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Contents

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SCREENING OF POTATO VARIETIES FOR MULTIPLE RESISTANCE TO *SYNCHYTRIUM ENDOBIOTICUM* IN THE WESTERN REGION OF UKRAINE

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Aim. To evaluate potato breeding material for resistance to pathotypes of *Synchytrium endobioticum* (Schilbersky) Percival (1909) known to be present in Ukraine (pathotypes 1(D_1), 11, 13, 18 and 22); to identify resistant registered and potential varieties for the usage in the national wart disease eradication programs and to recommend these selected (potential and registered) potato varieties for the breeding program targeted on the development of multiple resistance against pathotypes of *S. endobioticum* present in Ukraine. **Methods**. Evaluation of the potato breeding material and registered potato varieties for the resistance against common pathotype 1 (D_1) and four aggressive pathotypes of *S. endobioticum* (pathotypes 11, 13, 18 and 22) in climatic chamber and greenhouse tests of Ukrainian Scientific Research Plant Quarantine Station of Institute of Plant Protection NAAS (Boyany, Ukraine) following the Spieckermann and Glynne-Lemmerzahl methods (EPPO Standard PM7/28(2)). Field trials on naturally infected soils were conducted according to standard methods adapted to national requirements in the area of Chernivtsi, Zakarpattia and Ivano-Frankivsk regions. **Results.** 3,736 samples of potato breeding material from six breeding institutions of Ukraine were tested for resistance against *S. endobioticum* during 2011–2017 in the western region of the country. Among all samples tested, 3,389 were identified as resistant to the widely spread pathotype 1 in the preliminary climatic chamber and greenhouse tests, and 130 of them proved to be resistant under field conditions. Five out of 41 Ukrainian registered potato varieties (Bazys, Hlazurna, Solokha, Bozhedar and Santarka) were found to be resistant to all 5 pathotypes tested (1 (D_1), 11, 13, 18 and 22). **Conclusions**. The 130 samples of potato breeding material (which were found to be resistant against the common pathotype 1 of *S. endobioticum* in the laboratory, greenhouse as well as in the field trials) were recommended for the state variety registration and further usage in an eradication program to localize potato wart outbreaks of the western part of Ukraine. The screening tests revealed that the national breeding program targeted on resistance against *S. endobioticum* pathotype 11 was the most effective (49 % of samples tested proved to be resistant against this pathotype), whereas it was the least effective against pathotype 18, namely only 30 % of samples resistant. It was speculated that such a dissimilarity may be related to the differences in the genetic material used in the breeding process at various institutions, and which may be the subject of further analysis in order to improve the results of breeding programs. The already registered potato varieties Bazys, Hlazurna, Solokha, Bozhedar and Santarka which were found to have a multiple resistance to common pathotype 1 and four local aggressive pathotypes of *S. endobioticum* (11, 13, 18 and 22) were recommended for use in the breeding process as sources of resistance and also for the eradication programs in the western region of Ukraine, where *S. endobioticum* is mostly distributed (2409 hectares or 98 %).

Keywords: potato, wart disease, pathotypes, screening, resistance, breeding.

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INTRODUCTION

Potato is one of the most valuable and important agricultural crops, and ranks fourth in the world after corn,

wheat and rice in total volume [1]. In 2017, the total volume of harvested potato in Ukraine was 22 million tons and its cultivated area ca. 1.3 million ha [2]. These latter figures demonstrating the relevance of potato in national food security.

The potato plant is a host for a noxious obligate pathogen, namely the zoosporic chytrid fungus *Synchytrium endobioticum* (Schilbersky) Percival 1909, causing the so-called potato wart disease, which is subject to quarantine regulations in many countries of the world [3–6]. According to the data of the European and Mediterranean Plant Protection Organization (EPPO), as of January 2018 *S. endobioticum* is commonly found in 34 countries, including the Falkland Islands (Great Britain) and the Faroe Islands (Denmark) [7].

The quarantine status of the agent is conditioned by its ability to decrease the yield of the infected plants, even up to 100 %, which is especially notable for potato cultivation in small holdings [8]. This may be explained both by a high damaging ability of the agent but also by its abilities of adapting to unfavorable environmental conditions, including the formation of new and more aggressive pathotypes [6] and the extremely long survival, more than 46 years, of its thick-walled resting spores (winter sporangia) in the soil [9].

Long-term observations of spreading and damaging abilities of *S. endobioticum* in the territory of the European part of the continent demonstrated that most frequently the sources of aggressive pathotypes of the agent were manifested in the valleys of mountainous regions, located at the height of more than 400 m above sea level in the zone of continental climate, where in winter the ground freezes at least for several weeks, and the amount of precipitation within the vegetation period exceeds 600 mm [6]. It is also known that long-term cultivation of resistant varieties of potato, especially in case of a one-crop system, precedes the appearance of aggressive pathotypes of *S. endobioticum* [6, 8].

The above-mentioned conditions for development of new pathotypes of *S. endobioticum* prevail unfortunately in the mountainous areas of the western regions of Ukraine, where distribution of *S. endobioticum* has been registered since 1961. Whithin this part of the country five pathotypes have been observed till now: common pathotype 1(D_1), and four aggressive ones, namely pathotype 11 (the village of Maydan, Mizhhirya district, Lviv region), 13 (the town of Rakhiv, Zakarpattia region), 18 (the village of Yasinia, Rakhiv district, Zakarpattia region) and 22 (Bystrets, Verkhovyna district, Ivano-Frankivsk region) [8, 10] on a total area of 2409 hectares, which constitutes 98% of *S. endobioticum* distribution area in Ukraine [11]. So far the breeding of potato wart-resistant varieties is the only economically viable and efficient means of controlling this quarantine organism [12].

There are current data on global spreading of at least 39 pathotypes of *S. endobioticum,* although most breeding programs aim only at the most wide-spread ones namely pathotypes 1(D_1), 2, 6 and 18 [10]. Only several potato varieties with multiple resistance exists to date, but they were not widely introduced [13–15]. Evidently, a valuable acquisition of new potato varieties should be the combination of the feature of (multiple) resistance to (local) pathotypes of *S. endobioticum* and high indices of economically viable characteristics.

The aims of the study were 1) to evaluate potato breeding material for resistance to pathotypes of *Synchytrium endobioticum* (Schilbersky) Percival known to be present in Ukraine (pathotypes 1, 11, 13, 18 and 22); 2) to select potential new varieties on the basis of the results and 3) to identify multiple resistance in registered varieties for their use in national wart disease eradication programs and in national breeding programs.

MATERIALS AND METHODS

In 2011–2017, 3,736 potato samples of potato breeding material from six breeding institutions and 41 registered potato varieties from three breeding institutions in Ukraine were used in the study.

The evaluation of the potato breeding material and registered potato varieties for resistance to common and aggressive pathotypes of *S. endobioticum* was conducted under climatic chamber, greenhouse and field conditions using the following methods.

The method of infecting potato tubers with winter zoospores which were in a dormant state. The contamination of potato samples with zoospores from germinated winter zoosporangia of potato wart was conducted by the modified method of Spieckermann and Kothoff (1924) [2, 13] under greenhouse conditions. The soil samples, collected from infested fields, were each mixed with perlite in 1 : 1 ratio to obtain an average level of 40–50 winter zoosporangia per 1 g of soil. The mixture was placed into plastic containers (30 × 40 cm), and the potato varieties under investigation were planted therein. The experiment was performed in three repeats; potato varieties Poliska rozheva and Lorkh, susceptible to all presently known local

pathotypes of *S. endobioticum*, were used as a control. The containers were kept at 17–18 °C, and 70–80 % relative humidity (RH) in a 12/12 day/night regime; watered every three days, loosened once a week and the reaction of potato tubers present in the samples to infecting with potato wart was determined after 75 days (Fig. 1). For this reason, the plants were dug out of containers and the warts on all tubers of each experimental sample and control varieties of potato were counted. The results were deemed reliable if the not less than 75 % of control variety plants showed disease symptoms.

The method of infecting potato tuber sprouts with summer zoospores from freshly formed warts. The resistance of plants to summer zoospores of the pathogen, obtained from freshly formed warts, was evaluated by the method of Glynne-Lemmerzahl [2, 10] adapted as following [13]: a paper ring was fixed around the sprout part of a potato tuber using a warmed-up mixture of paraffine and vaseline (1 : 1) for this purpose. Distilled water was poured into the ring with the addition of 0.5 cc of the recent wart, containing summer zoospores of *S. endobioticum* (Fig. 2). The samples were incubated in the climatic chamber at 11 °C without any illumination to stimulate the infection process. Paper rings were removed from potato tubers 24 h later and the incubation was continued in the climatic chamber at 17–18 °C for 20 days in the darkness. After this period, the response of potato samples to being infected with the pathogen was determined (Fig. 3). Potato sprouts were analyzed under a microscope BioLight 300 (DELTA optical, Poland) to determine the degree of damage according to the following scale, adapted after [13, 16]:

1 point – necrotic tissue, rare sori (up to 5);

2 points – scattered sori (if exceeding 5);

3 points – dense sori without the deformation of a potato sprout;

4 points – dense sori with the deformation of a potato sprout;

5 points – deformation of a sprout, a wart.

The total score (M) of the potato variety damage was determined using the formula:

$$M = [1a+2b+3c+4d+5e]/n,$$

where a, b, c, d, e – number of tubers which received the relevant points for the damage; 1, 2, 3, 4, 5 – points for the damage; n – number of infected potato tubers of the experimental sample.

Fig. 1. Testing breeding material of potato under greenhouse conditions, using winter zoospores of *Synchytrium endobioticum*

Fig. 2. Infecting potato samples using summer zoospores of *Synchytrium endobioticum*

Fig. 3. Symptoms of potato wart after artificial infection of potato variety Poliska rozheva with summer zoospores of *Synchytrium endobioticum*

Fig. 4. Symptoms of wart disease after artificial infection of potato variety Poliska rozheva with winter zoospores of *Synchytrium endobioticum*

In case of determining the total score of the damage to be 1, 2 or 3, the experimental sample was considered to be resistant to *S. endobioticum* (R – resistant); points 4 or 5 – susceptible (S – susceptible).

Studying the resistance of potato breeding material to potato wart under field conditions. The evaluation and screening of breeding potato material and registered Ukrainian potato varieties under field conditions were conducted in natural infected soil in the areas of pathogen spreading in the western region of Ukraine: to common pathotype 1 (D_1) of *S. endobioticum*— in the village of Berehomet, Vyzhnytsia district, Chernivtsi region; to aggressive pathotypes – in the village of Maydan, Mizhhirya district, Lviv region (pathotype 11), in the town of Rakhiv (pathotype 13), in the village of Yasinia (pathotype 18), Rakhiv district, Zakarpattia region, and in the village of Bystrets (pathotype 22), Verkhovyna district, Ivano-Frankivsk region. The experiment was performed in three repeats; potato variety Poliska rozheva, susceptible to all the local pathotypes of *S. endobioticum*, was used as a control.

RESULTS AND DISCUSSION

Tests performed under climate chamber and greenhouse conditions in the years 2011–2017, aimed at evaluating the resistance of 3,736 potato samples to *S. endobioticum*, determined 3,389 samples to be resistant and 347 to be susceptible (Table 1, Fig. 4) to the pathogen (susceptible samples were excluded from further studies). Subsequently 130 resistant potential variety samples were admitted to the national field test program on the basis of resistance performance and their economically viable properties.

Screening 130 selected potential varieties from the tested breeding potato material, resistant to potato wart, under field conditions in the national test program. The resistance found under greenhouse and climatic chamber conditions of 130 potential potato varieties to common pathotype 1 of potato wart was confirmed by the field tests in the national test program which were conducted from 2011 to 2017 (Table 2).

Only three out of the 130 potential varieties under investigation (all three bred by the Institute for Potato Research, NAAS) showed resistance to all the local aggressive pathotypes (samples 08.40.14, 208ч.10 and F.15).

The remaining investigated samples differed in their response to the aggressive pathotypes of the pathogen, in particular, 64 samples were noted for their resistance to aggressive pathotype 11 (Mizhhirya); 59 samples –

Table 1. The results of the preliminary testing under greenhouse conditions of breeding potato material for resistance to common pathotype 1 of *Synchytrium endobioticum* (2011–2017)

Institution name	Number of potato samples		
	Total	Resistant	Susceptible
Institute for Potato Research, NAAS of Ukraine	1546	1368	178
Institute of Agriculture of the Carpathian Region, NAAS of Ukraine	205	191	14
Institute of Agriculture of Polissia, NAAS of Ukraine	274	262	12
Mountainous Scientific Division of the Institute of Agriculture of the Carpathian Region, NAAS of Ukraine	110	89	21
PJSC SPA "Chernihivelitkartoplia"	633	589	44
Polissia Experimental Department IP NAAS	968	890	78
Total	3736	3389	347

to pathotypes 13 and 22 (Rakhiv and Bystrets respectively); 39 samples – to pathotype 18 (Yasinia) (Table 2; Fig. 5–6).

In general, most positive results were demonstrated by the breeding program, aimed at obtaining potato varieties, resistant to aggressive pathotype 11 (49 % of investigated varieties demonstrated their resistance to this pathotype) and the most efficient – to pathotype 18 (30 % of resistant investigated varieties).

The results obtained were given to the institutions, wherefrom the breeding material had originated, with the indication of resistance characteristics to *S. endobioticum* and recommendations of their registration and stimulation of their further introduction in the spreading areas of *S. endobioticum* in Ukraine

Forty-one already registered Ukrainian potato varieties, selected by their economically viable properties, were also additionally studied for their resistance to common and local aggressive pathotypes of *S. endobioticum*, present in Ukraine.

According to our studies, all investigated varieties were resistant to common pathotype 1 (Table 3), whereas the control varieties Poliska rozheva and Lorkh were susceptible and showed clear disease symptoms.

24 varieties out of the 41 studied were also found to be resistant to aggressive pathotype 11 (Mizhhirya); 13 – to pathotype 13 (Rakhiv); 9 – to pathotype 18 (Yasinia) and 16 varieties to pathotype 22 (Bystrets).

Eight varieties (Oberih, Kimmeria, Chervona ruta, Fantasia, Poliske dzherelo, Vodohray, Obrii, Dobrochyn) were resistant to two aggressive pathotypes, six varieties were resistant to three aggressive pathotypes

Fig. 5. Potato variety Poliska rozheva, infected by common pathotype 1 of *Synchytrium endobioticum* (under field conditions, in the national test program) Berehomet, Vyzhnytsia district, Chernivtsi region)

Fig. 6. Potato breeding sample П08.86-11, infected by aggressive pathotype 18 of *Synchytrium endobioticum* (Yasinia) (under field conditions, in the national test program – the village of Yasinia, Rakhiv district, Zakarpattia region)

Table 2. The results of the state test of potato for resistance to local pathotypes of *Synchytrium endobioticum* (2011–2017)

Institution name	Total number of samples	Number (percentage) of samples, resistant to a specific pathotype of *Synchytrium endobioticum*				
		1 (D1) (comm.)	11 (Mizhhirya)	13 (Rakhiv)	18 (Yasinia)	22 (Bystrets)
Institute for Potato Research, NAAS of Ukraine	59	59 (100 %)	35 (59 %)	23 (39 %)	16 (27 %)	31 (53 %)
Institute of Agriculture of the Carpathian Region, NAAS of Ukraine	12	12 (100 %)	3 (25 %)	2 (17 %)	3 (25 %)	4 (33 %)
Mountainous Scientific Division of the Institute of Agriculture of the Carpathian Region, NAAS of Ukraine	17	17 (100 %)	6 (35 %)	3 (18 %)	4 (24 %)	5 (29 %)
PJSC SPA Chernihivelitkartoplia	8	8 (100 %)	4 (50 %)	3 (38 %)	4 (50 %)	3 (38 %)
Polissia Experimental Department IP NAAS	34	34 (100 %)	17 (50 %)	27 (79 %)	12 (35 %)	16 (47 %)
Total	130	130 (100 %)	64 (49 %)	59 (45%)	39 (30 %)	59 (45 %)

Table 3. The (degree of) response of existing, registered Ukrainian potato varieties, to infection with local pathotypes of *S. endobioticum* in the western region of Ukraine

No.	Variety name	Resistance/Susceptibility (the degree of response to infection)				
		Synchytrium endobioticum pathotypes				
		1 (D1) common	11 (Mizhhirya)	13 (Rakhiv)	18 (Yasinia)	22 (Bystrets)
Institute for Potato Research, NAAS of Ukraine						
1.	Bazys	R (1,0)*	R (1,6)	R (1,4)	R (2,0)	R (2,0)
2.	Hlazurna	R (1,4)	R (1,4)	R (1,4)	R (2,8)	R (2,0)
3.	Solokha	R (1,0)	R (1,4)	R (1,6)	R (2,0)	R (2,8)
4.	Kalynivska	R (1,6)	S (4,6)	R (2,0)	R (2,8)	R (2,0)
5.	Vernisazh	R (1,2)	R (1,6)	S (4,4)	R (1,4)	R (2,8)
6.	Khortytsia	R (1,4)	R (1,6)	R (1,2)	R (1,2)	S (4,4)
7.	Oberih	R (1,0)	R (1,2)	R (1,6)	S (4,6)	S (4,2)
8.	Kimmeria	R (1,2)	R (1,4)	R (1,4)	S (4,8)	S (4,6)
9.	Chervona ruta	R (1,4)	R (1,2)	R (1,6)	S (4,8)	S (4,4)
10.	Fantasia	R (1,2)	R (2,0)	S (4,6)	S (4,6)	R (2,8)
11.	Poliske dzherelo	R (2,8)	S (4,6)	R (2,0)	S (4,0)	R (2,0)
12.	Vodohrai	R (2,8)	R (2,8)	S (4,6)	S (4,8)	R (2,8)
13.	Obrii	R (1,6)	R (1,4)	S (4,4)	S (4,8)	R (2,0)
14.	Levada	R (1,6)	R (1,4)	S (4,4)	S (4,6)	S (4,4)
15.	Slovianka	R (2,8)	R (2,8)	S (4,6)	S (4,8)	S (4,6)
16.	Yavir	R (2,0)	S (4,4)	S (4,8)	S (4,8)	S (4,4)
17.	Lileia	R (2,8)	S (4,8)	S (4,6)	S (4,6)	S (4,8)
18.	Melodiia	R (2,0)	S (4,8)	S (4,6)	S (4,6)	S (4,4)
19.	Serpanok	R (2,8)	S (4,6)	S (4,6)	S (4,8)	S (4,4)
20.	Skarbnytsia	R (2,0)	S (4,6)	S (4,8)	S (4,6)	S (4,6)
21.	Zelenyi Hai	R (2,8)	S (4,8)	S (4,4)	S (4,8)	S (4,6)
Institute of Agriculture of the Carpathian Region, NAAS of Ukraine						
22.	Dyvo	R (1,8)	R (2,8)	R (2,8)	S (4,8)	R (2,0)
23.	Lehenda	R (2,0)	R (2,0)	S (4,6)	S(4,6)	S (4,4)
24.	Mukachivska	R (2,8)	S (4,6)	S (4,6)	S (4,8)	S (4,6)
25.	Oksamyt-99	R (2,0)	S (4,8)	S (4,8)	S (4,6)	S (4,8)
26.	Pikurovska	R (2,8)	S (4,6)	S (4,6)	S (4,8)	S (4,6)
27.	Uzhhorodska	R (1,8)	S (4,8)	S (4,6)	S (4,8)	S (4,4)
28.	Vira	R (2,8)	S (4,4)	S (4,6)	S (4,4)	S (4,6)
Polissia Experimental Department of the Institute for Potato Research, NAAS of Ukraine						
29.	Bozhedar	R (1,0)	R (1,8)	R (1,8)	R (2,0)	R (2,0)
30.	Santarka	R (1,8)	R (1,6)	R (2,8)	R (2,8)	R (2,0)
31.	Malynska bila	R (1,6)	R (2,8)	S (4,8)	R (2,8)	R (2,0)
32.	Partner	R (1,8)	R(2,0)	R (3,0)	S (4,6)	R (2,8)
33.	Dobrochyn	R (2,0)	R (2,8)	S (4,6)	S (4,8)	R (2,0)
34.	Poliska yuvileina	R (2,8)	R (2,8)	S (4,8)	S (4,8)	S (4,6)

End of Table 3

No.	Variety name	Resistance/Susceptibility (the degree of response to infection)				
		Synchytrium endobioticum pathotypes				
		1 (D1) common	11 (Mizhhirya)	13 (Rakhiv)	18 (Yasinia)	22 (Bystrets)
35.	Zaviia	R (2,0)	S (4,6)	S (4,6)	S (4,8)	R (2,8)
36.	Tyras	R (2,8)	R (2,8)	S (4,8)	S (4,6)	S (4,6)
37.	Zheran	R (2,8)	R (2,8)	S (4,6)	S (4,8)	S (4,6)
38.	Zvizdal	R (2,0)	R (3,0)	S (4,6)	S (4,8)	S (4,8)
39.	Dorohyn	R (2,8)	S (4,6)	S (4,8)	S (4,6)	S (4,6)
40.	Karlik	R (2,8)	S (4,6)	S (4,6)	S (4,8)	S (4,6)
41.	Teteriv	R (3,0)	S (5,0)	S (5,0)	S (5,0)	S (4,8)
Control	Poliska rozheva	S (4,8)	S (5,0)	S (5,0)	S (5,0)	S (4,8)
Control	Lorkh	S (4,8)	S (5,0)	S (5,0)	S (5,0)	S (4,8)

Note. "R" – resistant to *S. endobioticum* pathotypes; "S" – susceptible *S. endobioticum* pathotypes. * The degree of response to infection – the degree of resistance (between 1 and 3) or the degree of susceptibility (between 4 and 5).

(Kalynivska Vernisazh, Khortysia, Dyvo, Malynska bila, Partner) and five – to all four aggressive pathotypes (Bazys, Hlazurna, and Solokha, Bozhedar and Santarka).

Multiple resistance to common and all four aggressive pathotypes was noted for three potato varieties, bred by the Institute for Potato Research, NAAS (Bazys, Hlazurna, and Solokha) and two – bred by the Polissia Experimental Department of the Institute for Potato Research, NAAS (Bozhedar and Santarka). These varieties are recommended for the breeding process as resistance sources, to be included in seed multiplication programs and to be introduced in the infested areas in the western region of Ukraine.

