received morphogen callus lines	Received plants-regenerants
		Gurman variety	
MC (control)	100	64	136
MC+ FTM, μm			
10	200	68	139
20	200	62	119
30	200	54	106
40	200	49	78
50	200	43	61
60	200	38	41
70	200	36	37
		Tyras variety	
MC (control)	100	58	102
MC+ FTM, μm			
10	200	43	63
20	200	27	43
30	200	12	18
40	200	6	11
50	200	3	3
60	200	2	2
70	200	2	2

Thus, for Gurman variety on a control medium (without any phytotoxic metabolites) 136 regenerants were received, and on the medium which contained 10 μm FTM–139, 20–119, 30–106, 40–78, 50–61, and 60–41; on the medium with the highest concentration of 70 μm – only 37 plants were received. The same consistent pattern was true of Tyras variety.
The plants-regenerants, which were received on the selective medium, were cloned by a pot-culture method and were estimated on their resistance to *Fusarium oxysporum, Fusarium sambucinum.* By the method of artificial innoculation, 238 lines of self-clones and 825 lines which were received by the method of cell selection, were estimated. It should be mentioned that among the plants of each variety, which were received on a control medium (without FTM) and on selective ones, some lines with higher as well as with lower resistance were formed. It is necessary to admit that a cosiderable part of persistent forms had morphological changes as well. Perhaps it can be explained by a somaclonal variability of cells under cultivation conditions in vitro. The frequency of lines formation which are different on their resistance within each of the studied genetypes testifies to the fact that more persistent (on the level of plants-regenerants) lines are more frequently seen in descendants of the initially more resistant genotypes. Thus, among the regenerants of Gurman variety, the plants with a higher resistance level amounted to 43 %, as compared to the control. In Tyras variety the lines with a higher resistance did not exceed 12 % from the total amount of the received plants. On the average, from all the material, which was received by the method of somaclonal variability, 28,4 % of lines had resistance on the control level, 22,4 % of lines had resistance by 1–2 points more than a control one, which was 4–7 points depending on the variety. But there were no absolutely immune forms of potato as to fusarial wilt.
The application of phytotoxic metabolites of fungus *Fusarium oxysporum, Fusarium sambucinum* into selective medium resulted not only in higher resistance in plants-regenerants by 2-4 points, but also in the increase in the number of lines as compared to control. Thus, 38,7 % of the received lines had a higher resistance point as compared to an initial variety, and 41,4 % of plants had resistance rate on the control level.
The lines, which were characterized by a higher resistance, were further tested in field conditions on the atificial infection background with the purpose of studying the issue as to keeping the resistance level, which was achieved in the culture in vitro, in field conditions during 2014–2016.

Potato tubers were planted on the plot, which had already been infected with fusarial wilt. The infectious preparation (4g) of fusarium, which was received by pure culture propagation of *Fusarium oxysporum, Fusarium sambucinum* on the potato-oat medium, was applied into each planting hole.

The results (Table 2) testified that all the lines under research were infected by fusariosis to one degree or another.

Table 2. Variability of regenerants lines on economically valuable characteristics in field reproductions (2014–2016)

Variety	Line number	Yield, g/t	Starch content, %	Resistance point		
				2014	2015	2016
Gurman		1032	15.4	5	4.3	3.8
	3	1307	15.0	4.3	3.9	3.0
	4	1082	15.8	5.7	5.1	4.5
	5	664	15.2	6.3	6.0	5.6
	7	1305	15.7	5.7	5.4	5.0
	8	1140	15.9	7.7	6.8	4.9
	9	915	15.1	6.3	6.0	4.8
	12	1533	16.3	8.0	7.8	7.8
	16	970	15.2	6.6	5.0	3.9
	17	1087	15.5	5.2	5.0	4.5
	27	1234	15.7	6.7	6.1	4.8
	28	1670	16.6	7.8	6.5	6.0
	29	944	15.3	4.7	4.0	4.0
	34	1422	15.5	5.0	4.0	3.2
Tyras		995	12.2	2.8	1.7	0.9
	8	1360	12.2	4.1	3.6	2.9
	14	854	12.8	4.6	3.9	3.7

It is necessary to mention some increase in disease incidence during the years of research: in 2014 the amount of infected plants was 2.1–26.7 %, in 2016 it was 7.7–44 %. Visual estimation of tubers as to the degree of fusariosis infection has manifested a high resistance point (5–8), whereas control samples were more infected (resistance point was 1–5). As follows from the results of the research, we succeeded in separating the lines, which during three-year period were characterized by a higher resistance point as compared to the original varieties Gurman and Tyras. The received lines were estimated on their yield capacity and starch content. The lines deviations on economically valuable indices either increased or decreased as to characteristic value. Herewith, the deviation upward is 25–75 % depending on characteristic. The combination of maximal characteristic values was found in lines 12 and 28 of Gurman variety. The received lines were given to the selection breeding plot for further testing.

Conclusions

The variability among the potato sorts on their capability as to morphogenesis in vitro (callus – and regeneration characteristics) on the medium with different amount of phytotoxic metabolites *Fusarium oxysporum, Fusarium sambucinum* has been analyzed. As follows from the results of cell selection, the potato regenerants, which are resistant to selective agents in vitro and directly to the agents of fusarial wilt under condition of infectious background, have been received. We planned to estimate on genetic level callus cells (RAPD method), which are cultivated in selective conditions, as well as plants-regenerants which have been received as a result of cell selection and differ in higher resistance to *Fusarium oxysporum* and *Fusarium sambucinum.*

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