CONCLUSIONS

In 2011–2017, the preliminary climatic chamber and greenhouse tests showed 3,389 potato varieties to be resistant to the common pathotype 1 (D_1) of the potato wart pathogen *Sync*hytrium *endobioticum*. The national testing program under field conditions confirmed the resistance of 130 potential potato varieties, selected from the above mentioned resistant breeding material, to common pathotype 1 of *S. endobioticum*: the list of these potential varieties was given to the institutions, wherefrom the breeding material had originated, with the indication of resistance characteristics to *S. endobioticum* and recommendations of their registration and further introduction in the spreading areas of potato wart disease in Ukraine. In 2011–2017 the national breeding program targeted on resistance against *S. endobioticum* was the most effective against pathotype 11 (49 % of samples tested resistant), whereas it was the least effective against pathotype 18 (30% resistant). It was speculated that such a dissimilarity may be related to the differences in the genetic material used in the breeding process at various institutions, and which may be the subject of further analysis in order to improve the results of breeding programs. Testing for resistance of potential varieties, selected from the breeding material to common pathotype 1 and local (aggressive) pathotypes of potato wart, demonstrated that 64 samples were resistant to pathotype 11 (Mizhhirya); 59 samp-les – resistant to pathotype 13 (Rakhiv); 39 – resistant to pathotype 18 (Yasinia), and 59 samples – resistant to pathotype 22 (Bystrets). These samples were recommended for use in breeding programs and to be registered and cultivated in the potato wart infested areas in the western region of Ukraine. Noteworthy are three existing, registered Ukrainian potato varieties, bred by the Institute for Potato Research, NAAS (Bazys, Hlazurna and Solokha) and two varieties, bred by the Polissia Experimental Department of IP NAAS (Bozhedar and Santarka), which have multiple resistance both to common pathotype 1(D_1) and the four local aggressive pathotypes (11, 13,18 and 22) of *S. endobioticum*. These varieties are recommended for the breeding process as resistance sources, to be included in seed multiplication programs and to be introduced in the infested areas in the western region of Ukraine.

Відбір сортів картоплі з комплексною стійкістю до раку *Synchytrium endobioticum* (Schilbersky) Percival у західному регіоні України

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Мета. Оцінити селекційний матеріал картоплі на стійкість до патотипів рака картоплі *Synchytrium endobioticum* (Schilbersky) Percival, поширених в Україні (1(D_1), 11, 13, 18 та 22), та виділити стійкі сорти для впровадження у вогнищах хвороби і використання у селекційному процесі в якості джерел стійкості до збудника. **Методи.** Оцінку селекційного матеріалу на стійкість до звичайного 1(D_1) і агресивних патотипів збудника раку картоплі (11, 13, 18 та 22) проводили за методами Spieckermann та Glynne-Lemmerzahl (EPPO Standard PM7/28(2)) та польових умовах на природному інфекційному фоні у Чернівецькій, Закарпатській та Івано-Франківській області за загальноприйнятими методиками, адаптованими на національних потреб. **Результати**. Із тестованих впродовж 2011–2017 рр. у західному регіоні України 3736 зразків картоплі, отриманих від шести науково-дослідних та селекційних установ України, виділено 3389 зразків картоплі, стійких до звичайного патотипу 1(D_1) збудника раку *S. endobioticum* у попередньому випробуванні, та 130 – у державному польовому. Оцінено 41 сорт картоплі і виділено 5 сортів картоплі з комплексною стійкістю до 5 патотипів збудника (1(D_1), 11, 13, 18 та 22) (Базис, Глазурна, Солоха, Божедар і Сантарка). **Висновки.** Виділені за підсумками всіх випробувань стійкі проти звичайного патотипу збудника раку сортозразки картоплі рекомендовані до державної реєстрації із зазначенням характеристики стійкості проти збудника та подальшого районування у вогнищах хвороби в Україні. Відзначено, що впродовж 2011–2017 рр. найбільш результативною в Україні виявилась селекційна програма спрямована на одержання сортозразків картоплі стійких до ураження агресивним патотипом *S. endobioticum* 11 (49 % стійких зразків серед усіх тестованих) і найбільш складною – до патотипу 18 (30 %). Причиною такого стану речей може бути відмінність генетичного матеріалу установ-оригінаторів, залученого до селекційного процесу, що може бути предметом подальшого аналізу з метою виявлення перспективних джерел стійкості та корегування селекційних програм. Сорти картоплі Базис, Глазурна, Солоха, Божедар і Сантарка із комплексною стійкістю до звичайного патотипу 1(D_1) та чотирьох місцевих агресивних патотипів *S. endobioticum* (патотипи 11, 13, 18 та 22) рекомендовані для залучення в селекційний процес в якості джерел стійкості та впровадження у вогнищах раку картоплі у західному регіоні України.

Ключові слова: картопля, рак, патотипи, тестування, стійкість, селекція.

Отбор сортов картофеля с комплексной устойчивостью к раку *Synchytrium endobioticum* (Schilbersky) Percival в западном регионе Украины

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Цель. Оценить селекционный материал картофеля на устойчивость к патотипам рака картофеля *Synchytrium endobioticum* Schilbersky Perc., распространенных в Украине (1, 11, 13, 18 и 22), и выделить устойчивые сорта для внедрения в очагах болезни и использования в селекционном процессе в качестве источников устойчивости к возбудителю. **Методы.** Оценку селекционного материала и зарегистрированных сортов картофеля на устойчивость к обычному 1 (D_1) и агрессивным (11, 13, 18, 22) патотипам рака картофеля проводили по методу Spieckermann и Glynne-Lemmerzahl (EPPO Standard PM7/28(2)) и в полевых условиях на природном инфекционном фоне в Черновицкой, Закарпатской и Ивано-Франковской областях по общепринятым методам, адаптированных к национальным условиям. **Результаты.** Из тестированных на протяжении 2011–2017 гг. в западном регионе Украины 3736 образцов картофеля, полученных из шести научно-исследовательских и селекционных учреждений Украины, выделено 3389 образцов картофеля, устойчивых к обычному патотипу 1 (D_1) рака картофеля *S. endobioticum* в предварительном испытании и 130 – в государственном. Оценено 41 сорт картофеля и выделено 5 с комплексной устойчивостью ко всем местным патотипам возбудителя 1 (D_1), 11, 13, 18 и 22 (Базис, Глазурна, Солоха, Божедар и Сантарка). **Выводы.** Выделенные по результатам всех испытаний устойчивые против обычного патотипа 1(D_1) возбудителя рака картофеля сортообразцы картофеля рекомендованы для государственной регистрации и дальнейшего районирования в очагах болезни в Украине. Отмечено, что на протяжении 2011–2017 гг. наиболее результативной в Украине была селекционная программа, направленная на получение сортообразцов картофеля устойчивых к

заражению агрессивным патотипом *S. endobioticum* 11 (49 % устойчивых образцов среди всех испытуемых) и наиболее сложной – к патотипу 18 (30 %). Причиной такого положения дел могут быть отличия в генетическом материале, использованном в селекционном процессе различных учреждений, что может быть предметом дальнейшего анализа с целью выявления перспективных источников устойчивости и коррекции селекционных программ. Сорта картофеля Базис, Глазурна, Солоха, Божедар и Сантарка с комплексной устойчивостью к обычному патотипу 1(D_1) и четырем местным агрессивным патотипам *S. endobioticum* (11, 13, 18 та 22) рекомендованы для использования в селекционном процессе в качестве источников устойчивости и внедрения в очагах рака картофеля в западном регионе Украины.

Ключевые слова: картофель, рак, патотипы, оценка, устойчивость, селекция.

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CHARACTERIZATION OF AMINO ACID CONTENT OF GRAIN OF NEW WHEAT VARIETIES AND LINES

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Aim. To determine the formation of bound amino acids in grain of new wheat varieties and its biological value. **Methods.** Field, physical-chemical, computational, analysis. **Results.** The differences in amino acid composition of new varieties and lines of wheat were analyzed. It was established that the highest content of essential amino acids was in the grain of the Kulundynka variety (5.18 %) or 2.5 times higher compared to the standard (2.99 %). Their content in the grain of soft wheat, obtained by the hybridization of *Triticum aestivum* L./*Triticum spelta* L., was 1.4–1.5 times higher compared to the control. The grain of the soft variety Kulundynka had the highest biological value as the score of essential amino acids was not deficient and the remaining varieties were deficient in 2–5 amino acids. Only methionine was deficient in the grain of soft wheat lines (AAS = 64–74 %). **Conclusions.** The content of amino acids in soft wheat grain depends considerably on weather conditions, selective-genetic origin of the variety and the line. Glutamic acid, proline, and leucine were found to be most abundant. Out of nine samples of soft wheat tested, only the seed of the Kulundynka variety had a non-deficient amino acid score (91–298 %), and in the Pannonikus variety methionine was limited (49 %). The best balanced content of amino acids is present in the grain of non-spelt lines, obtained by hybridization of *Triticum aestivum* L. and *Triticum spelta* L., namely P 7 and LPP 1314. The grain of these lines has a non-deficient amino acid score, more methionine (AAS = 64–74 %), and supplies human daily requirement in the best way. The grain has a high index of complex estimation and metabolization coefficient for essential amino acids.

Keywords: amino acids, grain, soft wheat, variety.

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INTRODUCTION

According to the data of FAO experts, the developed countries with about 20 % of the world population supply about 50 % of the world production of wheat grain [1, 2].

It is possible to solve the problem of producing vegetative protein, valuable for bread baking and confectionary production, using grain of minor wheat varieties or introgressive lines due to higher content of protein and better balance in terms of essential amino acids [3, 4]. In addition, there are a great number of newly hybridized introgressive varieties and lines of wheat, the amino acid composition and biological value of which has not been studied in fine detail [5, 6].

One of the most important parameters of grain quality is the quantitative content of essential amino acids [7]. The information on the nutritional content of foods brings the knowledge to bear on the goals of food analysis and food science, may contribute to the establishment of policies on food production and storage, the evaluation of the nutritional status, the formulation of therapeutic diets and investigations into the relationships between diet, health and disease [8].

The essential amino acid parameter is not stable, and may change depending on wheat variety, weather conditions and agrotechnology [9, 10]. Therefore, the determination of amino acid composition of seed protein and its biological value in the grain of new varieties and lines becomes eminent.

MATERIALS AND METHODS

The experimental part of the work was conducted in the Laboratory of estimating the quality of grain and grain products at the Uman National University of Horticulture. The grain of soft winter wheat of the following varieties was used: Podolianka, Kokhana, Chornobrova with violet kernel, developed in Ukraine, and the varieties produced in other European countries, North America and Africa – Pannonikus (Austria), Emerino (Cyprus), white grain Kulundynka (Russia), Ac Meckinon (Canada) as well as the lines obtained by hybridization of *Triticum aestivum* and *Triticum spelta* – LPP 1314, P 7 (Ukraine). All varieties and lines were grown in the conditions of the Right-Bank Forest-Steppe of Ukraine in 2013–2015. The area-specific variety of soft winter wheat (national standard) Podolianka (st) was used as standard.

The experimental plot is located at Mankivsky natural farmland of the Medium Dnieper-Bug District in the Right-Bank Forest-Steppe with the Greenwich geographical coordinates of 48° 46'56,47" north latitude and 30° 14'48,51" east longitude. The altitude is 245 m. The soil of the experimental field is podzolic chernozem. The thickness of the soil profile, including P(h)k horizon, is 140–160 cm. The structure of soil within the profile is moderately dense, the granulometric composition is even. The degree of base saturation is 87–97 % with the medium acid reaction of the soil solution. The potential acidity fluctuates from 1.8 to 4.2 cmol/kg of soil. The maximal capacity of absorbing cations in the upper horizon is 29–32 cmol/kg of soil.

In 2012 and 2013, the weather conditions were characterized by a smaller amount of precipitation, with 178 and 209 mm of precipitation in April-July respectively, or 15–36 % less compared to the mean perennial index (277 mm). There was a sufficient amount of precipitation in 2014 and 2015. In April-July, there were 374, 292 and 271 mm of precipitation respectively, but their distribution was different. In 2013, there were only 13.3 mm, in 2015 – 45.8, and in 2014 – 140.8 mm of precipitation in the phase of stem elongation. Air temperature also had its impact on the growth and development of wheat varieties and lines. For instance, during the period of intense growth of the stem (stem elongation – earing) in 2013 it was unfavorable compared to the optimal temperature (9–16 °C), amounting to 18–21 °C. During the remaining years of the studies, the air temperature was optimal. During the period of grain ripening, the air temperature was below the optimal indices (22–25 °C), in addition, there were 65.6–143.6 mm of precipitation.

The predecessor crop was oat (*Avena sativa* L.), cultivated for green fodder. Wheat was grown without any fertilizers or protectors.

The content of bound amino acids was determined by the method of ion-exchange liquid chromatography with the analyzer for amino acids T-339 (Mikrotechna, Czech Republic, Prague).

The Amino Acid Score (AAS) was defined by the following formula [8] according to FAO/WHO:

$$A = \frac{Ac}{O} \times 100,$$

where A – amino acid score, %; Ac – actual content of amino acid, mg/g of grain; O – optimal content of amino acid, mg/g of grain.

The integral score was defined by the following formula:

$$A = \frac{Ac}{D} \times 100,$$

where A – amino acid score, %; Ac – actual content of amino acid, g/100 g of grain; D – daily requirement of this component by the organism of an adult, g.

The metabolization efficiency coefficient (MEC) of

$$MEC = \frac{\sum EA}{\sum NA},$$

essential amino acids was determined by the formula: where ΣEA – content of essential amino acids, %; ΣNA – content of non-essential amino acids, %.

The index of complex estimation (ICE) was determined by the formula:

$$ICE = \sqrt[n]{\frac{Ac_1}{O_1} \times \frac{Ac_2}{Ac_2} \times \ldots \times \frac{Ac_n}{O_n} \times \frac{P_1}{Ac_1} \times \frac{P_2}{Ac_2} \times \ldots \times \frac{P_n}{Ac_n}},$$

where Ac – actual value of the index; O – optimal value of the index; P – permissible value of the index; Ac/O – ratio, used for indices, the actual value of which should exceed the optimal one; P/Ac – ratio, used for indices, the actual value of which should be lower than the permissible level; *n* – number of indices, used in the model.

The statistical processing of the data was conducted in Microsoft Excel 2010 and STATISTICA 10. The interpretation of the impact level by the coefficient (thumb rule – Cohen): 0.02–0.13 – weak, 0.13–0.26 – medium, ≥0.26 – high.

The dispersion analysis was used to confirm or refute "null hypothesis". The method envisaged the value of coefficient "p", which demonstrated the probability of the respective hypothesis. In case of $p < 0.05$, the null hypothesis was refuted and the impact of the factor was reliable [11, 12].

RESULTS AND DISCUSSION

The sum of amino acids in the grain of soft wheat varieties varied from 10.55 % in the variety Ac Mackinnon to 17.47 % in the variety Kulundynka (Table 1).

In the grain of soft wheat lines, obtained by hybridization of *Triticum aestivum* L./*Triticum spelta* L., the sum of amino acids varied from 15.03 to 16.17 %, which was in general considerably higher as compared to the standard variety Podolianka (11.06 %, at 5 % Least Significant Difference, 5 % *LSD* = 0.68).

The content of essential amino acids was considerably higher compared to the standard (*LSD* = 0.21). The highest content of essential amino acids was in the grain of variety Kulundynka (5.18 %). The standard had 2.99 % essential amino acids.

We also found that the content of amino acids in wheat grain was strongly correlated with the variety and weather conditions (Fig.). The impact degree of the variety was the highest for essential amino acids – 0.71 and 0.93 – for non-essential acids. The degree of impact of weather conditions was 0.62.

The grain of other wheat lines was also characterized by high content of this group of amino acids. The content of essential amino acids in wheat lines, obtained by hybridization of *Triticum aestivum* L./*Triticum spelta* L., was from 4.17 to 4.51 % or 1.4–1.5 times higher compared to the control.

Table 1. The content of bound amino acids in the grain of some varieties and lines of wheat, mean for the period of 2013–2015, in %

Amino acid	Variety, line									
	Podolianka (st)	Kokhana	Emerino	Pannonikus	Ac Mackinnon	Kulundynka	Chornobrova	LPP 1314	P7	LSD_{05}
Val	0.48	0.52	0.52	0.47	0.54	0.66	0.53	0.68	0.63	*0.03*
Ile	0.38	0.42	0.54	0.49	0.41	0.75	0.43	0.65	0.45	*0.03*
Leu	0.59	0.69	0.68	0.70	0.76	0.98	0.66	0.85	0.77	*0.04*
Lys	0.37	0.41	0.40	0.56	0.43	0.71	0.47	0.54	0.61	*0.03*
Meth	0.06	0.07	0.07	0.10	0.08	0.15	0.07	0.08	0.09	*0.01*
Thre	0.33	0.37	0.34	0.54	0.37	0.74	0.36	0.47	0.58	*0.02*
Try	0.27	0.33	0.32	0.41	0.28	0.42	0.40	0.54	0.45	*0.02*
Phen	0.50	0.58	0.52	0.56	0.48	0.69	0.42	0.66	0.59	*0.03*
Σ_e	2.99	3.39	3.39	3.85	3.36	5.18	3.34	4.51	4.17	*0.21*
Ala	0.43	0.61	0.46	0.78	0.49	0.93	0.42	0.80	0.71	*0.03*
Arg	0.49	0.70	0.51	0.87	0.61	1.05	0.55	0.80	0.87	*0.04*
Asp	0.53	0.71	0.71	0.99	0.70	1.13	0.91	0.92	1.22	*0.04*
His	0.50	0.52	0.48	0.70	0.43	0.79	0.44	0.82	0.76	*0.03*
Gly	0.48	0.54	0.51	0.74	0.49	0.81	0.46	0.84	0.83	*0.03*
Glu	3.43	3.97	3.27	3.52	2.55	3.86	3.78	4.30	3.88	*0.16*
Pro	1.17	1.07	1.02	1.14	0.94	1.66	0.95	1.31	0.99	*0.06*
Ser	0.65	0.66	0.53	0.93	0.56	1.10	0.49	0.92	0.88	*0.04*
Thir	0.33	0.45	0.39	0.44	0.30	0.80	0.35	0.78	0.50	*0.02*
Cys	0.06	0.12	0.09	0.11	0.10	0.23	0.11	0.19	0.22	*0.01*
Σ_{ne}	8.07	9.36	7.96	10.18	7.19	12.29	8.47	11.65	10.87	*0.47*
Σ_s	11.06	12.75	11.35	14.03	10.55	17.47	11.81	16.17	15.03	*0.68*

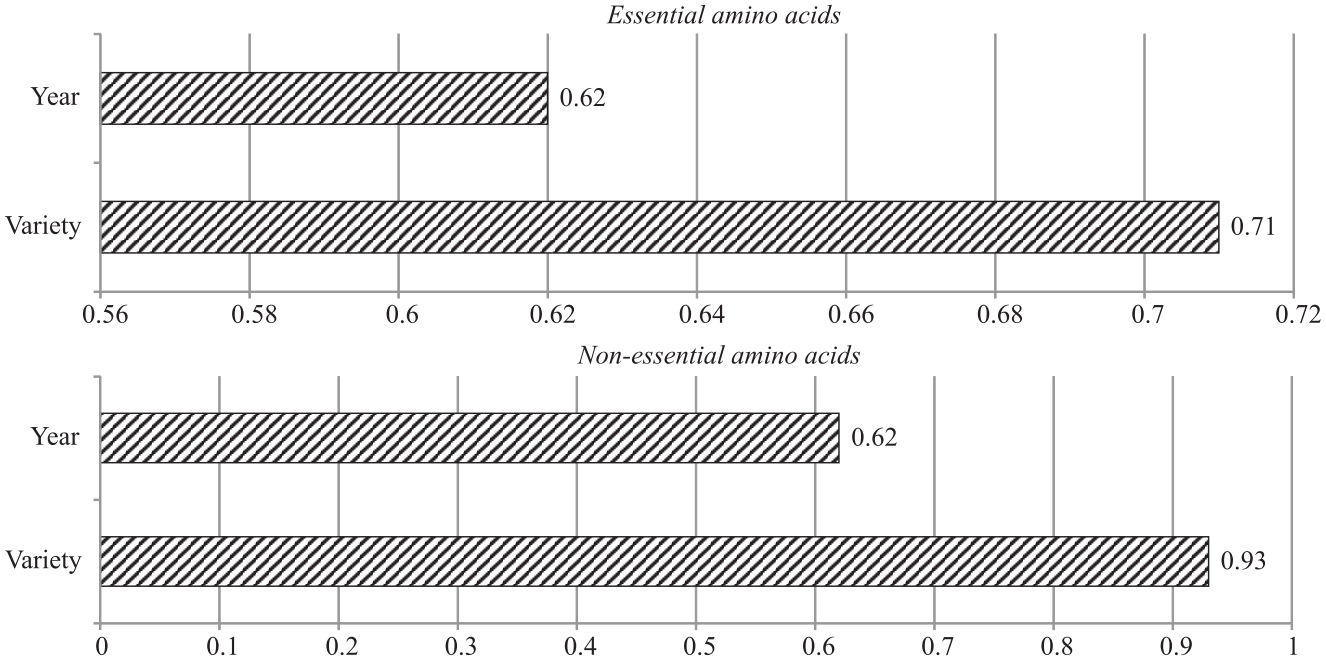

Fig. 1. The degree of impact of the factors under investigation on the content of amino acids

The main component of the amino acid composition of wheat grain is glutamic acid, the content varied from 2.55 to 4.30 % depending on the variety and line. The content of leucine and proline was higher as compared to other amino acids – from 0.59 % in Podolianka variety grain to 0.98 % in Kulundynka variety grain. The lowest indices were registered for the content of cystine, which varied from 0.06 to 0.23 %.

It is known that the content of protein or sum of amino acids does not correspond to high biological value of grain [13, 14]. In addition, the content of amino acids does not carry any information about meeting human organism requirements. Therefore, the value of amino acid score is calculated [14]. It is known that lysine and methionine are limiting amino acids in wheat protein in most varieties and lines, the amino acid score of which varied in our hands from 29 to 91 % (Table 2).

It was determined that at the accuracy of determining the content of amino acids in grain of about 5%, the score of 95% is considered to be non-deficient [15].

Thus, the protein of Kulundynka variety grain is the most balanced, as the score of essential amino acids is non-deficient, and the remaining varieties and lines are deficient in 2–5 more amino acids in addition to lysine

Table 2. The amino acid score of grain of varieties and lines of different wheat species (2013–2015), %

Variety, line	Amino acid							
	Meth + cys	Lys	Thre	Val	Ile	Leu	Try	Phen + thir
Podolianka (st)	29 ± 7^{c}	76 ± 5^{a}	76 ± 15^{c}	88 ± 15^{b}	88 ± 12^{b}	107 ± 2^{a}	144 ± 123^{c}	162 ± 6a
Kokhana	44 ± 5^{b}	84 ± 8^{b}	85 ± 18^{c}	96 ± 11^{b}	95 ± 15^{b}	128 ± 12^{b}	165 ± 128^{c}	206 ± 35^{b}
Emerino	34 ± 11^{c}	73 ± 9^{b}	63 ± 21^{c}	81 ± 29^{c}	97 ± 37^{c}	103 ± 35^{c}	138 ± 91^{c}	170 ± 14a
Pannonikus	49 ± 17^{c}	112 ± 7^{a}	122 ± 30^{c}	85 ± 26^{c}	113 ± 35^{c}	121 ± 19^{b}	212 ± 174^{c}	189 ± 28b
Ac Mackinnon	44 ± 8^{b}	90 ± 16^{b}	85 ± 7^{a}	98 ± 9^{a}	93 ± 16b	149 ± 33^{c}	130 ± 90^{c}	159 ± 32^{c}
Kulundynka	91 ± 12^{b}	144 ± 15^{b}	169 ± 19^{b}	120 ± 13^{b}	172 ± 8^{a}	187 ± 28^{b}	210 ± 164^{c}	298 ± 30^{b}
Chornobrova	43 ± 10^{c}	97 ± 16^{b}	82 ± 6^{a}	96 ± 10^{b}	97 ± 15^{b}	126 ± 28^{c}	185 ± 126^{c}	157 ± 28^{b}
LPP 1314	64 ± 10^{b}	112 ± 16^{b}	106 ± 9^{a}	125 ± 9^{a}	148 ± 10^{a}	165 ± 32^{c}	240 ± 150^{c}	291 ± 52^{c}
P 7	74 ± 17^{c}	120 ± 33^{c}	133 ± 24^{b}	114 ± 30^{c}	102 ± 26^{c}	141 ± 25^{c}	237 ± 200^{c}	208 ± 27^{b}

Table 3. The mean amino acid content (%) of essential amino acids per 100 g of grain of some varieties and lines of wheat determined over the period 2013–2015

Amino acid	Variety, line								
	Podolianka (st)	Kokhana	Emerino	Pannonikus	Ac Mackinnon	Kulundynka	Chornobrova	LPP 1314	P 7
Val	19 ± 4[b]	21 ± 3[b]	21 ± 3[b]	19 ± 6[c]	22 ± 2[a]	26 ± 3[b]	21 ± 2[b]	28 ± 3[a]	25 ± 7[c]
Ile	20 ± 3[b]	21 ± 4[b]	27 ± 2[a]	25 ± 8[c]	21 ± 4[b]	38 ± 2[a]	22 ± 4[b]	33 ± 2[a]	23 ± 6[c]
Leu	13 ± 4[c]	15 ± 3[b]	15 ± 3[b]	15 ± 7[c]	17 ± 2[a]	22 ± 2[a]	14 ± 2[b]	19 ± 2[b]	17 ± 5[c]
Lys	10 ± 2[b]	10 ± 2[b]	10 ± 1[a]	14 ± 3[c]	11 ± 1[a]	18 ± 3[b]	12 ± 2[b]	13 ± 1[a]	15 ± 5[c]
Meth	3 ± 1[c]	4 ± 1[b]	4 ± 1[c]	6 ± 1[b]	5 ± 2[c]	9 ± 2[c]	4 ± 1[b]	5 ± 1[b]	5 ± 1[b]
Thre	14 ± 3[c]	16 ± 4[c]	15 ± 3[c]	23 ± 6[c]	16 ± 2[a]	32 ± 4[b]	15 ± 2[a]	20 ± 2[a]	25 ± 5[b]
Try	35 ± 10[c]	42 ± 5[b]	40 ± 2[a]	52 ± 9[c]	35 ± 5[b]	53 ± 15[c]	51 ± 3[a]	68 ± 4[a]	57 ± 13[c]
Phen	12 ± 4[c]	13 ± 2[b]	12 ± 2[b]	13 ± 4[c]	11 ± 2[b]	16 ± 4[c]	10 ± 1[a]	15 ± 2[b]	14 ± 5[c]
Ala	7 ± 1[a]	9 ± 2[b]	7 ± 1[a]	12 ± 3[c]	8 ± 1[a]	14 ± 2[b]	7 ± 1[a]	13 ± 1[a]	11 ± 2[b]
Arg	8 ± 2[b]	12 ± 3[c]	9 ± 2[b]	14 ± 1[a]	10 ± 1[a]	18 ± 2[a]	9 ± 1[a]	13 ± 1[a]	15 ± 3[c]
Asp	5 ± 1[c]	6 ± 1[b]	6 ± 1[b]	9 ± 2[b]	6 ± 1[a]	10 ± 2[b]	8 ± 1[a]	8 ± 1[a]	10 ± 2[b]
His	24 ± 5[c]	25 ± 1[a]	23 ± 2[a]	33 ± 4[b]	21 ± 1[a]	38 ± 2[a]	21 ± 2[a]	40 ± 2[a]	37 ± 7[b]
Gly	14 ± 4[c]	16 ± 1[a]	15 ± 4[c]	21 ± 2[a]	14 ± 1[a]	24 ± 5[c]	14 ± 2[a]	24 ± 1[a]	24 ± 3[b]
Glu	26 ± 2[a]	30 ± 1[a]	24 ± 2[a]	26 ± 2[a]	19 ± 3[b]	29 ± 3[a]	28 ± 1[a]	32 ± 1[a]	29 ± 1[a]
Pro	26 ± 2[a]	24 ± 4[b]	23 ± 4[b]	26 ± 6[c]	21 ± 4[b]	37 ± 2[a]	21 ± 4[b]	30 ± 2[a]	22 ± 4[b]
Ser	8 ± 1[b]	8 ± 2[b]	6 ± 2[c]	12 ± 1[a]	7 ± 1[b]	13 ± 1[a]	6 ± 1[b]	12 ± 1[a]	11 ± 2[b]
Thir	8 ± 2[b]	11 ± 2[b]	9 ± 2[b]	10 ± 4[c]	7 ± 1[a]	19 ± 1[a]	9 ± 2[b]	18 ± 2[a]	12 ± 5[c]
Cys	4 ± 1[b]	7 ± 2[b]	5 ± 2[c]	6 ± 3[c]	6 ± 2[c]	13 ± 1[a]	6 ± 2[c]	11 ± 3[c]	13 ± 4[c]

Note. a – insignificant variation (V = 0–10 %); b – medium variation (V = 10–20 %); c – significant variation (V = ≥20 %).

and methionine. The amino acid score of tryptophane and phenylalanine was non-deficient in grain of all the varieties and lines of wheat.

In the studies of Graciela Caire-Juvera, Francisco A. et al. [8] the amino acid score of lysine for grain products of wheat was 15–54 %, for methionine – 41–47 %. However, this index was estimated for children aged 1–2 y.o., whose requirement in amino acids is higher compared to adults, therefore, it is lower.

The calculations demonstrated that 100 g of grain of varieties and lines of wheat species meet the biological requirement of an adult in tryptophane the most (35–68 %) (Table 3). The lowest integral score of 100 g of grain meets the requirement in methionine – for 3–6 % depending on the varieties and lines of wheat, and for the rest of amino acids – for 4–40 %. The biological requirement was met in the best way by 100 g of grain of varieties Kulundynka (9–53 %), P 7 and LPP 1314 lines – for 5–68 % depending on the amino acid.

Table 4. The metabolization efficiency coefficient and the index of complex estimation of the content of essential ami-no acids in the grain of varieties and lines of wheat, 2013–2015

Variety, line	MEC	ICE
Podolianka (st)	0.38 ± 0.06[b]	0.83 ± 0.16[c]
Kokhana	0.36 ± 0.04[b]	0.99 ± 0.13[b]
Emerino	0.43 ± 0.03[a]	0.96 ± 0.09[a]
Pannonikus	0.38 ± 0.07[b]	1.12 ± 0.33[c]
Ac Mackinnon	0.47 ± 0.04[a]	0.95 ± 0.02[a]
Kulundynka	0.42 ± 0.03[a]	1.57 ± 0.17[b]
Chornobrova	0.40 ± 0.04[a]	0.98 ± 0.11[b]
LPP 1314	0.39 ± 0.03[a]	1.37 ± 0.12[a]
P 7	0.38 ± 0.05[b]	1.27 ± 0.31[c]

Note. a – insignificant variation (V = 0–10 %); b – medium variation (V = 10–20 %); c – significant variation (V = ≥20 %).

The highest metabolization coefficient of essential amino acids was in the grain of varieties Kulundynka (0.42), Emerino (0.43) and Ac Mackinnon (0.47) or 11–24 % higher as compared to the control (0.38) (Table 4). As for grain of other soft wheat varieties, this coefficient varied from 0.36 to 0.40.

ICE index characterizes the levels of several indices compared to the optimal values. If ICE = ≤1, the actual value of indices is below the optimal one, ICE = 1 – actual values correspond to the optimal ones, ICE = ≥ ≥1 – actual values exceed the optimal ones.

The highest index of complex estimation (ICE) of the content of essential amino acids was registered in the grain of varieties Pannonikus (1.12), Kulundynka (1.57) and P 7 (1.27), LPP 1314 (1.37) lines. The lowest index was in the grain of Podollianka variety – 0.83. ICE in other varieties was from 0.95 to 0.98.

CONCLUSIONS

The content of amino acids in wheat grain depends the most on selective-genetic origin of the variety and the line. Out of nine samples of soft wheat, only the grain of Kulundynka variety had a non-deficient total amino acid score. In the variety Pannonikus, methionine (AAS = 49 %) and valine (AAS = 81 %) appeared to be limited as the content of amino acid was lower compared to the index of the ideal product.

The best-balanced content of amino acids is present in the grain of non-spelt lines P 7 and LPP 1314, obtained by hybridization of *Triticum aestivum* L./ *Triticum spelta* L. The grain of these lines has a non-deficient amino acid score and supplies the human daily requirement in the best way. This grain has 1.1–1.3 times higher content of glutamic, 1.6–1.8 times higher content of arginine, 1.7 times – that of glycine, 1.3–1.4 times – leucine, and 1.3–1.4 times – valine compared to the standard (Podolianka variety). The grain has a high index of complex estimation for essential amino acids.

It is recommended to use Kulundynka variety, lines P 7 and LPP 1314, in the breeding of wheat varieties, as they have a non-deficient score of essential amino acids in grain.

Характеристика амінокислотного складу зерна нових сортів і ліній пшениці

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Мета. Визначити формування зв'язаних амінокислот у зерні різних сортів пшениці та його біологічну цінність. **Методи.** Польовий, фізико-хімічний, розрахунковий, аналізування. **Результати.** Проаналізовано відмінності амінокислотного складу нових сортів і ліній пшениць. Встановлено, що найвищий вміст есенційних амінокислот був у зерні сорту Кулундинка (5,18 %) або більше в 2,3 рази порівняно зі стандартом (2,99 %). У зерні ліній пшениці м'якої, отриманих гібридизацією *Triticum aestivum* L./*Triticum spelta* L., їх вміст в 1,4–1,5 рази більший порівняно з контролем. Зерно пшениці м'якої сорту Кулундинка має найвищу біологічну цінність, тому що скор есенційних амінокислот бездефіцитний, а решта сортів мають дефіцит 2–5 амінокислот. У зерні ліній пшениці м'якої лише метіонін був у дефіциті (амінокислотний скор 64–74 %). **Висновки.** Вміст амінокислот у зерні м'якої пшениці істотно залежить від погодних умов, селекційно-генетичного походження сорту та лінії. Глутамінова кислота, пролін і лейцин – основні амінокислоти зерна. З дев'яти досліджених зразків пшениці, лише зерно сорту Кулундинка мали бездефіцитний амінокислотний скор (91–298 %), а в сорту Паннонікус вміст метіоніну був у дефіциті (49 %). Найкраще збалансований вміст амінокислот у зерні неспельтоподібних ліній, отриманих шляхом гібридизації *Triticum aestivum* L./*Triticum spelta* L., а саме P 7 і LPP 1314. У зерні цих ліній є бездефіцитний амінокислотний скор, крім метіоніну (64–74 %), найкраще забезпечує добову потребу людського організму. Зерно має високий показник комплексного оцінювання та коефіцієнт ефективності метаболізму для незамінних амінокислот.

Ключові слова: амінокислоти, зерно, пшениця м'яка, сорт.

Характеристика аминокислотного состава зерна новыхсортов и линий пшеницы

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Цель. Определить формирование связанных аминокислот в зерне новых сортов пшеницы мягкой и его биологическую ценность. **Методы.** Полевой, физико-химический, расчетный анализ. **Результаты.** Проанализированы различия аминокислотного состава новых

сортов и линий пшеницы. Установлено, что высокое содержание эссенциальных аминокислот был в зерне сорта Кулундинка (5,18 %) или более в 2,3 раза по сравнению со стандартом (2,99 %). В зерне линий пшеницы мягкой, полученных гибридизацией *Triticum aestivum* L./*Triticum spelta* L., их содержание в 1,4–1,5 раза больше по сравнению с контролем. Зерно пшеницы мягкой сорта Кулундинка имеет самую высокую биологическую ценность, так как скор эссенциальных аминокислот бездефицитный, а остальные сорта имеют дефицит 2–5 аминокислот. В зерне линий пшеницы мягкой только метионин был в дефиците (аминокислотный скор 64–74 %). **Выводы.** Содержание аминокислот в зерне пшеницы мягкой в значительной степени зависит от погодных условий, селекционно-генетического происхождения сорта и линии. Было установлено, что глутаминовая кислота, пролин и лейцин основные аминокислоты. Из девяти образцов исследованных образцов пшеницы мягкой только зерно сорта Кулундинка имело бездефицитный показатель аминокислот (91–298 %), а в сорте Паннониkус метионин был дефицитный (49 %). Наилучше сбалансированное содержание аминокислот в зерне неспельтоидных линий, полученных гибридизацией *Triticum aestivum* L./ *Triticum spelta* L., а именно P 7 и LPP 1314. Зерно этих линий имеет бездефицитный аминокислотный скор, кроме метионина (64–74 %), наибольше удовлетворяет суточную потребность человеческого организма ими. Зерно имеет высокий показатель комплексной оценки и коэффициента эффективности метаболизации для незаменимых аминокислот.

Ключевые слова: аминокислоты, зерно, пшеница мягкая, сорт.

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CULTIVATION OF POTATO LEAFROLL VIRUS (PLRV) IN MAMMALIAN CONTINUOUS CELL LINES

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Aim. To use the ability of potato leafroll virus (PLRV) to infect and multiply in mammalian continuous cell lines to purify PLRV isolates from the vegetative plant material, and to study the pathogenicity of those isolates for plants (after culturing in mammalian continuous cell line), to investigate morphological, physical-chemical, biological and antigen properties of PLRV isolates from mammalian cells and to study an alternative diagnostic method – the neutralization test in the mammalian continuous cell lines. **Methods**. The methods of cultivating animal viruses in the mammalian continuous cell line, microscopical biochemical, and serological methods, the method of artificial nutrition of aphids are detailed under Material and Methods. **Results.** It was demonstrated that successful cultivation of PLRV in mammalian continuous cell line allowed obtaining pure virus isolates from potato plants and aphids and preserving them for a long time (over a period of 7 years). The cultivation of PLRV in the mammalian continuous cell line did not impact its pathogenic properties and allowed transmitting the virus to plants. Continuous cells lines of pig embryonic kidney (PEKV), of kidney Syrian hamster (BHK-21), of testicles of piglets (PTP), of kidneys of the bull (MDBC), and of carcinoma rabbit kidney (RK-13) were found to be sensitive to PLRV, Continuous cell lines of human (HeLa, Hep-2 and of African green monkey kidney (Vero) were not infected by the virus. The infectious activity of PLRV in the sensitive continuous cell lines was 20–8.5 lg TCD_{50}/ml depending on the cell line. The isolates of PLRV were resistant to lipid-dissolving solvents, multiplied in a pH range from 4.0 till 10.0 and were thermoresistant at 50 ºC in the absence of bivalent ions of magnesium, TIP was in the range of 60–65 ºC under our experimental conditions. The optimal temperature for the reproduction of PLRV in the cell culture was c. 24 °C. The use of neutralization test in the mammalian continuous cell line allowed isolation in pure culture and identification of PLRV reliably in a time span of c. 14 days. **Conclusions**. It was proven that PLRV can be cultivated in the mammalian continuous cell lines of PEKV, BHK-21, PTV, MDBK and RK-13. It was established that the cultivation of PLRV in these continuous cell lines did not impact its biological, pathogenic, antigenic and physical-chemical properties. The identification of pure cultures of PLRV obtained in mammalian cells can be reliably performed by the use of neutralization reaction.

Keywords: phytopathogenic viruse, mammalian cell culturing, neutralization test.

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INTRODUCTION

Potato leafroll virus (PLRV), a representative of the *Polerovirus* genus, *Luteoviridae* family, is a single stranded RNA-virus, of which the sequence of nucleotides is completely determined [1]. The virions of PLRV are isometric, 23 to 25 nm in size and its pure RNA has ratio of A_{260}/A_{280} of 1.78. The virus remains infectious when diluted up to 10^{-4} in sap from infected plants, and in sap j after 5–10 days at 2 ºC, the virus temperature inactivation point (TIP) in crude sap when heated for 10 minutes is 70 to 80 °C [2].

PLRV is an economically important phytopathogenic virus mainly infecting potato (*Solanum tuberosum)*. Apart from potato, PLRV is able to infect about 40 plant species from different families: *Amaranthaceae, Cucurbitaceae, Chenopodiaceae, Cruciferae, Com-*

positae, Labiatae, Portulaceae, Nolanaceae and *Solanaceae* [3].

PLRV often occurs in potato plants along with other viruses, and to our knowledge there are no data in the scientific literature regarding the method of obtaining its isolates from plants with a mixed infection into pure culture. The above mentioned characteristics of PLRV complicate its detection and identification and hinders the development of fully specific diagnostic methods. The routine diagnosis of PLRV involves the application of immunological methods. These include the now outdated double immunodiffusion in gel, enzyme-linked immunosorbent assay (ELISA), the immunochromatographic assay detection of the virus (lateral flow immunochromatographic assay), Luminex × MAP® Technology – a novel method of analyzing different antigens, which combines immunological, fluorescent methods and laser technologies and allows the simultaneous detection of several viruses in plant material [4, 5]. Detecting virus by the method of reverse transcription polymerase chain reaction (RT-PCR) is ranked the first among the genetic molecular methods for the detection and identification of RNA viruses [6, 7].

Multiplication of PLRV is done using potato plants or indicator plants such as *Datura stramonium* L. and *Physalis angulata* L., onto which the virus is transmitted from a stock of known infected plants using grafting or aphids. After the multiplication period, which takes about 30 days, the leaves of infected plants are used to obtain PLRV preparations.

Cultivation and multiplication of PLRV can also be performed in protoplasts of tobacco or potato mesophyll. It envisages obtaining a culture of protoplasts, infecting it with the purified and concentrated preparation of PLRV using poly-L-ornithine and incubating protoplasts at permanent illumination and temperature [8].

In 2009 we detected a phenomenon of productive potato leafroll virus infection in mammalian continuous cell lines. The procedure to obtain an infected mammalian cell is, in short, as follows: To isolate PLRV vegetative parts of potato plants with the of PLRV (reference strain 879 from the Institute's phytopathogenic viruse collection) are used, in which the presence of the virus was confirmed by electron microscopy (EM) and ELISA. Plants samples are clarified with chloroform and placed in culture vials with cultures cells of pigs embryonic kidney (PEKV). The inoculated culture vials with the continuous cell line are incubated in a thermostat at 37 °C for appearance of virus cytopathic effect (CPE). Degenerative changes of PEKV showed appearance and gradual increase in number of single rounded cells with enhanced refractivity. The infected cells are moved away from glass. Areas without cells increase in size up to complete destruction of cell monolayer. On average the detection of CPE due to PLRV infection takes 5 to 15 days.

After adaptation of PLRV to the continuous cell line via four passages, the new system «virus-cell» is used for the accumulation of viral biomass. The EM of virus preparations after ultracentrifugation demonstrated aggregates of whole isometric particles and absence of empty capsids. After the manifestation of a CPE, the virus was identified using the reverse transcription polymerase chain reaction (RT-PCR) with the primer pair sense-5'-CgCgCTAACAgAgTTCAgCC and antisense-5'-gCAATgggggTCCAACTCAT, that should yield a 336 bp product, corresponding to the RNA of PLRV [6].

The aims of our present work were: 1) to isolate PLRV isolates, circulating in Ukraine's territory (Chernihiv's region); 2) to investigate physical-chemical, biological and antigen properties of PLRV isolates; 3) to study the pathogenicity of PLRV for plants, after it was multiplied and cultured in mammalian cells; 4) to investigate an alternative method of PLRV identification, namely the neutralization test in the continuous mammalian cell line.

MATERIALS AND METHODS

Potato material (18 plants with symptoms of leafroll and three aphids samples (per sample 5–10 *Myzus persicae* aphids, directly collected from plants) selected in the fields of Chernihiv's region, and the reference strain of PLRV, kept in the virology laboratory of Institute in potato clone G 879, were used to conduct the virological research.

Plants and aphids samples were clarified with chloroform (1 : 4) and placed in culture vials with cultures cells of pigs embryonic kidney (PEKV). The multiplication of virus was done in these cell cultures which were grown in growth medium 199 («BioTestLab», Kyiv, Ukraine) at the culture vials. Prior to introducing the virus, the nutrient medium was drained, and the cell monolayer was rinsed twice with a 0.9 % NaCl solution and Hank's solution. An amount of 0.5 ml of the virus suspension was introduced per vial and placed in an incubator at 37 °C to contact for one hour. After the contact the cell monolayer was washed with Hank's solution, the solution was removed and the monolayer of

cells further incubated at 37 °C up to a stage of 75 % CPE, subsequently the cultures were stored deep frozen at –18 °C in triplicate. Pure populations of PLRV were obtained via three times cloning of viruses by the method of limiting dilutions with subsequent three-times cloning by Melnick's method of plaque technique [9]. Virus accumulated in the previous dilution, was used for each subsequent passage.

The isolation, concentration and purification of PLRV from the infected mammalian cell lines was performed as follows: Two parts of virus suspension were added to one part of chloroform with subsequent hand shaken homogenization for 30 min. Then the mixture was kept for 12–18 h at 4 °C, and subsequently centrifuged for 20 min at 1500 g. To the supernatant ammonium sulfate was added until saturation of 50 % and the suspension kept at 4 °C for 1 h. The precipitate was removed by centrifugation at 5–10 °C for 20 min at 1500 g, resuspended in 0.9 % NaCl, and dialyzed against the same solution for 16–18 h. Control of purity of virus preparations was done spectrophotometrically using a spectrophotometer (SF-46, Leningrad, USSR) by measuring the ratio of A_{260}/A_{280}, and by electron-microscopy using a transmission electron microscope (UEMB-100V, Sumy, USSR).

For the hyperimmunization of rabbits purified and concentrated virus-containing suspensions of PLRV were used. To the prepared antigen, used for subcutaneous administration, adjuvant Montanide ISA 25 (SEPPIC, France) was added according to the manufacturer's instructions. The immunization of rabbits was done according to the scheme, developed by us. Immunization of rabbits is carried out through five-time administration of concentrated virus antigen in turns subcutaneously with adjuvant Montanide ISA 25 in an amount of 1 mg of protein/2 ml intracutaneously without adjuvant along spinal column to 8–10 points in amount of 1 mg of protein/1 ml with an interval between introductions of 7, 3, 4, 3 days respectively.

We did not determine genetic markers of PLRV when cultured in mammalian cells, but we analysed resistance of virus isolates to lipid-dissolving solvents [11], the sensitivity to certain media at different pH values, thermo-resistance [12] and thermal stability in order to properly identify the isolates as PLRV.

The stability of isolates of PLRV at various pH values of the solution (0,1 M Na_2CO_3, 0.1 M $Na_3C_6H_5O_7$ and their combinations) was studied in a BHK-21 cell line. For this purpose, the virus isolates LT, LB, LS at a dose of 1 $lgTCID_{50}$ were kept in the solution with a pH value of 2.0, 3.0, 4.0, 7.2, 10.0 and 11.0 respectively at room temperature for 10 minutes. After that, in all samples, the pH was adjusted to 7.2. The sensitivity of the virus to the acid and alkaline pH values was determined by the difference between the titre of the virus isolates as compared to that of the PLRV control strain (pH 7.2). The experiment was performed in three replications.

Thermo-resistance was studied via determining the infectious activity of the three PLRV isolates in cell culture after heating them at 50 °C for 1 h in the presence of 1 M of a $MgCl_2$ solution and without it. The experiment was performed in three replications.

The impact of temperature on the functioning of the PLRV-animal cell system was studied in the BHK-21 cell line, infected with isolate LT at a dose of 1 $lgTCID_{50}$. The incubation was conducted at 2, 10, 24 and 37 °C. The degeneration changes in the monolayer, the time of their occurrence and infectious activity of the virus were noted. The experiment was performed in three replications.

The antigenic affinity between virus isolates was established in the cross reaction of virus neutralization using a stable dose of the virus (100 $TCID_{50}$) and antiserum to PLRV isolates (20 neutralizing doses) and 10-times dilutions of the virus with a stable dose of antiserum (20 neutralizing doses). Normal rabbit serum in 1 : 5 dilution and blood serum, obtained from the culture of BHK-21 cells, were used for the control. The antigenic affinity was calculated by the formula [9]:

$$A = 100\sqrt{r_1 \times r_2},$$

r_1 = *heterologous titer/homologous titer, for strain 1;*

r_2 = *heterologous titer/homologous titer, for strain 2.*

To study biological properties of PLRV the sensitivity of different cultures of mammalian cells to PLRV, we conducted the studies of virus replication in continuous cell lines (from the Institute's continuous cell line collection) such as: acontinuous cell line of pigs embryonic kidney (PEKV), of kidney Syrian hamster (BHK-21), of testicles of piglets (PTP), of kidneys of the bull (MDBC), of rabbit kidney carcinoma (RK-13), of human (HeLa, Hep-2) and of African green monkey kidney (Vero). Reference strain 879 of PLRV was adapted to the mammalian continuous cell lines with 4 passages.

To study the pathogenicity of PLRV for plants, after it was multiplied and cultured in mammalian cells, the method of Rochow [13] was used to transmit the virus to plants using aphids (*Myzus persicae* Sulz.), which were fed through an artificial membrane (Fig. 1).

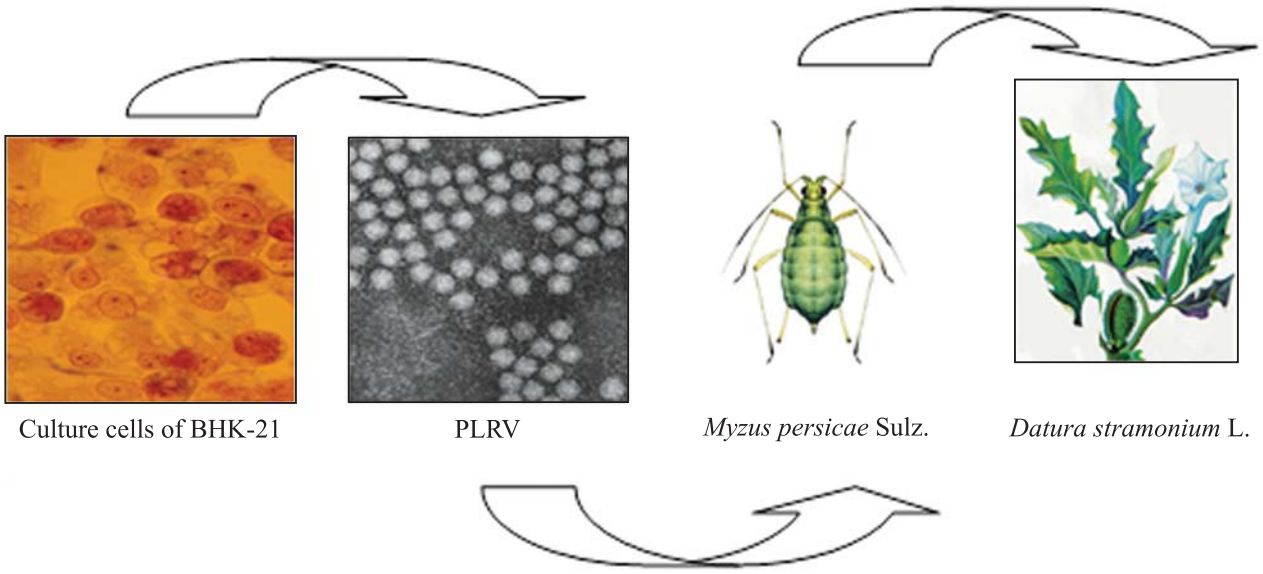

Fig. 1. The scheme of transmitting PLRV by aphids from the infected continuous mammalian cell line to plants-indicators

We have made a 'aphid nursery' in order to obtain "sterile" (not infected) clones of aphids. After consecutively obtaining four generations of *M. persicae* on *Brassica pekinensis* (Lour.) Rupr. plants (under greenhouse conditions, 18–25 °C, natural light), the insects are deemed free from PLRV. After five weeks of replication, homogeneous colonies of *M. persicae* were obtained. "Sterile" aphids (10–20 insects from the replication nursery) were fed for 24 h via a "sandwich" of membranes of *Parafilm M* (52 × 52 mesh), fixed at the end of a small glass tube with the size of 15 mm × × 5 cm. To that end 0.1 µl of the PLRV preparation solution (obtained in the BHK-21 cell line and cleared with chloroform, concentrated by centrifugation via a 20 % sucrose cushion at 37,000 g) was introduced between the membranes. Negative control was a similar combination with 5 % saccharose solution only. For the experiment we used 5 passage reference strain 879 in the BHK-21 cell line.

After artificial nutrition, aphids were transmitted in batches of 10 insects on five indicator plants of *Datura stramonium* and five indicator plants of *Physalis angulata (*under greenhouse conditions, 18–25 °C, …% relative humidity (RH) and natural light*)*. One day later the aphids were killed with insecticide.

To study an alternative method of PLRV identification, namely the neutralization test, two samples of potato leaves (on 1 sheet with 10 plants) of variety Suvenir Chernihivsky with mild mosaic symptoms and variety Tyras without any disease symptoms. Samples were selected in the hydroponic greenhouse of Scientific Production Association "Chernihivelitkartoplia".

A pure culture of PLRV reference strain obtained via the PEKV cell line, was used to obtain the reference antiserum.

The preparations, obtained from both samples of potato leaves, were used to infect the PEKV cell line and incubated in the thermostat at 37 °C till the manifestation of the features of cytopathic effect of the virus, which appeared on the third day.

The samples were typed in the serological neutralization test in the PEKV cell line. Prior to that, the titer of the obtained reference antiserum was determined to be 1:256 in the neutralization test.

To identify the isolated viruses in the neutralization test, 0.5 ml, containing 100 $TCID_{50}$ of virus antigen in 0.1 ml, was mixed with 0.5 ml of reference serum to PLRV, containing 20 neutralizing doses per milliliter. After incubating the mixture at 37 °C for 60–90 min, 0.2 ml was introduced into a test tube to which 0.8 ml of the supporting medium was added before. Control and experiment test tubes were further incubated at 37 °C and the results registered on the 4^{th} and 7^{th} day.

RESULTS AND DISCUSSION

Three isolates (LB, LS and LT) of PLRV were obtained from 18 symptomatic potato and 3 aphid samples after being cultured in the PEKV cell line (Table 1).

PLRV isolates were extracted in 3, 4 and 5 passages, their infection titers were 6.5–8.5 lg $TCID_{50}$/ml and typed with rabbit serum to the reference strain 879 in the PEKV cell line, wherethe neutralization test estab-

lished their homology to the reference strain 879 of PLRV

All three isolates of PLRV, when multiplied in the cell culture under agar cover, formed small plaques of 1 mm in diameter on days 3–4.

The LB, LS, LT isolates of PLRV were kept at –18 °C in a domestic freezer and did not lose their infectivity over a period of 7 years and maintained in a pure culture by passaging them in PEKV and BHK-21 cell cultures.

Virus isolates, extracted from plant samples and aphids, were found to be resistant to lipid-dissolving solvents (ether, chloroform) which demonstrated the absence of a lipid-containing envelope in them.

As seen from the results, presented in Table 2, at different pH values the infectious titer almost did not change in the range from 4 to 10.0, decreased by 4 lg TCD_{50}/ml at pH 3.0, and at pH 2.0 there was complete inactivation.

The study of thermal stability of extracted PLRV isolates established that the thermal TIP in the culture of PEKV after heating for 10 min was in the range from 60 ºC to 65 ºC. It may be that the difference in TIP values was conditioned by different chemical composition of the media, where the viruses were placed while heated, although we did not test this supposition.

While studying the thermal resistance, the infectivity of the three PLRV isolates, heated without 1 M of the solution of $MgCl_2$, did not change compared to the unheated control, and in the presence of 1 M of $MgCl_2$, it decreased by 2–3.5 lg $TCID_{50}$/ml, which demonstrated the absence of stabilization of virions with bivalent cations of magnesium (Table 3).

The results of the determination of temperature impact are presented in Table 4.

There was a noted considerable slowing down of PLRV replication at temperatures below 24 °C. For instance, after 7 days of incubation at 2 and 10 °C, there were no degenerative changes observed in the monolayer, and the infectious titer of PLRV decreased to 2 lg $TCID_{50}$/ml. At 24 °C on the 3rd and 4th day after the inoculation, single round cells were observed. The destruction of 75 % of the monolayer was observed after 7 days. On the 3rd and 4th day, the infectious activity was 4.5–5.5 lg $TCID_{50}$/ml respectively, on the 7th day it was 6.5 lg $TCID_{50}$/ml. At the optimal temperature of incubation, *i.e.* 37 °C, the cytopathic action of the virus developed already after 24 h, and the infectious titer of the virus was 7.5 lg $TCID_{50}$/ml.

The antigenic affinity of the three PRLV isolates from three different sources, namely aphids, leaves and tubers of potato, was 100 % *i.e.* they were serologically identical.

PLRV had a cytopathic effect on the PTP cell line 12–24 h after inoculation, for the PEKV and the BHK-21 cell lines it was 24–48 h after inoculation. The cytopathic effect was visible as symptoms of degeneration in the cell culture in the form of single rounded cells with increased refractivity, in increasing numbers. The affected cells came loose from glass, and clear empty spots appeared in the monolayer, increasing in size up to complete and visible destruction of cell groups.

Table 1. The three isolates of PLRV extracted from plant samples and aphids used in our study

Source of extracting the isolate	Code of the isolate	Passage of extracting	Infection titer (lg TCID50/ml
Potato tubers, Skarb variety	LB	4	6.5 ± 0.12
Potato leaves, Suvenir Chernihivsky variety	LS	3	8.5 ± 0.18
Aphids from potato leaves, Tyras variety	LT	5	7.0 ± 0.10

Table 2. The impact of pH value of the solution on the infectivity of three PLRV isolates

PLRV isolates	Virus titer (lg TCD_{50}/ml) by variants					
	pH 7.2	pH 2.0	pH 3.0	pH 4.0	pH 10.0	pH 11.0
LT	8.5 ± 0.14	0	4.0 ± 0.12	8.0 ± 0.12	8.0 ± 0.12	6.0 ± 0.14
LB	8.5 ± 0.10	0	4.5 ± 0.14	8.5 ± 0.18	8.0 ± 0.12	5.5 ± 0.10
LS	8.0 ± 0.12	0	4.0 ± 0.10	8.0 ± 0.14	7.0 ± 0.24	6.0 ± 0.18

After 48–96 h, destructive changes were also observed in the cultures of the RK-13 and MDBK cell lines, here the cells also formed symplasts.

No degenerate changes were detected in the cultures of HeLa, Hep-2 and Vero cells after consecutive passaging, PLRV did not multiply in these cell lines.

The infectious activity of PLRV in the PTP cell line was 7.5-8.5 lg $TCID_{50}$/ml, in the BHK-21 cell line – 6.0-8.5 lg $TCID_{50}$/ml, in the PEKV cell line – 7.5–8.5 lg $TCID_{50}$/ml.

Twenty-four days after the 10 *M. persicae* aphids per PLRV isolate had been feeding for 24 h and were killed by insecticides there was a noted manifestation of interveinal chlorotic zones on old and young leaves in *P. angulata* plants and a delay in growth of *D. stramonium* plants, which indicated successful transmission of PLRV with the preservation of virus pathogenicity after long-term cultivation in mammalian cell culture.

We also studied the possibility of using the mammalian cell culture for PLRV diagnostics in plant samples.

The neutralization of viruses with the reference serum of rabbit blood demonstrated PLRV infection in potato leaves of varieties Suvenir chernihivsky and Tyras. The virological analysis with the mammalian cell culture takes c. 10 days. Thus, it was established that the application of neutralization test – a method, previously not applicable for identification of phytoviruses – in the continuous mammalian cell culture allows isolation and identification of PLRV in potato plants reliably.

Table 3. Thermal resistance of three PLRV isolates in the cell culture of PEKV

PLRV isolates	Virus titer (lg TCD_{50}/ml)		
	Control (without heating)	Heating at 50 ºC, 1 h	Heating at 50 ºC, 1 h, 1 M, solution of $MgCl_2$
LT	8.5 ± 0.12	8.5 ±	5.0 ±
LB	8.0 ± 0.12	8.0 ±	5.0 ±
LS	6.5 ± 0.14	6.5 ±	4.0 ±

CONCLUSIONS

It was demonstrated that the cultivation of PLRV in some mammalian continuous cell lines allowed isolation of pure virus isolates from potato plants and aphids and preserving them for a long time (up to 7 years).

The investigated three isolates of PLRV were resistant to lipid-dissolving solvents, were multiplying in media with pH values from 4.0 till 10.0 and were thermo-resistant at 50 ºC in the absence of bivalent ions of magnesium; TIP was in the range of 60–65 ºC under our experimental conditions. The optimal temperature for the replication of PLRV was c.24 °C.

It was established that continuous cell line lines of PEKV, BHK-21, PTP, MDBK and RK-13 were sensitive to PLRV. The human cell lines HeLa and Hep-2, and a primate cell line (Vero) were not infected by the virus. The infectivity of PLRV in the sensitive cell cultures was 2.0–8.5 lg $TCID_{50}$/ml depending on the cell culture.

The cultivation of PLRV in continuous mammalian cell lines did not impact its pathogenic and other physic-chemical properties and allowed transmitting the virus to plants.

The application of neutralization test can be reliably used to identify pure isolates of PLRV obtained from mammalian continuous cell lines.

Table 4. The impact of temperature on PLRV multiplication in the cell culture of BHK-21

Incubation temperature, °C	Degenerate changes in the monolayer of the cell culture	Period of observations, days	Infectious titer of the virus (lg TCD_{50}/ml)
2	not observed	7	2.0 ± 0.12
10	not observed	7	2.0 ± 0.12
24	single round cells	3	4.5 ± 0.12
24	single round cells	4	5.5 ± 0.12
24	destruction of 75 % monolayer	7	6.5 ± 0.12
37	destruction of 75 % monolayer	1	7.5 ± 0.12

Культивування вірусу скручування листя картоплі в культурах клітин ссавців

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Мета. Використати явище продуктивної інфекції вірусу скручування листя картоплі в культурах клітин ссавців для виділення ізолятів ВСЛК з рослинного матеріалу, вивчення патогенності ВСЛК для рослин при культивуванні в культурі клітин ссавців, дослідження морфологічних, фізико-хімічних, біологічних й антигенних властивостей ізолятів ВСЛК та випробування альтернативного методу діагностики – реакції нейтралізації в культурі клітин ссавців. **Методи**. Використано загальноприйняті методи культивування вірусів тварин в культурі клітин ссавців, біохімічні методи, серологічні методи, метод штучного живлення попелиць. **Результати**. Показано, що культивування ВСЛК в культурі клітин ссавців робить можливим виділення чистих ізолятів вірусу з рослин картоплі та попелиць та їх підтримання тривалий час. Культивування ВСЛК в культурі клітин ссавців не впливає на його патогенні властивості та дозволяє передавати вірус на рослини. Перещеплювані лінії культур клітин нирки ембріона свині (СНЕВ), нирки сірійського хом'яка (ВНК-21), тестикул поросят (ПТП), нирки бика (MDBK) та карциноми нирки кроля (RK-13) виявилися чутливими до ВСЛК. Інфекційна активність ВСЛК в чутливих культурах клітин становила 2,0–8,5 lg $ТЦД_{50}/см^3$ в залежності від культури клітин. Ізоляти ВСЛК стійкі до ліпідорозчинників, до середовищ із значеннями pH від 4,0 до 10,0 та терморезистентні при 50 ºС за відсутності двовалентних іонів магнію, ТТІ знаходиться в межах 60–65 ºС. Оптимальною температурою для репродукції ВСЛК в культурі клітин є +24 °С та вище. Використання реакції нейтралізації в культурі клітин ссавців дозволяє швидко та надійно діагностувати ВСЛК у рослинах картоплі. **Висновки**. Доведено, що ВСЛК можна культивувати в культурах клітин ссавців СНЕВ, ВНК-21, ПТП, MDBK, RK-13. Встановлено, що культивування ВСЛК у цих культурах клітин не впливає на його біологічну активність, патогенні, антигенні, та фізико-хімічні властивості. За результатами досліджень рекомендовано використання реакції нейтралізації для ідентифікації ВСЛК.

Ключові слова: фітопатогенний вірус, культивування клітин ссавців, реакція нейтралізації.

Культивирование вируса скручивания листьев картофеля в культурах клеток млекопитающих

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Цель. Использовать явление продуктивной инфекции вируса скручивания листьев картофеля в культурах клеток млекопитающих для выделения изолятов ВСЛК из растительного материала, изучение патогенности ВСЛК для растений при культивировании в культуре клеток млекопитающих, исследования морфологических, физико-химических, биологических и антигенных свойств изолятов ВСЛК и использование альтернативного метода диагностики – реакции нейтрализации в культуре клеток млекопитающих. **Методы**. Использованы общепринятые методы культивирования вирусов животных в культуре клеток млекопитающих, биохимические методы, серологические методы, метод искусственного питания тлей. **Результаты**. Показано, что культивирование ВСЛК в культуре клеток млекопитающих делает возможным выделение чистых изолятов вируса из растений картофеля и тлей, их поддержание длительное время. Культивирование ВСЛК в культуре клеток млекопитающих не влияет на его патогенные свойства и позволяет передавать вирус на растения. Перевиваемые линии культур клеток почки эмбриона свиньи (СПЭВ), почки сирийского хомяка (ВНК-21), тестикул поросят (ПТП), почки быка (MDBK) и карциномы почки кролика (RK-13) оказались чувствительными к ВСЛК. Инфекционная активность ВСЛК в чувствительных культурах клеток составляла 2,0–8,5 lg $ТЦД_{50}/см^3$ в зависимости от культуры клеток. Изоляты ВСЛК устойчивы к липидорастворителям, к среде со значениями pH от 4,0 до 10,0 и терморезистентны при 50 °С в отсутствие двухвалентных ионов магния, ТТИ находится в пределах 60–65 °С. Оптимальной температурой для репродукции ВСЛК в культуре клеток является 24 °С и выше. Использование реакции нейтрализации в культуре клеток млекопитающих позволяет быстро и надежно диагностировать ВСЛК в растениях картофеля. **Выводы.** Доказано, что ВСЛК можно культивировать в культурах клеток млекопитающих СПЭВ, ВНК-21, ПТП, MDBK, RK-13. Установлено, что культивирование ВСЛК в этих культурах клеток не влияет на его биологическую активность, патогенные, антигенные и физико-химические свойства. По результатам исследований реко-

мендовано использование реакции нейтрализации для идентификации ВСЛК.

Ключевые слова: фитопатогенный вирус, культивирование клеток млекопитающих, реакция нейтрализации.

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FORMATION OF SEED PRODUCTIVITY AND SOWING QUALITIES OF RED CLOVER SEED DEPENDING ON THE EFFECT OF LIME, MINERAL AND WATER-SOLUBLE FERTILIZERS

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Aim. Development of efficient fertilization of red clover seed sowings with mineral, lime and microfertilizers in order to optimize plant nutrition during their vegetation period to obtain stable seed yields with high sowing and yield qualities. **Methods.** field, visual, measuring, weight, quantitative, method of a test sheaf, laboratory, mathematical-statistical. **Results.** The paper presents the results of optimizing the nutrition of red clover seed sowings on the basis of the rational application of quick-acting lime ($Ca(OH)_2$ – 0.5 of the rate by hydrolytic acidity, mineral ($N_{30}P_{60}K_{60}$) and water-soluble fertilizers, which ensured seed yield increase 1.8–2.0 times at the level of 0.35–0.40 t/ha. **Conclusions.** The most effective combination of the basic fertilization with mineral fertilizers ($N_{30}P_{60}K_{60}$) and lime fertilizers ($Ca(OH)_2$) at the rate of 0.5 by hydrolytic acidity applied under the cover crop using water-soluble fertilizer (plantafol – 1.0 kg/ha) and boric fertilizers (H_3BO_4 – 0.8 kg/ha) at the shooting stage of the second cut of red clover and molybdenum fertilizers ($(CNH_4)_2MoO_4$ – 0.3 kg/ha) in spring at the beginning of its regrowth.

Keywords: red clover, seed sowings, yield, lime, mineral and water-soluble fertilizers.

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INTRODUCTION

The intensification of field and meadow feed production envisages the use of perennial grasses, which ensure high yield of green mass and high-quality hay. These requirements are met by red clover, which, along with alfalfa, is the most common forage crop, solving the problems of producing vegetative protein and increasing the fertility of soils. Here red clover is one of the most reliable and high-yield crops, especially by the amount of obtained forage protein [1, 2, 3, 4].

In the Forest-Steppe of Ukraine red clover takes about 50 %, and in Polissia – 15–20 % of the area of legume grass sowings. While growing the latter for seeds, a considerable amount of nutrients is removed from soil: at the yield of seeds of 0.35 t/ha and straw 4.5 t/ha, the total removal of nitrogen (N) is 60, phosphorus (P_2O_5) – 55, potassium (K_2O) – 94, calcium (CaO) – 89 kg/ha [5, 6], thus the fertilization for seed sowings of this crop, in particular, the application of new forms of micro- and water-soluble fertilizers and quick-acting calcium-containing fertilizers impacts the seed productivity of red clover considerably.

MATERIALS AND METHODS

The experiments were conducted in the experimental farm "Bohonytske" of the Institute of Feed Research and Agriculture of Podillia, NAAS, in the crop rotation of the department of seed development and innovation transfer in 2011–2013. Gray forest soil was used with the following indices: pH – 4.8–5.2, hydrolytic acidity – 2.73–3.04 mg-eq. per 100 g of soil, the sum of absorbed alkali 12–13 mg-eq. per 100 g of soil, in the arable layer of soil (0–20 cm) the content of humus was 1.91–2.40%, easily hydrolyzed nitrogen (N) by Kornfeld 7.5–10.0, mobile forms of phosphorus (P_2O_5) by Chirikov and potassium (K_2O) respectively 15–19, 10.3–12.5 mg per 100 g of soil.

The cover crop, protecting the sowing of red clover from weeds, winds, cold, and heat, was spring barley, Lofant variety, with the norm of sowing of 3 million

seeds for germination per 1 ha. The norm of sowing red clover, Sparta variety, is 7 million seeds for germination per 1 ha of certified seeds. The area of the registered area was 25 sq.m., the number of repeats – 3 times.

Phosphorus-potassium fertilizers in the form of granulated superphosphate and potassium chloride and lime fertilizers in the form of $CaCO_3$ (defecate) and slaked lime – hydrated lime ($Ca(OH)_2$) on grey forest soils were introduced in autumn under the main tillage of soil according to the scheme of studies. Nitrogen fertilizers (ammonium nitrate) were introduced in spring under the cover crop.

Water-soluble fertilizer – plantafol – was introduced according to the scheme of experiment in the phase of shooting (1 kg/ha) and in the bud phase of red clover (1 kg/ha). By its composition plantafol contained N – 5 %, P_2O_5 – 15 %, K_2O – 45 %, B – 0.02 %, Fe – 0.01 %, Mn – 0.05 %, Zn – 0.05 %, Cu – 0.05 %. Here copper, iron, magnesium, zinc in plantafol were chelates in the EDTA form (ethylenediaminotetraacetic acid). In addition, the experiments used molybdenum fertilizers ($(CNH_4)_2MoO_4$ – acid ammonium molybdate) – 0.3 kg/ha in spring at the beginning of regrowth of red clover and borium (H_3BO_4 – boric acid) – 0.8 kg/ha at the shooting stage of the second cut of red clover for seeds. Except for variants under study in the experiment, agroequipment was common for these zonal conditions.

A structural analysis of yield was conducted prior to harvesting by determining the number of generative shoots and ripe heads per 1 sq.m. in six places of each variant. The mass of seeds in grams and their number were defined in 30 randomly selected heads of red clover. The number of seeds was calculated in 10 heads. In addition, the experiment determined the mass of 1,000 seeds, energy and germination of seeds, percentage of pollinated flowers in one head.

RESULTS OF INVESTIGATIONS

A relevant agrotechnical technique in farming seeds of red clover is liming acid soils. A high content of seeds may be obtained only on neutral and slightly acid soil. Red clover plants are most sensitive to acid reaction of soil at the initial stages of development during the sowing year. Acid soils have aluminum and magnesium in the amount of over 3 mg per 100 g of soil, which have a toxic effect on young shoots of plants. Their action is decreased by liming. Acid medium inhibits the activity of nodule bacteria, plants are poorly developed due to the disruption of nitrogen exchange in them. The introduction of lime fertilizers even prior to sowing red clover increased its seed productivity considerably [5, 6, 7]. Increased acidity of soil inhibits the improvement of seed performance, limits a positive action of other elements of cultivation technology. Liming acid soils improves the supply of phosphorus for plants, enhances winter-resistance, promotes better growth of vegetative organs, blossoming occurs better and with higher amount of viable pollen, which conditions the growth in seed yield [8, 9].

Liming can decrease acidity from pH 5.0 to 6.2–6.5 and thus increase the yield of red clover, though here the latter is also sensitive to over-liming and high content of salts [10].

According to the results of the studies red clover develops optimally at pH 6.0–6.5, accumulating about 300 kg/ha of nitrogen in soil, and forms the yield of seeds up to 0.5–0.6 t/ha in case of following the requirements of other technological operations. At pH 4.0–5.0 this crop can grow and develop, but it accumulates only 80–100 kg/ha of nitrogen and forms the yield of seeds of 0.15–0.20 t/ha [5].

The results of studies, conducted in 2011–2013, demonstrated that the introduction of lime fertilizers in the form of $CaCO_3$ (defecate) and $Ca(OH)_2$ (slacked lime – hydrated lime) – 0.5 of the rate by hydrolytic acidity prior to ploughing under the cover crop had a considerable impact on the yield of seeds of red clover, Sparta variety (Table).

The yield of red clover seeds on the plots, where liming was conducted using quickly-acting soluble lime fertilizers in the form of $Ca(OH)_2$ in combination with the application of mineral fertilizers in the dose of $N_{30}P_{60}K_{60}$ under the cover crop (factor A) on average for 2011–2013 was 291 kg/ha, which was 115 kg/ha more compared to the variant, where neither mineral nor lime fertilizers were introduced (control) and 63 kg/ha more compared to the plots with the introduction of mineral fertilizers only. After the introduction of calcium fertilizers in the form of $CaCO_3$ in combination with the application of mineral fertilizers in the dose of $N_{30}P_{60}K_{60}$ (factor A) this index was considerably lower and amounted to 266; 90; 38 kg/ha respectively. It demonstrated high efficiency of quickly-acting lime fertilizers in the form of slacked lime ($Ca(OH)_2$) during the first year after their application.

To obtain high stable yields of red clover seeds, the plants should be provided both with macro- and mi-

The yield of red clover seeds depending on the action of lime, mineral and water-soluble fertilizers, kg/ha

Foliar application, Factor B	Years			Average
	2011	2012	2013	
No fertilizers –control				
No additional nutrition	245	115	167	176
Plantafol – 1 kg/ha at the shooting stage	258	131	189	193
Variant 2 + Mo at the beginning of regrowth	280	135	194	203
Variant 2 + B at the shooting stage	297	141	201	213
Variant 2 + Mo + B	322	147	209	226
Plantafol – 1 kg/ha at the bud stage	297	137	197	210
Variant 2 + variant 6	313	139	201	218
Average	287	135	194	205
$N_{30}P_{60}K_{60}$				
No additional nutrition	288	179	217	228
Plantafol – 1 kg/ha at the shooting stage	307	198	241	249
Variant 9 + Mo at the beginning of regrowth	315	201	267	261
Variant 9 + B at the shooting stage	323	203	278	268
Variant 9 + Mo + B	349	207	301	286
Plantafol – 1 kg/ha at the bud stage	321	201	263	262
Variant 9 + variant 13	324	203	270	266
Average	318	199	262	260
$Ca(OH)_2 + N_{30}P_{60}K_{60}$				
No additional nutrition	346	215	312	291
Plantafol – 1 kg/ha at the shooting stage	356	225	341	307
Variant 16 + Mo at the beginning of regrowth	357	234	358	283
Variant 16 + B at the shooting stage	385	241	369	332
Variant 16 + Mo + B	399	254	401	351
Plantafol – 1 kg/ha at the bud stage	376	230	360	322
Variant 16 + variant 20	380	233	368	327
Average	371	233	344	316
$CaCO_3 + N_{30}P_{60}K_{60}$				
No additional nutrition	304	209	286	266
Plantafol – 1 kg/ha at the shooting stage	322	218	309	283
Variant 23 + Mo at the beginning of regrowth	336	224	322	294
Variant 23 + B at the shooting stage	359	226	333	306
Variant 23 + Mo + B	373	232	356	320
Plantafol – 1 kg/ha at the bud stage	352	220	317	296
Variant 23 + variant 27	359	224	325	303
Average	344	222	321	296
HIP_{05}				
A	8.4	8.1	6.9	
B	11.1	10.7	9.1	
AB	7.3	7.0	6.0	

croelements: borium, molybdenum, magnesium, zinc, cobalt, iron, sulfur. Microfertilizers promote intense accumulation of organic substances, increase in winter-resistance of plants and resistance to diseases, increase growth and accelerate development, improve the quality of products [6]. Thus, on the second year of life for red clover the scheme of studies should include foliar application of water-soluble fertilizers (plantafol – 1 kg/ha) and borium and molybdenum fertilizers (factor B).

When water-soluble fertilizers are introduced, plants receive nutrients via leaves. When introduced onto the plant, they are capable of causing considerable changes in the growth and development of plants. Water-soluble fertilizers get involved into the metabolism of substances, increase the level of vital activity, save water for plants, and activate microbiological processes. It is efficient to use water-soluble fertilizers with microfertilizers.

For instance, borium (B) enhances the intensity of photosynthesis, regulates pollination and settlement, improves carbohydrate and protein exchange, activates the activity of enzymes, has positive impact on the processes of cell division, enhances resistance to diseases. Also, borium improves synthesis and transfer of carbohydrates, especially sugars from the leaves to the organs of fruit-bearing and roots [6].

Molybdenum (Mo) is an irreplaceable component of many enzymes. It participates in carbohydrate, nitrogen, and phosphorus exchange, synthesis of vitamins and chlorophyll, increases the intensity of photosynthesis, is included to the composition of enzymes of nitroreductase, which takes part in the oxidation of nitrates to ammonium in plants. An important part is attributed to molybdenum in the processes of nitrogen fixation from the atmosphere by nodule and free bacteria [6].

The application of water-soluble fertilizers at the shooting stage (1 kg/ha) ensured the yield of seeds of 193 kg/ha on average during the years of studies (2011–2013) when they were introduced in the bud phase, the yield of red clover seeds was somewhat higher and amounted to 210 kg/ha or 17 kg/ha more compared to the introduction in the shooting phase and 34 kg/ha more compared to the variant with no additional nutrition. The combination of additional introduction of microfertilizers (Mo, B) in the control (variant 5) promoted a considerable growth in the yield of seeds (on average by 50 kg/ha in 2011–2013.) The introduction of plantafol at the shooting stage for red clover (1 kg/ha) and its additional application in the bud phase (variant 7) promoted the growth of seed performance. The combination of introduction in these phases compared to the application at the stage of shooting or in the bud phase was more efficient and promoted the increase in the yield of red clover by 25 and 8 kg/ha respectively.

The introduction of water-soluble fertilizers during red clover vegetation at the background of applying mineral fertilizers ($N_{30}P_{60}K_{60}$) under the cover crop promoted further considerable growth in the seed performance of red clover. In particular, the application of plantafol (1 kg/ha) at the shooting stage at the background of $N_{30}P_{60}K_{60}$ increased the yield of seeds by 21 kg/ha. The application of molybdenum and borium fertilizers and their combination at this background promoted the increase in this index by 12, 19, 58 kg/ha respectively. The introduction of plantafol (1 kg/ha) in the bud phase ensured the yield of red clover seeds at the level of 262 kg/ha (variant 13), which was 12 kg/ha more compared to its application at the shooting stage at the background of $N_{30}P_{60}K_{60}$ (variant 9). The combination of the introduction of water-soluble fertilizers at the shooting stage and in the bud phase at the background of mineral fertilizer did not promote a considerable growth of its seed performance (variant 14).

The application of water-soluble fertilizers at the background of introduction of mineral fertilizers ($N_{30}P_{60}K_{60}$) and liming with quickly-acting lime fertilizers ($Ca(OH)_2$) promoted further considerable growth of the yield of red clover seeds. In particular, the introduction of Plantafol in the dose of 1 kg/ha at the shooting stage at the abovementioned background (variant 16) ensured the yield of red clover seeds at the level of 307 kg/ha on average during 2011–2013. At this background the introduction of additional molybdenum (0.3 kg/ha) and borium (0.8 kg/ha) fertilizers and their combination promoted the formation of the yield of seeds respectively 316; 332; 351 kg/ha (variants 17, 18, 19) or respectively by 16; 25; 41; 60 kg/ha more compared to the variant without introduction of water-soluble fertilizers (variant 15).

The application of water-soluble fertilizers in the bud phase of red clover at the background of liming using slacked lime and mineral fertilizer $N_{30}P_{60}K_{60}$ was efficient and ensured the formation of yield at the level of 322 kg/ha on average in 2011–2013 (variant 20), which was 15 kg/ha more compared to the application of plantafol at the shooting stage (variant 16).

Water-soluble fertilizers and microfertilizers, introduced at the background of mineral fertilizers and liming using calcium fertilizers in the form of $CaCO_3$

which are hard for plants to get during the first years after the introduction or which are less efficient (variants 23–28) and ensured the formation of the yield of seeds in the investigated variants 283–320 kg/ha, which was 17–54 kg/ha more compared to the variant without additional nutrition (variant 22) and 8–10% less compared to the variants, where quickly-acting lime fertilizers were introduced (variants 15–21).

A similar phenomenon was observed in terms of seed quality. The highest germination of seeds (on average by variants) (94–95 %) was obtained in the variants with liming, whereas in the plots without fertilizers it was 91–92 % on average during the years of studies, while in the variant with the introduction of mineral fertilizers only it was 91–93 %. Liming also impacted the mass of 1,000 of seeds. The largest mass of 1,000 seeds (1.73 g) was noted in the variant with slacked lime in combination with mineral fertilizers ($N_{30}P_{60}K_{60}$), while in the variant where the main fertilization using lime and mineral fertilizers was not conducted it was 1.60 g.

Thus, the highest yield of red clover seeds (351 kg/ha) on average in 2011–2013 was obtained in the variant with the introduction of quickly-acting lime fertilizers ($Ca(OH)_2$) – 0.5 of the rate by hydrolytic acidity and mineral fertilizers ($N_{30}P_{60}K_{60}$) under the cover crop with the application of molybdenum fertilizers (0.3 kg/ha) in spring at the regrowth of red clover in combination with the introduction of water-soluble (plantafol – 1 kg/ha) and borium (0.8 kg/ha) fertilizers at the shooting stage of the crop.

CONCLUSIONS

The introduction of quickly-acting lime fertilizers in the form of $Ca(OH)_2$ (hydrated lime – slacked lime) on gray forest soil at 0.5 of rate by hydrolytic acidity prior to ploughing under the cover crop in combination with the application of mineral fertilizers in the dose of $N_{30}P_{60}K_{60}$ ensured the yield of red clover seeds in conditions of 2011–2013 at the level of 291 kg/ha, which was 115 kg/ha more compared to the plots without fertilizers and 63 kg/ha more compared to the plots, where only mineral fertilizers were introduced. At the introduction of calcium fertilizers in the form of $CaCO_3$ (defecate) these indices were 9–12 % lower.

The application of mineral fertilizers ($N_{30}P_{60}K_{60}$) under the cover crop of red clover promoted the increase in the yield of seeds by 52 kg/ha or by 23 % compared to the plots, which were not fertilized.

The most effective combination is uniting the basic fertilization with mineral fertilizers ($N_{30}P_{60}K_{60}$) and lime fertilizers ($Ca(OH)_2$) at 0.5 of the rate by hydrolytic acidity applied under the cover crop using water-soluble fertilizer (plantafol – 1.0 kg/ha) and boric fertilizers (H_3BO_4 – 0.8 kg/ha) at the shooting stage of the second cut of red clover and molybdenum fertilizers ($(CNH_4)_2MoO_4$ – 0.3 kg/ha) in spring at the beginning of regrowth of red clover, which ensured the yield of seeds at the level of 351 kg/ha or 50 % more compared to the plots without fertilizers with high sowing qualities of seeds (germination of 94–95 %, mass of 1,000 seeds – 1.73 g).

Формування насіннєвої продуктивності та посівних якостей насіння конюшини лучної залежно від дії вапнякових, мінеральних та водорозчинних добрив

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Мета. Розробка раціонального удобрення насіннєвих посівів конюшини лучної мінеральними, вапняковими та мікродобривами з метою оптимізації живлення рослин протягом їх вегетації з ціллю отримання стабільних урожаїв насіння з високими посівними та врожайними властивостями. **Методи.** Польовий, візуальний, вимірювальний, ваговий, кількісний, метод пробного снопа, лабораторний, математично-статистичний. **Результати.** Висвітлено результати оптимізації живлення насіннєвих посівів конюшини лучної на основі раціонального застосування швидкодіючих вапнякових ($Ca(OH)_2$ – 0,5 норми за гідролітичною кислотністю, мінеральних ($N_{30}P_{60}K_{60}$) та водорозчинних добрив, що забезпечило підвищення врожаю насіння в 1,8–2,0 рази на рівні 0,35–0,40 т/га. **Висновки.** Найбільш ефективним є поєднання основного удобрення мінеральними ($N_{30}P_{60}K_{60}$) та вапняковими добривами ($Ca(OH)_2$) в 0,5 нормі за гідролітичною кислотністю, внесених під покривну культуру із застосуванням молібденових добрив ($(CNH_4)_2MoO_4$ – 0,3 кг/га) весною на початку відростання конюшини лучної водорозчинних (Плантафолу – 1,0 кг/га) і борних добрив (H_3BO_4 – 0,8 кг/га) у фазу стеблування другого укосу.

Ключові слова: конюшина лучна, насіннєві посіви, урожай, вапнякові, мінеральні та водорозчинні добрива.

Формирование семенной продуктивности и посевных качеств семян клевера лугового в зависимости от действия известковых, минеральных и водорастворимых удобрений

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Цель. Разработка рационального удобрения семенных посевов клевера лугового минеральными, известковыми и микроудобрениями с целью оптимизации питания растений в течение их вегетации с целью получения стабильных урожаев семян с высокими посевными и урожайными свойствами. **Методы.** Полевой, визуальный, измерительный, весовой, количественный, метод пробного снопа, лабораторный, математически-статистический. **Результаты.** Представлены результаты оптимизации питания семенных посевов клевера лугового на основе рационального применения быстродействующих известковых ($Ca(OH)_2$ – 0,5 нормы по гидролитической кислотности, минеральных ($N_{30}P_{60}K_{60}$) и водорастворимых удобрений, что обеспечило повышение урожая семян в 1,8–2,0 раза в уровне 0,35–0,40 т/га. **Выводы.** Наиболее эффективным является сочетание основного удобрения минеральными ($N_{30}P_{60}K_{60}$) и известковыми удобрениями ($Ca(OH)_2$) в 0,5 норме по гидролитической кислотности, внесенных под покровную культуру с применением молибденовых удобрений ($(CNH_4)_2MoO_4$ – 0,3 кг/га) весной в начале отрастания клевера лугового, водорастворимых (Плантафол – 1,0 кг/га) и борных удобрений (H_3BO_4 – 0,8 кг/га) в фазу стеблевания второго укоса.

Ключевые слова: клевер луговой, семенные посевы, урожай, известковые, минеральные и водорастворимые удобрения.

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PHYSICAL-CHEMICAL COMPOSITION AND TECHNOLOGICAL PROPERTIES OF DEMINERALIZED MILK WHEY RECEIVED BY MEMBRANE METHODS

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Aim. To investigate the composition and properties of the samples of cheese and acid milk whey, obtained in industrial conditions using a combination of nanofiltration and electrodialysis methods. **Methods.** Determination of physical-chemical indices using standard methods, study of functional-technological properties of demineralized whey by common methods. **Results.** It was established that there was high efficiency of applying membrane methods for processing of secondary resources in current conditions of raw materials source, which are presented by different kinds of milk whey, formed during cheese production. It was determined that processing of different kinds of whey using the combination of nanofiltration and electrodialysis methods led to a considerable decrease in the content of ash compared to the initial whey. The level of demineralization of cheese whey may amount to 90 %, that of acid whey – 75 %. In addition to dry kinds of whey, liquid demineralized whey is of some interest for practical application, which may be used during the production of sour-milk and milk-containing drinks due to a high content of dry substances. It was found that the increase in protein content in dry demineralized whey, obtained using the complex of membrane methods of processing, led to a considerable increase in its foam-forming, moisture-retaining, fat-retaining and emulsifying abilities compared to milk whey, obtained by a traditional technology. **Conclusions.** It was established that dry demineralized whey, obtained by a combination of nanofiltration and electrodialysis methods, had better organoleptic and physical-chemical indices compared to dry whey. The investigated industrial samples were remarkable for improved functional and technological properties which allows using them in the formulations of other food products.

Keywords: nanofiltration, electrodialysis, combined membrane methods, physical-chemical indices, demineralization level, dry demineralized whey, functional-technological properties.

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INTRODUCTION

At present the developed countries have accepted a concept of the best available technologies, which means modernization of all the production with the purpose of minimizing a negative effect on environment via maximal processing of raw materials and by-products of production, reducing expenses on reagents and water, ensuring the possibility of water recirculation at enterprises. Membrane processes were proven to be successful in solving these tasks [1].

The introduction of membrane technologies at milk-processing enterprises allows enhancing the efficiency and cost effectiveness due to the economy of energy resources, more complete use of raw material resources, expanding the assortment, receiving additional profit [2].

Among modern membrane technologies, including reverse osmosis, microfiltration, ultrafiltration, nanofiltration, and electrodialysis, in Ukraine ultrafiltration, nanofiltration and electrodialysis found their practical application.

During nanofiltration (NF) there is concentration of dry substances up to 18–22 % which makes it rea-

sonable to use it with the purpose of reducing energy resources compared to whey evaporation in vacuum. Besides, from the practical standpoint the optimal variant of NF-processing is maximal removal of mineral salts and lactic acid from different kinds of milk whey with the most complete retaining of valuable whey components – proteins and lactose, and, as a result, obtaining concentrates, the technological indices of which allow using them in the production of other products [3–6].

Electrodialysis (ED) allows increasing the target indices of demineralization up to 90 %, which is especially promising for processing of salty cheese, acid and caseic milk whey [7, 8]. Any kind of whey with the application of demineralization of different level (50, 70, 90 % and above) may be standardized by physical-chemical composition and organoleptic indices, it is possible to achieve the category of quality which allows using it in baby food [2].

However, achieving a high level of demineralization is accompanied with a considerable increase in energy expenses, which is economically not substantiated [9]. Taking into consideration the fact that usually not more than 70–80 % of salts are practically removed, electrodialysis is widely used in industrial conditions while desalinizing various kinds of milk whey [10-12].

To increase efficiency, electrodialysis is combined with other membrane methods of separation [2, 13]. In particular, the combination of nanofiltration and electrodialysis is recommended not only to enhance the efficiency of whey processing technology, to economize energy resources, but also to reduce the impact of high temperatures on thermolabile components of milk whey which, at the end, enhances the biological value and improves technological properties of obtained products [14]. Such demineralized dry whey has better taste, physical-chemical characteristics and functional-technological properties compared to dry whey, obtained by traditional technology.

Taking the abovementioned into consideration, one may assume that the use of a complex of membrane methods allows increasing the quality of milk whey processing in conditions of a dairy enterprise compared to their separate application. This technology was successfully implemented at some dairy enterprises.

MATERIALS AND METHODS

Cheese and acid whey, obtained during the production of cheese or lactic cheese and the corresponding kinds of whey after nanofiltration and electrodialysis, were used in the work. Demineralization of milk whey was conducted at experiment electrodialysis (MEGA, Czech Republic) and nanofiltration equipment (GEA, Denmark). Dry samples were obtained by drying the corresponding kinds of whey on spray dryer.

The mass content of moisture in dry products was defined by the standardized method, which is based on the ability of the product to lose free moisture while drying at constant temperature – (102 ± 2) °C. The arithmetic mean value of the results of two parallel measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.06 %.

The mass content of ash was determined by the method, based on ashing 2.8–3.2 g of dry product at the temperature of (525 ± 25) °C. The mass content of ash in percentage *(X)* was calculated according to the formula:

$$X = \frac{(m_1 - m_2)}{m} \cdot 100, \qquad (1)$$

where m_1 – mass of a pot with the ashes of the product after ashing, g; m_2 – mass of an empty pot after calcination, g; m – mass of the weighed quantity of product, g; 100 – coefficient of transferring grams into percentage.

The arithmetic mean value of the results of two parallel measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.1 %.

The mass content of fat was determined by the standardized acid method, based on extracting fat from dry products under the impact of concentrated sulphuric acid and isoamil alcohol with further centrifugation and measuring the volume of fat in the calibrated part of butyrometer. The arithmetic mean value of the results of two parallel measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.5 % on condition that the results were within one lowest graduation mark of the butyrometer.

The mass content of lactose was determined by the standardized iodometric method in the weighed quantity of the product of 3.0 g. The arithmetic mean value of the results of two parallel measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.2 %.

The acidity of dry whey was determined by the standardized titrimetric method using 0.1 mol/cu dm. The arithmetic mean value of the results of two parallel

measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.5 %.

The solubility index was determined in one cubic centimeter by the method, based on measuring the volume of insoluble precipitation in the restored sample of dry whey after centrifugation at 8,000 rpm for 5 min. The arithmetic mean of the results of two parallel measurements of one sample was accepted as the final result, the permissible differences between them could not exceed 0.1 %.

The foam-forming capability of dry samples was determined by the relative increase in their solution volumes after shaking. For this purpose, a chemical glass was added weighed 25 g of dry product, and 225 g of distilled water with the temperature of 20 °C. The samples of 250 cu cm were shaken for 5 min at the frequency of shaker rotation of 800 rpm. After shaking the volume of liquid fraction and the volume of obtained foam were measured by a measuring glass cylinder. The foam-forming capability (*C*, %) was determined by the formula:

$$C = \frac{V_f \cdot 100}{V_m}, \quad (2)$$

where, V_f – volume of foam after shaking, cc; V_m – the initial volume of the mixture prior to shaking, cc; 100 – coefficient of transferring into percentage.

The arithmetic mean value of the results of two parallel measurements was accepted as the final result after rounding down to the first decimal figure.

The moisture-retaining capability of dry products was determined by the increase in the mass of wet precipitate after centrifugation. A previously weighed centrifugal tube was introduced a weighed quantity in the amount of 1 g and 3 cc of distilled water. The mixture was mixed for 1 min. Then the tube was centrifuged at 8,000 rpm for 15 min. The liquid, which was above the precipitate, was poured out, the tube was turned over the filtration paper and left undisturbed for 10 min (to remove the remaining water) and weighed. The moisture-retaining capability (*MRC*, %) was calculated by the formula:

$$MRC = \frac{C–B}{B–A} \cdot 100, \quad (3)$$

where, *A* – mass of an empty centrifugal tube, g; *B* – mass of centrifugal tube with the weighed quantity of dry matter, g; *C* – mass of centrifugal tube with precipitation after centrifugation, g.

The arithmetic mean value of the results of two parallel measurements was accepted as the final result after rounding down to the first decimal figure.

The fat-retaining capability of the investigated products was estimated using the emulsion solutions with refined oil. A previously weighed centrifugal tube was introduced a weighed quantity of dry product in the amount of 1 g and 3 cc of refined oil. The mixture in the tube was mixed for 1 min. Then the tube was centrifuged at 8,000 rpm for 15 min. The liquid, which was above the precipitate, was poured out, the tube was turned over the filtration paper and left undisturbed for 10 min (to remove the remaining refined oil) and weighed.

The fat-retaining capability (*FRC*, %) was calculated by the formula:

$$FRC = \frac{C–B}{B–A} \cdot 100, \quad (4)$$

where, *A* – mass of an empty centrifugal tube, g; *B* – mass of centrifugal tube with the weighed quantity of dry matter, g; *C* – mass of centrifugal tube with precipitation after centrifugation, g.

The emulsifying capability of the investigated products was estimated using the emulsion solutions with refined oil. The chemical glass with the volume of 500 cc was introduced 7 g of dry product and 100 cc of distilled water. The mixture was mixed using the mixer at 4,000 rpm for 5 min with subsequent addition of 100 cc of refined oil and the mixing was continued at 8,000 rpm for 5 min. The emulsion was poured in equal parts into 4 calibrated centrifugal tubes with the volume of 10 cc and centrifuged at 2,000 rpm for 5 min. The emulsifying capability (*EC*, %) was calculated by the formula:

$$EC = \frac{V}{V_1} \cdot 100, \quad (5)$$

where, *V* – volume of the liquid above the precipitate, cc; V_1 – total volume of centrifugal tube (10 cc); 100 – coefficient of transferring into percentage.

The arithmetic mean value of the results of two parallel measurements was accepted as the final result after rounding down to the first decimal figure.

The mathematical processing of the results was conducted by methods of statistical analysis and standard algorithms of Microsoft Excel programs. The experiments were conducted in three repeats. The results were deemed to be reliable at $P < 0.05$.

RESULTS AND DISCUSSION

Our previous studies established that during electrodialysis the mass share of ash in the initial whey decreased in the range from 0.56–0.71 % to 0.02–0.08 % after electrodialysis, depending on the kind of whey and the initial content of ash therein. The maximal decrease in the content of mineral salts in cheese milk whey was achieved using nanofiltration at the level of 40 % [9, 10]. Regardless of different levels of demineralization, there was the most considerable decrease noted in the content of monovalent ions which led to improving organoleptic properties of dry whey [10]. This whey may be considered to be full value raw material during the production of other food products – cooked sausages, yogurts, ice-cream, cheese paste, cheeses, *etc*.

Taking the abovementioned into consideration, there was a study of the impact of combined application of membrane methods of processing whey on the composition and technological properties of the end products. Being the most common by-products of milk processing, formed during the production of cheese and sour-milk cheese, cheese and acid milk whey are usually processed by drying. Thus, the most attractive and economically grounded method is a possibility of improving the consumer properties of such dry products due to a high content of complete whey proteins therein.

The organoleptic and physical-chemical indices of liquid and dry products of processing cheese and acid whey were determined. It is noteworthy that in addition to dry kinds of whey, liquid demineralized whey with the mass share of dry substances of ≈ 20 % is of some interest for practical application, for instance, for the production of sour-milk beverages. As noted above, the decrease in the content of ash in whey improves its taste properties considerably. The data, presented in Table 1, demonstrate that after electrodialysis the indices of the mass share of ash (1) decreased in cheese and acid whey by 21.2 and 62.7 % and after the treatment using both methods – by 9.6 and 14.7 % respectively, compared to the initial content in the initial whey. The same tendency was remarked regarding the acidity indices as well: the values decreased for cheese and acid whey (after electrodialysis) 1.8 times and 4.2 times and 1.2 times and 2.7 times respectively, after the combination of treatment methods. Therefore, the decrease in the content of salts and lactic acid leads to improving organoleptic and physical-chemical indices of the end products.

It was established that during the treatment of whey with nanofiltration or during complex treatment with nanofiltration and further electrodialysis, the content of dry substances in liquid concentrate increased to 19... 20 %. This intermediate product of whey processing is full value raw material and may be used for normalization of milk mixtures while producing other milk products, sour milk beverages, *etc*.

It is obvious that the application of any method of processing whey or their combination allows improving the properties of the initial raw material considerably due to decreasing the content of ash (Table 2). For instance, after consecutive treatment using the methods of nanofiltration and electrodialysis, the indices of ash in dry whey (NF/ED) decreased 2.8 times in case of using cheese whey as the initial raw material and 3 times – in case of acid whey. The demineralization level during electrodialysis may reach 86.5 % for cheese whey and 95.8 % for acid whey, and during the treatment using the combination of methods – up to 90 % and 75 %.

To estimate the possibility of using dry demineralized whey (NF/ED), there was a determination of its function-

Table 1. The physical-chemical indices of liquid products of processing cheese and acid whey after different methods of treatment

Index	Whey initial		Liquid concentrate after nanofiltration (NF)		Dilute after electrodialysis (ED)		Liquid concentrate combined method of treatment (NF/ED)	
	cheese	acid	cheese	acid	cheese	acid	cheese	acid
Mass share of dry substances, %	6.67	5.77	19.43	15.56	6.04	5.48	20.03	17.5
Mass share of ash, %	0.52	0.75	1.0	1.1	0.41	0.28	0.47	0.64
Mass share of lactose, %	4.50	4.02	15.20	10.43	4.90	5.0	6.71	5.92
Mass share of fat, %	< 0.1	< 0.1	0.1	0.1	< 0.1	< 0.1	0.1	0.1
Titrated acidity, 0T	14.5	75.6	10.0	120	8.0	18	12.0	28

al-technological indices – foam-forming (2), moisture-retaining (3), fat-retaining (4) and emulsifying (5) properties (Table 3). These properties characterize the ability of whey proteins to participate in surface phenomena and are most widely used while obtaining products on the basis of foam-like and emulsion systems. It is evident that such differences are possible due to the increase in protein content in dry demineralized whey.

The presented data demonstrate that the highest indices of moisture-retaining and fat-retaining capability were found for cheese whey, obtained by the combination of treatment methods, namely, 32.5 % and 120 % respectively. It is quite evident that it is due to the increased content of protein and the ability of whey proteins to bind water, emulsify and retain fats, dry cheese demineralized whey has better technological properties. A similar regularity was noted for foam-retaining capability as well.

Summarizing the abovementioned, one may assume that dry demineralized whey may be used as full value

Table 2. The characteristics of dry milk whey after treatment with different membrane methods

Name of indices	Dry whey (traditional technology)		Dry demineralized whey				
	whey cheese	acid whey	cheese after nanofiltration (NF)	cheese after electrodialysis (ED)	acid after electrodialysis (ED)	cheese combination of treatment methods (NF/ED)	acid combination of treatment methods (NF/ED)
Mass content of dry substances, %	97.00	95.19	97.87	95.06	94.52	97.00	94.80
Mass content of ash, %	7.27	8.29	5.10	1.55	2.82	2.63	2.72
Mass content of lactose, %	74.5	73.03	76.20	82.60	79.93	65.92	63.73
Mass content of fat, %	1.50	1.6	0.5	0.5	1.0	1.0	1.0
Mass share of protein, %	12.57	11.10	15.86	8.98	9.77	27.02	26.10
Titrated acidity, 0T	14.0	75.0	9.5	8.0	25	12.0	18.0
Index of solubility cc of wet precipitate	0.3	0.5	0.45	0.1	0.2	complete solubility	0.1
Level of demineralization, %	–	–	43.35	86.5	95.8	90.0	75.0
Organoleptic indices							
Consistence	Fine powder						
Taste and smell	Sweetish-salty taste	Sour-salty taste	Sweetish taste	Neutral taste with sweetish flavor	Neutral taste with slight sour flavor	Sweet milk taste	Milk taste with slight sour flavor
Color	Light yellow color						

Table 3. The functional-technological properties of dry whey

Name of product	Foam-forming ability, %	Moisture-retaining ability, %	Fat-retaining ability, %	Emulsifying ability, %
Dry cheese whey (control)	5.8 ± 0.6	12.2 ± 0.1	83.0 ± 0.6	27.0 ± 0.4
Dry acid whey (control)	4.3 ± 0.2	14.6 ± 0.1	79.0 ± 0.2	29.0 ± 0.1
Dry cheese whey (NF/ED), demineralization level 90 %	15.6 ± 0.2	32.5 ± 0.01	120.0 ± 0.2	33.0 ± 0.2
Dry acid whey (NF/ED), demineralization level 75 %	11.9 ± 0.1	27.4 ± 0.02	107.3 ± 0.1	31.8 ± 0.1

replacement of dried skimmed milk and dry whey in the formulations of other food products with the purpose of improving their consumer and functional-technological properties.

RESULTS

It was established that there was high efficiency of applying membrane methods for processing of secondary resources of milk raw materials in current conditions of raw materials source, which are presented by milk whey, formed during cheese production. It was determined that processing of different kinds of whey using the combination of nanofiltration and electrodialysis methods led to a considerable decrease in the content of ash compared to the initial whey. The level of demineralization of cheese whey may amount to 90 %, that of acid whey – 75 %. In addition to dry kinds of whey, liquid demineralized whey is of some interest for practical application, which may be used during the production of sour-milk and milk-containing drinks due to a high content of dry substances. It was found that the increase in protein content in dry demineralized whey, obtained using the complex of membrane methods of processing, led to a considerable increase in its foam-forming, moisture-retaining, fat-retaining and emulsifying abilities compared to milk whey, obtained by a traditional technology.

CONCLUSIONS

It was established that dry demineralized whey, obtained by a combination of nanofiltration and electrodialysis methods, had better organoleptic and physical-chemical indices compared to dry whey. The investigated industrial samples are remarkable for improved functional and technological properties which allows using them in the formulations of other food products.

Фізико-хімічний склад та технологічні властивості сироватки молочної демінералізованої, отриманої мембранними методами

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Мета. Провести дослідження складу та властивостей зразків підсирної та кислої молочних сироваток, отриманих в промислових умовах із застосуванням комбінації методів нанофільтрації та електродіалізу. **Методи.** Визначення фізико-хімічних показників за стандартними методами, функціонально-технологічні властивості сироватки демінералізованої за загальноприйнятими методиками. **Результати**. Відмічено високу ефективність застосування мембранних методів для переробки вторинних ресурсів в існуючих умовах сировинної бази, якими на сьогоднішній день є різні види сироватки молочної, що утворюються під час виробництва сирів. Встановлено, що обробка різних видів сироватки із використанням комбінації методів нанофільтрації та електродіалізу призводить до значного зменшення вмісту золи у порівнянні з вихідною сировиною. Рівень демінералізації підсирної сироватки може досягати 90 %, кислої сироваток – 75 %. Крім сухих видів сироватки певну зацікавленість для практичного застосування має рідка демінералізована сироватка, яка завдяки високому вмісту сухих речовин може використовуватися під час виробництва кисломолочних та молоковмісних напоїв. Встановлено, що зі збільшенням вмісту білка у сухій демінералізованій сироватці, отриманій за допомогою комплексу мембранних методів обробки, її піноутворююча, вологоутримуюча, жироутримуюча та емульгуюча здатність у порівнянні із сироваткою молочною, отриманою за традиційною технологією, істотно зростає. **Висновки.** Встановлено, що суха демінералізована сироватка, одержана із використанням комбінації методів нанофільтрації та електродіалізу, має кращі органолептичні та фізико-хімічні показники у порівнянні з сироваткою сухою. Досліджені промислові зразки характеризуються покращеними функціонально-технологічними властивостями, що дозволяє використовувати їх під час виробництва інших харчових продуктів.

Ключові слова: нанофільтрація, електродіаліз, комбіновані мембранні методи, фізико-хімічні показники, рівень демінералізації, сироватка демінералізована суха, функціонально-технологічні властивості.

Физико-химический состав и технологические свойства сыворотки молочной деминерализованной, полученной мембранными методами

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Цель. Провести исследования состава и свойств образцов подсырной и кислой молочных сывороток, полученных в промышленных условиях с применением комбинации методов нанофильтрации и электродиализа. **Методы.** Определение физико-химических показателей за стандартными методами, функционально-технологические свойства сыворотки деминерализованной по общепринятым методикам. **Результаты**. Отме-

чено высокую эффективность применения мембранных методов для переработки вторичных ресурсов молочного сырья в существующих условиях сырьевой базы, которыми на сегодняшний день является сыворотка молочная, которая получается при производстве сыров. Установлено, что обработка различных видов сыворотки с использованием комбинации методов нанофильтрации и электродиализа приводит к значительному уменьшению содержания золы по сравнению с исходным сырьем. Уровень деминерализации подсырной сыворотки может достигать 90 %, кислой сыворотки – 75 %. Кроме сухих видов сыворотки определенную заинтересованность для практического применения имеет жидкая деминерализованная сыворотка, которая благодаря высокому содержанию сухих веществ может использоваться при производстве кисломолочних и молокосодержащих напитков. Установлено, что с увеличением содержания белка в сухой деминерализованной сыворотке, полученной с помощью комплекса мембранных методов обработки, ее пенообразующая, влагоудерживающая, жироудерживающая и эмульгирующая способность по сравнению с сывороткой молочной, полученной по традиционной технологии, существенно возрастает. Выводы. Установлено, что сухая деминерализованная сыворотка, полученная с использованием комбинации методов нанофильтрации и электродиализа, имеет лучшие органолептические и физико-химические показатели по сравнению с сывороткой сухой. Исследованные промышленные образцы характеризуются улучшенными функционально-технологическими свойствами, что позволяет использовать их при производстве пищевых продуктов.

Ключевые слова: нанофильтрация, электродиализ, комбинированные мембранные методы, физико-химические показатели, уровень деминерализации, сыворотка деминерализованная сухая, функционально-технологические свойства.

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IMPACT OF FEEDING MALE RATS F_2 WITH DIFFERENT DOSES OF GERMANIUM CITRATE ON THE CONTENT OF TRACE ELEMENTS IN THEIR TISSUES AND ORGANS

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Aim. To determine the impact of the dose of germanium citrate on the distribution and concentration of the trace elements Fe, Zn, Cu, Co, Mn in tissues and organs of male F_2 rats. **Methods**. Standard physiological, biochemical (including atomic absorption spectrometry), clinical and statistical methods were applied. **Results**. It was established that there were changes in the content of Fe, Zn, Cu, Co, Mn in soft tissues and their distribution in liver, kidneys and lungs of male F_2 rats. It was demonstrated that these were mostly changes in organ-tissue specific functioning of some physiological systems, for instance, hepatorenal and respiratory systems of the organism as induced with a few exceptions independent of the different doses of germanium (10, 20 and 200 μg/kg of bodyweight). The differences were most apparent in kidneys and less in liver and lungs. The doses of 10, 20 and 200 μg Ge, and those of Fe – 20 and 200 μg – caused higher concentrations of Cu, Co, Mn and Zn in muscle tissues. The differences in the weight of liver, kidneys and lungs of rats of experimental and control groups were determined in order to eliminate intergroup differences and to obtain the absolute content of the investigated trace elements in liver, kidneys and lungs. The mentioned differences were more expressed for the absolute content of Cu in liver and for Mn in kidneys and lungs. **Conclusions**. Long-term introduction of oral aqueous germanium citrate into the organism of a F_2 generation of rats at 10, 20 and 200 μg Ge kg-1 of the bodyweight is characterized by the changes in the content of Cu, Co, Mn, Fe, Zn both per one unit of soft tissue weight and their absolute content in the internal organs. The biological effect of germanium citrate is expressed more in the high dose of 200 μg Ge/kg of the bodyweight, conditioning the increase in the content of Cu (from 51 to 95 %) and Zn from 22 to 78 % in all the investigated tissues of rats of this group. There was a decreased level of Co in liver at the effect of 20 and 200 μg Ge, and at the effect of all the administered doses in kidneys and lungs. The level of Mn increased by 27.7; 74.0 and 23.4 % in groups II, III and IV respectively in the muscle tissues of male F_2 rats at the effect of all the administered doses of Ge, Co 20 and 200 μg, Fe 10 and 20 μg, and Zn 10 and 200 μg Ge, which testifies to the differences in the regulatory impact of NGeC on the level of investigated trace elements in the muscle tissues of rats.

Keywords: trace elements, soft tissues, muscle.

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INTRODUCTION

It is known that the uptake and incorporation of trace elements in the organism of humans and animals is determined by a number of physiological mechanisms, including synergetic or antagonistic interactions. These interactions have been firmly established for most macro- and trace elements, and they are normalized in nutrition [1–3]. The regulatory impact of physiologically active, but insufficiently studied elements, among which germanium takes a prominent place, on organ-tissue distribution of other minerals, is presently actively studied in biology, medicine, and veterinary science [3, 4]. Organic formulations of macro- and trace elements (nanoaquachelates) as obtained by nanotechnology methods receive special attention [5, 6]. The

biological role of these organo-compounds and their interaction with other macro- and trace elements and their impact on their distribution in the organism and its organs are actively studied as well. In particular, our earlier research [7, 8] showed that the administration of germanium citrate, obtained via the erosive and explosive ablation with electro-impulse, caused a number of biological effects in the organism of rats. This may have found its cause in the fact that germanium (Ge) has an immuno-stimulating effect, enhances transport and transfer of O_2 and ensures the decrease of hypoxia at tissue level [3, 4]. Certain organic forms of Ge have negatively charged oxygen ions that can scavenge free damaging hydrogen ions and minimize their damage to cells and tissues [9]. A notable characteristic of organic forms of Ge is that they are removed fast with urine from the organism, which indicates its low accumulation in tissues [4, 10]. Different concentrations and duration of Ge intake affect physiological-biochemical processes in the organism differently, including their influence on the level of macro- and trace elements in tissues [3, 6, 7]. Recent experimental studies on physiological mechanisms of the effect of different doses of germanium citrate on organ-tissue and systemic level [6, 7, 9] and preparations, including Astrogerm, Germatranol, Germavit, elaborated on the basis of this chelate complex, stimulated a profound investigation of this compound on the intake of such vital elements as Cu, Co, Mn, Fe, Zn in the organism, the results of which are reported in this article.

MATERIALS AND METHODS

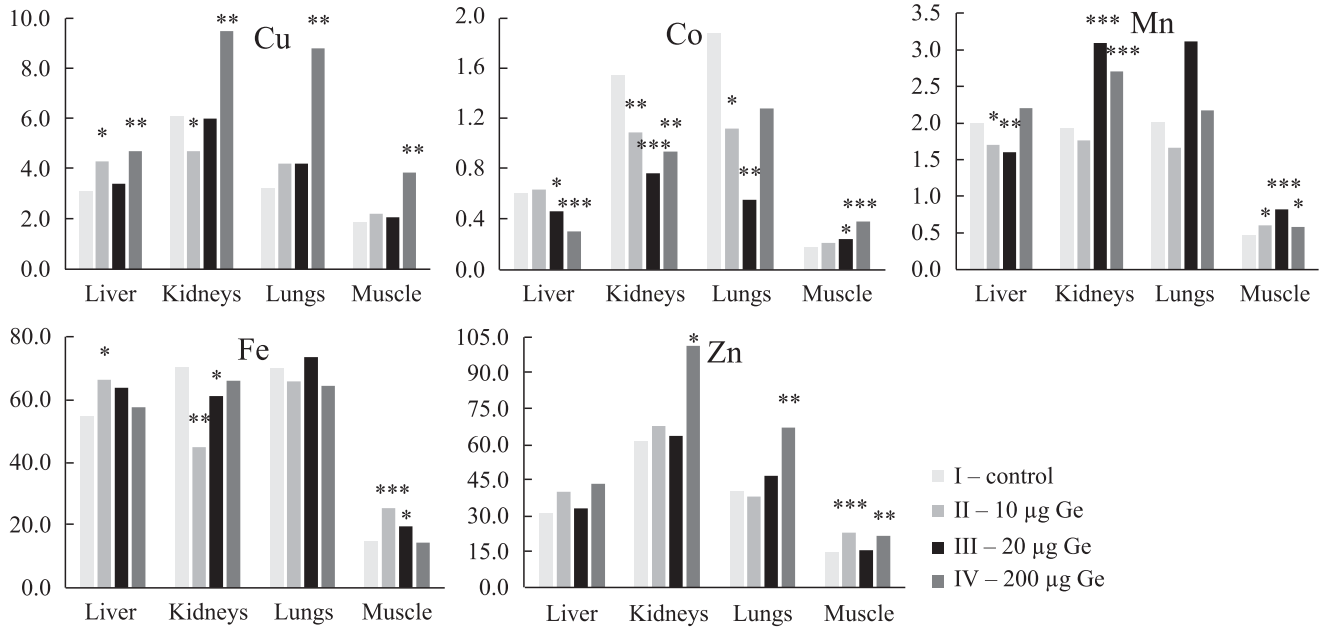
The studies were conducted using white laboratory male F_2 rats, divided into one control (I) and three experimental (II, III, IV) groups, 4 animals in each. Contrary to the control group, the rats of experimental groups daily received the addition of nanogermanium citrate (NGeC), manufactured by the nanotechnology method [11, 12], with drinking water, calculated as 10 (experimental group II), 20 (III) and 200 (IV) μg Ge/kg of the bodyweight. Feeding female rats of generations F_0 and F_1 with germanium citrate in the mentioned doses during their ontogenesis and pregnancy, and feeding young rats of respective groups F_1 and F_2 with milk of the germanium-fed mothers was conducted. The effect of germanium citrate on the organism of young F_2 rats was revealed at the stages of embryonic, fetal and preweaning period of development via mothers' blood and milk as well as via absorption in the digestive tract after the start of independent consumption of feeds and water. At the age of 4–4.5 months, 4 male rats from each group were decapitated after narcosis to study their internal organs. The content of Fe, Zn, Cu, Mn, Co in homogenates of tissues of liver, kidneys, lungs and femoral muscle was determined after dry ashing in the muffle furnace at 450–500 °C and dissolving the mineral residue in 10 % HCl. The trace elements were detected during the period of burning their acid solutions in the acetylene flame, using an atomic absorption spectrophotometer SF-115 PC (Selmi, Ukraine) with the software for concentration calculation, as described in [13]. The obtained results were statistically processed using MS Excel and determining the mean values (M), and their deviations, where standard deviation = standard error of the mean (± m SD), and the probability degree by Student's coefficient ($P \leq 0.05$). The obtained mean results of the experimental groups were compared against those of the control group.

RESULTS AND DISCUSSION

The analysis of the obtained results indicated unevenly directed changes in the content of the investigated trace elements in the tissues of internal organs and muscle tissue of F_2 males depending on the dose of NGeC. In particular, a higher content of Cu was 38.7 and 51.6 % detected in liver tissues of rats at 10 ($P < 0.05$) and 200 ($P < 0.01$) μg Ge/kg of the bodyweight, and the elevated Fe of 21.3 % at 10 μg ($P < 0.05$) with the preservation of this tendency for Fe also for males receiving 20 – 16.7 % and less so for those receiving 200 μg – 5.3 % (Figure).

The content of Co decreased by 23.3 ($P < 0.05$) and 50 % ($P < 0.001$) in liver tissues of males receiving 20 and 200 μg. The content of Mn decreased also by 15 and 20 % at the dose of 10 and 20 μg Ge. No significant differences in Mn content were detected in the tissues of liver, kidneys and lungs of F_2 rats with all dose rates applied, which was also noted by other researchers [2, 3]. It is known that Mn is found in all the tissues and liquids of the organism without considerable organ-, species- or age-related differences. The increase in Mn concentration in the liver of rats was noted at the effect of 5 pg Ge in the form of sodium germanate [3]. There was a confirmed impact of this compound on the mineral exchange via functioning of the main regulatory enzymes, activated by Mn – hydrolases, kinases, decarboxylases.

In the processes of absorption from the intestines, Mn competes with Co for the binding sites, whereas the mechanisms of absorption of Mn and Fe are similar and there is no competition [2]. The differences in Co



The content of trace elements in the tissues of male F_2. Note: Statistically significant differences from the control group I in Figure and Table are indicated as *– $p \leq 0.05$; ** – $p \leq 0.01$, *** – $p \leq 0.001$

content in the tissues of internal organs, noted for F_2 rats, were also established for male F_1 rats at the age of 2–2.5 months. In particular, the content of Co in liver tissues of male F_1 rats at the doses of 20 and 200 μg Ge was 40–50 % lower as compared to the control group [7]. The mentioned regularity of Co content in liver tissues of F_1 and F_2 rats at the effect of these doses of NGeC in male F_1 rats was also preserved for Co content in lung tissue of animals of generation F_2. In particular, the effect of germanium was revealed in the decrease by 40.3 and 70.4 % in the content of Co in lung tissues of rats of groups II ($P < 0.05$) and III ($P < 0.01$) and the decrease by 32 % – group IV.

A significantly lower content of Co (by 29.4; 50.3 and 39.2 %) was found in the tissues of kidneys of male F_2 rats of all the experimental groups, Cu and Fe – at the effect of 10 and 20 μg Ge at the background of a higher level of Mn ($P < 0.001$) in groups III and IV and Cu ($P < 0.01$) and Zn ($P < 0.05$) in male rats of group IV, which received 200 μg Ge. NGeC had a more visible inhibiting effect on the content of Fe and Co in the tissues of kidneys and on the content of Co in liver tissues at the effect of 20 and 200 μg Ge, and as for Mn – 10 and 20 μg Ge in liver, which may impact the hematopoietic ability of the organism of male F_2 rats as follows: it is known that Co enhances the intake of Fe and synthesis of hemoglobin, stimulating erythropoiesis. Co negatively affects the synthesis of proteins and repair of S-S group sulphur bridges, that operate in the processes of blocking and detoxification of poisonous elements in the organism [2, 3].

A less clear regulatory effect of NGeC compared to the liver tissue on the content of the investigated elements was noted for the tissues of lungs, but the content of Zn and Cu in lung tissue was also 66.9 and 175 % higher ($P < 0.05$; $P < 0.01$) for male rats of group IV. It is remarkable that the content of Cu in lung tissue of male rats of group IV was 27.5 % higher ($P < 0.01$) against the control compared to the tissues of liver, kidneys and muscles. It may indicate a stimulating effect of NGeC in the dose of 200 μg Ge on the intensity of Cu metabolism in the organism and as a result, the level of this microelement in the internal organs and muscles of rats.

The increase in the content of Cu in the tissues of liver, kidneys, lungs and muscles of rats may condition enhanced redox processes and supply of these tissues with O_2, which was also noted by other researchers [3, 14, 15, 16]. It is known that a high level of Cu in the tissues of the organism stimulates the processes of antioxidant protection with the participation of its incorporation into enzymes involved in these processes.

The content of trace elements in the tissues of femoral muscle of rats was found to be 2–10 times different from that of the tissues of internal organs. For instance, a higher content (by 27.7; 74.0 and 23.4 %) of Mn for males of all the experimental groups was noted in the

samples of muscle tissues at the effect of low (10 μg), medium (20 μg) and high (200 μg) doses of Ge, and Zn by 56.3 and 46.7 % – 10 ($P < 0.001$) and 200 μg ($P < 0.01$), see Table. The content of Fe was also considerably higher in the muscle tissues at the effect of 10 and 20 μg Ge, and as for Cu – 200 μg ($P < 0.01$).

The absolute content of the trace elements Cu, Co, Mn, Fe and Zn was also studied in the liver, kidneys and lungs in relation to the weight of these organs. The weight of the organs, except for kidneys in group IV, in males of all experimental groups showed a tendency to decrease in the range from 7.3 to 22.6 % which was statistically significant at least for liver in group II ($P < 0.01$) and III ($P < 0.05$), and for lungs in group III ($P < 0.05$) (Table). In particular, a lower content (from 35.7 to 56.7 %) of Co was found in the liver of rats in groups III ($P < 0.01$) and IV ($P < 0.001$) and for Mn in groups II and III ($P < 0.001$). To the contrary, a higher level of Zn was found in animals of group IV ($P < 0.05$). The absolute content of Cu in the liver of male rats of group IV increased from 34.9 % ($P < 0.05$).

The absolute content of the investigated trace elements in the kidneys of rats mainly preserved the direction of differences between the control and experimental groups relative to their level in mg/kg of the weight of the tissue (Figure). The absolute content of Mn in the kidneys of males of group II at the effect of a low (10 μg) dose of NGeC, however, was 15.5 % lower as compared to that of the control. It may be conditioned by the impact of lower indices of Mn content in these tissues and a smaller weight of this organ by 7.3 % in rats of group II. The absolute content of Mn in kidneys of males of groups III and IV was 47.8 and 54.8 % higher respectively ($P < 0.001$) at an insignificantly increased level (by 10.7 %) of the weight of this organ as compared to that of the control. A statistically significant higher absolute content of Mn in the kidneys of males of group III and IV was demonstrated at the expressed impact of 20 and 200 μg Ge of NGeC

The absolute content of trace elements in some internal organs of male F_2 rats at the age of 4 months, (mean, M and standard deviation, ± m; number of rats, $n = 4$)

Groups	Organ weight, g	Content of the microelements, μg				
		Cu	Co	Mn	Fe	Zn
Liver						
Control – I	8.97 ± 0.46	27.5 ± 3.82	5.43 ± 0.27	17.9 ± 0.81	489.6 ± 27.7	489.6 ± 27.7
Experimental/dose of Ge, μg						
II – 10	6.94 ± 0.45**	29.5 ± 3.03	4.39 ± 0.35	12.0 ± 0.52***	459.5 ± 23.1	278.4 ± 22.5
III – 20	7.59 ± 0.39*	25.6 ± 4.45	3.49 ± 0.18**	11.9 ± 0.36***	483.6 ± 26.0	251.4 ± 33.5
IV – 200	7.92 ± 0.26	37.1 ± 2.59	2.35 ± 0.12***	17.5 ± 0.75	455.1 ± 28.0	344.2 ± 63.2*
Kidneys						
Control – I	1.78 ± 0.08	10.8 ± 0.61	2.72 ± 0.14	3.43 ± 0.13	124.9 ± 5.1	109.2 ± 17.7
Experimental/dose of Ge, μg						
II – 10	1.65 ± 0.07	7.7 ± 0.46**	1.77 ± 0.12**	2.90 ± 0.04**	73.9 ± 6.3***	111.7 ± 15.3
III – 20	1.64 ± 0.04	9.8 ± 0.60	1.25 ± 0.06***	5.07 ± 0.09***	100.1 ± 3.4**	104.4 ± 9.9
IV – 200	1.97 ± 0.04	18.8 ± 0.97***	1.83 ± 0.13**	5.31 ± 0.15***	129.9 ± 7.4	199.8 ± 10.1*
Lungs						
Control – I	1.55 ± 0.09	5.0 ± 0.33	2.88 ± 0.33	3.11 ± 0.11	108.4 ± 10.6	62.2 ± 3.69
Experimental/dose of Ge, μg						
II – 10	1.35 ± 0.09	5.6 ± 1.19	1.50 ± 0.01*	2.24 ± 0.20*	88.7 ± 7.8	51.4 ± 7.64
III – 20	1.30 ± 0.06*	5.4 ± 0.74	0.72 ± 0.22**	4.04 ± 1.07	95.4 ± 7.5	60.8 ± 9.05
IV – 200	1.36 ± 0.08	11.9 ± 1.19**	1.72 ± 0.39	2.95 ± 0.14	87.5 ± 6.1	91.3 ± 7.29*

on the increase in Mn content in kidneys. This effect is also confirmed by the increase ($P < 0.001$) in Mn content in the tissues of kidneys (in mg/kg) of males from groups III and IV (Figure).

Statistically significant differences in the absolute content of the investigated trace elements were preserved in the lungs of rats of experimental groups similarly to those as per one unit of tissue weight in mg/kg, presented in Figure. The detected changes demonstrated that the absolute content of Mn in the lungs of group II males showed a significant decrease similarly to the liver and kidneys. It was conditioned both by the lower level of this element in the lung tissue and by the tendency to the decrease in the weight of this organ (12.1 %) compared to the control. However, a lower index of the weight of lungs in animals of group IV had no considerable impact on the significant increase in the absolute content of Zn in this organ, as the content of Zn in the lung tissues of rats of this group was 46.9 % higher ($P < 0.01$). It should be noted that a high (200 μg) dose of Ge conditioned a significant increase in the absolute content of Zn in the liver, kidneys and lungs, Cu – in the kidneys and lungs, Mn – in the kidneys.

CONCLUSIONS

Thus, the introduction of germanium citrate for 120–135 days, obtained via electric impulse ablation and its administration with drinking water in the amounts of 10, 20 and 200 μg Ge/kg bodyweight into F_2 rats is characterized by the changes in the content of Cu, Co, Mn, Fe, Zn both per unit of soft tissue weight and their absolute content in the entire internal organs. The biological effect of germanium citrate is more expressed in the high dose of 200 μg Ge/kg than in the lower doses conditioning the increase in the content of Cu from 51 to 95 % and Zn from 22 to 78 % in all the investigated tissues of rats. There is a decrease of Co by 40.3 % in the liver at 20 and by 70 % at 200 μg Ge, and at all the administered doses from 27 to 50 % in kidneys and 33–310 % in lungs. The level of Mn increased from 23.4 to 74 % respectively in the muscle tissues of male F_2 rats at all administered doses of Ge, for Fe it was respectively for the doses of 10 ($P < 0.001$) and 20 μg ($P < 0.05$), and finally for Zn respectively for the doses of 10 ($P < 0.001$) and 200 μg Ge ($P < 0.01$). There was a statistically significant increase in the content of Cu and Zn in all the investigated tissues and organs of F_2 rats at the effect of 200 μg Ge, which may indicate enhanced accumulation of Cu and Zn in the organism of rats at long-term (F_0-F_1-F_2) intake of germanium citrate with drinking water.

Вплив випоювання різних доз германію цитрату на вміст мікроелементів у тканинах та органах самців щурів F_2

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Мета. З'ясувати вплив дози германію цитрату на розподіл Fe, Zn, Cu, Co, Mn у тканинах та органах щурів-самців F_2. **Методи.** Фізіологічні, біохімічні, клінічні, статистичні. **Результати.** Встановлені зміни вмісту Fe, Zn, Cu, Co, Mn у м'яких тканинах та їх розподілу у печінці, нирках і легенях самців щурів F_2. Показано, що ці зміни зумовлюються в більшій мірі органо-тканинними особливостями функціонування окремих фізіологічних систем організму, зокрема гепато-ренальної і дихальної, а в меншій – дозою Германію (10, 20 і 200 мкг/кг м. т.). Більше виражені зміни вмісту цих елементів встановлені для нирок за дії всіх застосованих доз, а менше – печінки і легень. У тканинах м'яза відзначено позитивний вплив германію цитрату на вміст Cu, Co, Mn і Zn за дії 10, 20 і 200 мкг Ge, а Fe – 20 і 200 мкг. Встановлені різниці маси печінки нирок і легень щурів дослідних і контрольної груп, що нівелювало міжгрупові відмінності абсолютного вмісту досліджених мікроелементів у печінці, нирках і легенях. Вказані відмінності більше виражені для абсолютного вмісту Cu у печінці, Mn у нирках і легенях. **Висновки**. Тривале надходження в організм щурів F_2 з водою германію цитрату в кількості 10, 20 і 200 мкг Ge/кг м. т. характеризується змінами вмісту Cu, Co, Mn, Fe, Zn як на одиницю маси м'яких тканин, так і абсолютного вмісту їх у внутрішніх органах. Біологічна дія германію цитрату більше виражена у дозі 200 мкг Ge/кг м. т., що зумовлює підвищення вмісту Cu (від 51 до 95 %) і Zn (від 22 до 78 %) у всіх досліджених тканинах щурів цієї групи на тлі зниження рівня Co у печінці за дії 20 і 200 мкг Ge, а нирках і легенях – за дії всіх застосованих доз. У тканинах м'язів самців F_2 вірогідно зростав вміст Mn (на 27,7; 74,0 і 23,4 % в II, III і IV групах відповідно) за дії всіх застосованих доз Ge, Co – 20 і 200 мкг, Fe – 10 і 20 мкг, а Zn 10 і 200 мкг Ge, що свідчить про відмінності регуляторного впливу HGeЦ на рівень досліджених мікроелементів у тканинах м'язів щурів.

Ключові слова: наноматеріали, внутрішні органи, м'язи.

Влияние выпаивания разных доз германия цитрата на содержание микроэлементов в тканях и органах самцов крыс F_2

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Цель. Выяснить влияние дозы германия цитрата на распределение Fe, Zn, Cu, Co, Mn в тканях и органах крыс-самцов F_2. **Методы**. Физиологические, биохимические, клинические, статистические. **Результаты**. Установлены изменения содержания Fe, Zn, Cu, Co, Mn в мягких тканях и их распределения в печени, почках и легких самцов крыс F_2. Показано, что эти изменения обусловлены в большей степени органо-тканевыми особенностями функционирования отдельных физиологических систем организма, в частности гепато-ренальной и дыхательной, а в меньшей – дозой германия (10, 20 и 200 мкг/кг м. т.). Более выраженные изменения содержания этих элементов установлены для почек при действии всех примененных доз, а меньше – печени и легких. В тканях мышцы отмечено положительное влияние германия цитрата на содержание Cu, Co, Mn и Zn при действии 10, 20 и 200 мкг Ge, а Fe – 20 и 200 мкг. Установленные различия массы печени, почек и легких крыс опытных и контрольной групп сглаживали межгрупповые различия абсолютного содержания исследованных микроэлементов в печени, почках и легких. Указанные различия более выражены для абсолютного содержания Cu в печени, Mn – в почках и легких. Выводы. Длительное поступление в организм крыс F_2 с водой германия цитрата в количестве 10, 20 и 200 мкг Ge/кг м. т. характеризуется изменением содержания Cu, Co, Mn, Fe, Zn как на единицу массы мягких тканей, так и абсолютного содержания их во внутренних органах. Биологическое действие германия цитрата больше выражено в дозе 200 мкг Ge/кг м. т., и приводит к повышению содержания Cu (от 51 до 95 %) и Zn (от 22 до 78 %) во всех исследованных тканях крыс этой группы на фоне снижения уровня Co в печени при действии 20 и 200 мкг Ge, а почках и легких – при действии всех примененных доз. В тканях мышц самцов F_2 достоверно возрастало содержание Mn (на 27,7; 74,0 і 23,4 % во II, III и IV группах соответственно) при действии всех примененных доз Ge, а Co – 20 и 200 мкг, Fe – 10 и 20 мкг, Zn – 10 и 200 мкг Ge, что свидетельствует о различиях регуляторного влияния HGeЦ на уровень исследованных микроэлементов в тканях мышц крыс.

Ключевые слова: наноматериалы, внутренние органы, мышцы.

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FORMATION OF EROSION RESISTANCE OF GRAY FOREST SOILS IN THE CONDITIONS OF CARPATHIAN REGION

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Aim. To study the impact of perennial grasses mixtures on the formation of erosion resistance of gray forest soils of different degradation degree and their unmodified analogues in conditions of long-term permanent experiment. **Methods.** Field, laboratory, assessment and comparison. **Results.** It was established that lupine-cereal grass mixtures increased erosion resistance of slope soils considerably within fifteen years. The sum of soil structural aggregates was improved from satisfactory into good category. There were positive changes in the number of water-resistant aggregates as well. The studies confirmed a positive impact of legume-grasses on the density and porosity of soil. Soil density was the lowest when the slope was laid down in perennial lupine and its mixture with cereal grasses. The intensification of soil erosion and the approximation of the illuvial horizon level to the surface resulted in the compaction of upper soil layers to 1.29–1.44 g/cc. General porosity of soil correlated with its density which did not exceed optimal values in poorly eroded soils in upper layers. The studies proved the impact of the ways of laying down the slope in grass and the level of soil degradation on its humidity and water permeability. In the experiment conditions, the increase in soil degradation led to the decrease in its moisture. The decrease in the content of humidity in crop field took place at the expense of higher intensity of the growth and water consumption of lupine-cereal grasses in comparison with natural mixed herbs, especially in the periods with a small amount of precipitation. The difference in the upper soil layers was in the range of 0.2–0.5 %. It was established that the highest values of soil water permeability were formed on lupine-cereal grass mixtures in all the variants of degraded soils. **Conclusions.** Laying down slope lands in perennial lupine in combination with cereal grasses promotes the formation of their higher resistance to erosion processes and restoration of fertility. Perennial legume-cereal mixtures ensure the improvement of structural-aggregate state, total density and porosity of soil, enhance its water supply and water permeability.

Keywords: gray forest soils, degradation, erosion resistance.

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INTRODUCTION

The degradation processes, caused by the impact of water erosion, are widely spread on the slope lands of the Carpathian region. The inconsistence of the land use structure and crop rotation, the non-compliance of soil-protecting technologies of cultivating agricultural crops, the violation of zonal norms of general and field-protecting forest cover lead to the decrease in soil erosion resistance and enhance erosion processes. In particular, in conditions of Lviv region in the zone of small (Lviv) Polissia on the agricultural land, the development of water erosion processes of different intensity takes place on the area of 47,446 ha, wind erosion – 25,091 ha, in the zone of west Forest-Steppe – 146,055 and 15,790 ha respectively. In the Subcarpathian region, 50,314 ha and in the Carpathians – 56,790 ha of lands are subjected to destructive impact of water. According to the results of studies of the Institute of Agriculture of the Carpathian Region NAAS, the highest index of erosion-ecological tension of agricultural lands (the ratio of lands, subjected to the impact of erosion processes, to the total area of agricultural lands) was noted on the arable land in all soil-climatic zones of the region. It was 0.26 in Polissia, 0.37 – in the Forest-Steppe, 0.24 – in the Subcarpathian region,

0.49 – in the Carpathians. The lowest values were recordedon hayfields and meadows [1, 2]. It conditions a considerable aggravation of ecologic situation and a sharp decrease in ecologic restoration and productive functions of soils. Along with the loss of humus layer and nutrients there is a considerable change in physical-chemical and water-physical properties of soils and its heat regime [1, 2].

Taking into consideration economic aspects, the introduction of meadow in the system of soil-protecting agriculture is a cheap and reliable method of protecting soils, enhancing their erosion resistance and restoring the soil fertility [1, 3–6]. Perennial grasses and grass mixtures enhance the performance of ecosystem, stabilize its functioning, improve physical properties, enrich soil with nitrogen, phosphorus, and calcium [2, 7].

MATERIALS AND METHODS

The studies were conducted in conditions of long-term permanent experiment of the Institute of Agriculture of the Carpathian Region.

The experiment was started in 2003 on the slope of the southern-western exposition, its length was 100 m and the steepness – 11°. There were two factors under investigation. The variants of the first factor were the areas of different degrees of degradation – heavily, medium and poorly eroded soils and their unmodified analogues, the variants of the second factor– perennial grasses: perennial lupine (pure sowing); clover-cereal grass mixture; lupine-cereal grass mixture; perennial cereal grasses (pure sowing); natural overgrowth. The cereal component consists of the following perennial grasses: awnless bromegrass, meadow brome, timothy grass.

The location of variants was sequential, there were three repeats, the area of the experimental plot was 320 sq.m., that of the registration plot – 160 sq.m., the total area under the experiment – 1,20 ha.

The arable soil layer of different degradation degree was characterized by the following agrochemical indices: content of humus (according to Turin) – 1.4–1.7 %, mobile phosphorus and potassium – 125–205 and 50–112 mg per 1 kg of soil respectively, pH (KCl) – 5.2–6.0, hydrolytic acidity – 2.3–2.5 mg-eq per 100 g of soil, the sum of absorbed alkali – 4.4–5.3 mg-eq per 100 g of soil, the content of base-hydrolyzed nitrogen is 60–85 mg/kg of air-dry soil.

RESULTS AND DISCUSSION

The aim of our studies was to investigate the impact of meadow-reclamation events on the erosion resistance of gray forest soils in conditions of sufficient humidity.

It was proven that the fields of perennial grasses on erosion-hazardous and eroded soils decrease the surface runoff, promote its diffusion due to the formation of dense turf, ensure the formation of water-resistant

Table 1. Structural-aggregate composition of gray forest surface-clay soil (2015)

Way of laying down with grass	Size of structural aggregates in mm, content in %									Sum of macroaggregates	C_{str} $C_{water\ resistance}$	Estimation of structural state of soil
	>10	10..7	7..5	5..3	3..2	2..1	1..0.5	0.5..0.25	<0.25			
Poorly eroded												
Lupine-cereal mixture	16.4	6.0	6.8	12.2	9.8	17.7	4.4	6.4	20.3	63.2	1.72	Good
			3.2	2.1	4.4	16.7	7.4	7.1		40.9	0.65	Satisfactory
Natural overgrowth	20.5	6.6	6.8	14.6	10.6	14.2	3.7	5.0	17.9	61.6	1.60	Good
			4.1	5.4	3.8	12.8	5.9	5.5		37.5	0.61	Unsatisfactory
Heavily eroded												
Lupine-cereal mixture	17.2	7.6	7.6	12.3	8.4	14.4	3.2	6.0	23.3	59.5	1.47	Satisfactory
			3.3	3.7	4.0	11.1	6.8	5.9		34.8	0.58	Unsatisfactory
Natural overgrowth	27.4	7.3	7.5	12.4	8.4	14.6	3.0	5.0	14.3	58.3	1.40	Satisfactory
			6.1	6.9	2.5	10.8	4.2	5.3		35.8	0.61	Unsatisfactory

Note. The numerator – structural-aggregate state, denominator – water resistance of soil aggregates.

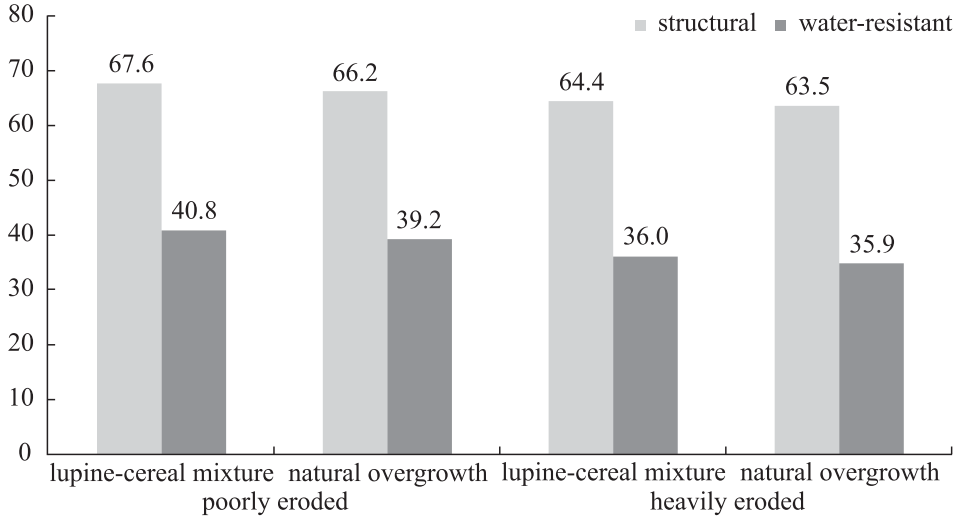

Fig. 1. The content of structural and water-resistant aggregates depending on the meadow-reclamationevents and the degree of slope erosion, % for 2015

structure, enhance water permeability of soil and protect the surface from the destructive action of rain drops [2, 7].

In our studies, the analysis of experimental data as of 2015 demonstrated a considerable positive impact of perennial grasses on the structural-aggregate state and water resistance of soil aggregates (Table 1). It was established that, depending on the erosion degree, the sum of soil structural aggregates in the variants of lupine-cereal grass mixture was in the range of 63.3–59.5 % and exceeded the variant of natural overgrowth by 1.6 and 1.2 %. There were 3.6 and 3.3 % less structural aggregates on heavily eroded soil compared to poorly eroded soils. There was domination of structures with the size of 1...2 mm – 14.2–17.7 % and 3...5 mm, the content of which was at the level of 12.2–14.6 %. The fraction of aggregates of 0.5...1 mm was the least – 3.0–4.4 %. Under natural overgrowth of eroded lands, the content of structure-free soil aggregates (under 0.25 mm) was 2.4 and 9.0 % less compared to the fields of the mixture of lupine and cereal grasses.

Water resistance of aggregates is a relevant characteristic of erosion resistance of soil [8]. In our studies the content of water-resistant aggregates under grasses was 34.8–35.8 % on heavily eroded soils, and 37.5–40.9 % on poorly eroded analogues.

The calculations demonstrated that the coefficient of structuredness ($C_{str.}$) for the poorly eroded soil was 1.60–1.72, and that for heavily eroded soil – 1.40–1.47. The coefficient of water resistance ($C_{water\ resistance}$) of soil aggregates was 0.61–0.65 and 0.58–0.61 respectively.

The estimation of structural state demonstrated that good structural state of soil aggregates is formed on the fifteenth year after the start of the experiment (0.25–10 mm) in the range of 63.5–67.6 %. This shows a clear tendency towards its improvement.

The changes in the number of water-resistant aggregates were less evident. Water-resistant structure was satisfactory only on lupine-cereal grass mixture in conditions of poorly eroded soil (Fig. 1).

Soil density is a relevant index of erosion resistance of soils. Optimal indices of the density promote a favorable ratio between solid, liquid and gas phases of soil, ensuring the most efficient consumption and use of moisture, with the formation of good conditions for the development of the root system of plants.

Our studies confirm a positive impact of grass mixtures on soil density (Table 2). In all the experiment variants, the root system of perennial grasses and the absence of impact of the movement of agricultural equipment promotes the optimization of this index. It was also promoted by the plant cover of grasses which created a barrier for soil compaction by rain drops.

The density of poorly eroded surface clay soil was 1.22–1.36 g/cc. The lowest soil density was found in the upper layers in the variants of the fields of lupine-cereal mixture – 1.22–1.27 g/cc.

The intensification of soil erosion and the approximation of the illuvial horizon level to the surface resulted in the compaction of upper soil layers to 1.29–1.44 g/cc. Soil density was lower when the slope was laid down in perennial lupine and its mixture with cereal grasses.

Table 2. Physical properties of soil of different erosion degree under grasses (2015)*

Soil layer, cm	Density of soil structure, g/cc*			Soil porosity, %		
	Way of laying down with grass					
	perennial lupine	lupine-cereal mixture	natural overgrowth	perennial lupine	lupine-cereal mixture	natural overgrowth
Poorly eroded soils						
0–10	1.25	1.22	1.23	51.2	52.0	52.0
10–20	1.30	1.27	1.31	50.0	51.2	49.6
20–30	1.31	1.30	1.36	50.0	50.4	48.1
0–30	1.29	1.27	1.30	50.4	51.2	49.9
Heavily eroded soils						
0–10	1.30	1.29	1.31	50.0	50.4	49.6
10–20	1.37	1.35	1.40	48.1	48.9	47.0
20–30	1.40	1.37	1.44	47.2	48.3	45.7
0–30	1.36	1.34	1.38	48.4	49.2	47.4

* The density of soil structure was defined prior to the second cutting.

Table 3. The level of humidity in crop field depending on the slope erosion and its laying down with grasses, 2015, %

Soil layer, cm	Way of laying down with grass			
	Poorly eroded		Heavily eroded	
	lupine-cereal grasses	natural overgrowth	lupine-cereal grasses	natural overgrowth
Prior to the first cut				
0–10	19.2	19.6	18.9	19.8
10–20	19.6	19.9	19.2	19.3
20–30	18.0	18.8	17.8	17.8
30–40	18.7	19.2	18.1	18.7
40–60	19.0	19.3	18.7	19.0
60–80	19.6	20.4	19.0	19.1
80–100	20.0	20.9	19.5	19.5
0–30	18.9	19.4	18.6	19.0
0–100	19.2	19.7	18.7	19.0
Prior to the second cut				
0–10	6.6	6.9	5.8	6.3
10–20	5.9	6.1	5.2	5.6
20–30	5.4	5.5	5.0	5.2
30–40	5.2	5.2	5.1	5.2
40–60	6.1	5.9	5.3	5.7
60–80	13.5	13.8	13.1	13.0
80–100	15.3	15.0	14.8	14.8
0–30	6.0	6.2	5.3	5.7
0–100	8.3	8.3	7.8	8.0

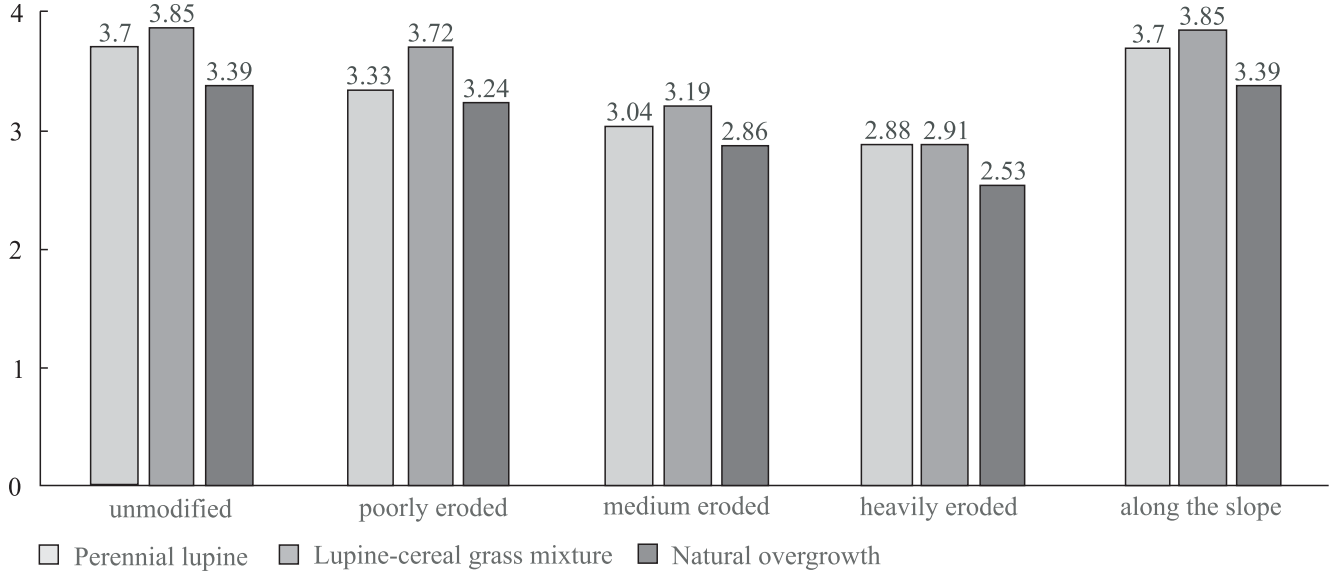

Fig. 2. The estimation was performed prior to the second cutting. Water permeability of soil, mm/min during the first hour

General porosity of soil correlated with its density which did not exceed optimal values in poorly eroded soils in upper layers – less than 50 % (Table 2). In conditions of heavy erosion in the 0–10-cm soil layer, it was 49.6–50.4 % and in the 0–30 cm layer it was 47.4–49.2 %. Porosity was 2–4 % higher in poorly eroded soil.

Soil moisture has a considerable impact on soil structure and thus on their erosion resistance. Plant cover promotes interception of precipitation, even accumulation of snow which ensures the improvement of water indices of fertility and decrease in erosion progress.

Humidity in crop field was determined prior to the first and second cutting of grasses. The results of the studies demonstrated (Table 3) which way of laying down the slope with grass and the degree of soil erosion had impact on humidity in crop field. In conditions of 2015, the enhanced development of lupine-cereal grass mixture promoted increased water consumption and decrease in soil moisture compared to the grasses in the variants of natural overgrowth which was 18.6–19.4 % in the 0–30 cm soil layer and 18.7–19.7 % in the 0–100 cm layer depending on the erosion degree.

In general, as of the time of the first cutting of grasses, the moisture of eroded soil was sufficient for the formation of their high performance. A considerable amount of precipitation promoted the latter.

The number of precipitations after the first cutting of grass, which was not high compared to perennial grasses, conditioned a sharp decrease in soil moisture (Table 3). For instance, its content in the layers down to 60 cm did not exceed 6.9 %. Higher intensity of the growth of lupine-cereal grasses compared to natural overgrowth conditioned a decrease in the content of humidity in crop field. The difference in the upper layers was in the range of 0.2–0.5 %.

Starting with the depth of 60 cm, the content of moisture in creased and was 13.0–15.3 %. Soil moisture of a one-meter-deep layer was at the level of 7.8–8.3 %.

Water permeability of soil determines the completeness of absorbing water of atmospheric precipitation and impacts the degree of water supply of soil and the development of erosion processes. Waters, flowing from the field surface, cause ablation and erosion of soil.

Drought conditions in summer of 2015 impacted water permeability of soil. It was 3.39–3.85 mm/min on average on the slope depending on the grass. The highest values were formed on lupine-cereal grass mixtures in all the variants of degraded soils. The analysis of changes in this index by degradation variants demonstrated its decrease in all the areas of perennial grasses from unmodified analogues to heavily eroded soil, amounting to 3.70–2.88 in the fields of perennial grasses, 3.85–2.91 – for lupine-cereal grass mixture, and 3.39–2.53 in the natural state during the first hour (Fig. 2).

The decrease in water permeability in conditions of enhanced soil ablation occurs due to the deterioration of water resistance of its structure, which conditions fast colmatati on of soil pores by dispersed particles. In heavily eroded soils water permeability decreased by 22–24 % compared to unmodified analogues.

CONCLUSIONS

Laying down slope lands in perennial lupine in combination with cereal grasses promotes the formation of their higher resistance to erosion processes and restoration of fertility. In these conditions, a good structural state of aggregates (0.25–10 mm) is formed on poorly eroded soil in the range of 63.5–67.6 % and there is a clear tendency towards its improvement on heavily eroded analogues. Perennial legume-cereal mixtures ensure the improvement of general density and porosity of soil, its water supply and permeability.

Формування протиерозійної стійкості сірих лісових ґрунтів в умовах Карпатського регіону

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Мета. В умовах довготривалого стаціонарного досліду дослідити вплив комплексів багаторічних трав на формування протиерозійної стійкості сірих лісових ґрунтів різного ступеня деградованості та їх незмитих аналогів. **Методи.** Польовий, лабораторний, розрахунково-порівняльний. **Результати.** Встановлено, що люпино-злакові травосумішки за п'ятнадцятирічний період істотно підвищували протиерозійну стійкість схилових ґрунтів. Покращувалась сума структурних агрегатів і, згідно градації оцінювання структурного стану, на варіантах досліду переходила з категорії задовільної в добру. Проявлялись позитивні зміни й кількості водостійких агрегатів. Проведеними визначеннями підтверджено позитивний вплив бобово-злакових трав на щільність та шпаруватість ґрунту. Щільність ґрунту була найменшою при залуженні схилу люпином багаторічним та його сумішкою із злаковими травами. Посилення змитості ґрунту та підвищення до поверхні рівня ілювіального горизонту спричинювало ущільнення верхніх пластів ґрунту до 1,29–1,44 г/см^3. Загальна шпаруватість ґрунту корелювала з його щільністю, яка на слабозмитих ґрунтах у верхніх шарах не виходила за оптимальні значення. Дослідженнями доведено вплив способів залуження схилу та рівня деградованості ґрунту на його вологість та водопроникність. В умовах досліду зростання деградованості ґрунтів призводило до зниження їх вологості. Зменшення вмісту польової вологи відбувалось і за рахунок вищої інтенсивності росту та водоспоживання люпино-злакових трав в порівнянні до природного різнотрав'я, особливо в періоди з невеликою кількістю опадів. Різниця у верхніх шарах була в межах 0,2–0,5 %. Встановлено, що найвищі значення водопроникності формувались на люпино-злакових травосумішках по всіх варіантах деградованих ґрунтів. **Висновки.** Залуження схилових земель багаторічним люпином у сумішці зі злаковими травами сприяє формуванню їх високої стійкості до ерозійних процесів і відновленню родючості. Багаторічні бобово-злакові комплекси забезпечують покращення структурно-агрегатного стану, загальної щільності та шпаруватості ґрунту, поліпшують його вологозабезпеченість та водопроникність.

Ключові слова: сірі лісові ґрунти, деградація, протиерозійна стійкість.

Формирование противоэрозионной устойчивости серых лесных почв в условиях Карпатского региона

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Цель. В условиях длительного стационарного опыта исследовать влияние комплексов многолетних трав на формирование противоэрозионной устойчивости серых лесных почв разной степени деградованости и их незмитих аналогов. **Методы.** Полевой, лабораторный, расчетно-сравнительный. **Результаты.** Установлено, что люпино-злаковые травосмеси по пятнадцатилетний период существенно повышали противоэрозийную устойчивость склоновых почв. Улучшалась сумма структурных агрегатов и, согласно градации оценки структурного состояния, на вариантах опыта переходила из категории удовлетворительного в хорошую. Проявлялись положительные изменения и количества водостойких агрегатов. Проведенными определениями подтверждено положительное влияние бобово-злаковых трав на плотность и скважность почвы. Плотность почвы была наименьшей при заложенные склона люпина многолетним и его смеси со злаковыми травами. Усиление змитости почвы и повышения к поверхности уровня илювиального горизонта вызывало уплотнения верхних слоев почвы к 1,29–1,44 г/см^3. Общая скважность почвы коррелировала с его плотностью, на слабозмитих почвах в верхних слоях не выходила за оптимальные значения. Исследованиями доказано влияние способов заложенные склона и уровня деградованости почвы на его влажность и водопроницаемость. В условиях опыта рост деградованости почв приводило к снижению их влажности. Уменьшение содержания поле-вой влаги происходило и за счет более высокой интенсивности роста и водопотребления люпино-злаковых

трав в сравнении с природного разнотравья, особенно в периоды с небольшим количеством осадков. Разница в верхних слоях была в пределах 0,2–0,5 %. Установлено, что высокие значения водопроницаемости формировались на люпино-злаковых травосмеси по всем вариантам деградированных почв. **Выводы.** Заложенные склоновых земель многолетним люпином в сумишци со злаковыми травами способствует формированию их высокой устойчивости к эрозионных процессов и восстановлению плодородия. Многолетние бобово-злаковые комплексы обеспечивают улучшение структурно-агрегатного состояния, общей плотности и скважности почвы, улучшают его влагообеспеченность и водопроницаемость.

Ключевые слова: серые лесные почвы, деградация, про-тивоэрозионная устойчивость.

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VIABILITY OF SPERM CELLS OF BOARS AT THE ADDITION OF ULTRA FINE SILICA TO CRYOPRESERVATION AND DEFROSTING MEDIA

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Aim. In modern conditions the priority task of preserving biological diversity is increasing the role of agriculture in its maintenance. Within the system of long-term preservation of genetic resources of farm livestock, urgent improvement is needed in the field of media for dilution, cryopreservation and storage of genetic material of animals; technological elements of cryopreservation of genetic material of animals and biotechnological means of obtaining embryos from cryopreserved genetic material outside of the organism. **Methods.** It is known that the medium for boar sperm cryopreservation is added the following components: hydrophilic extract of oaken silkworm pupa, aqueous extract of propolis, bovine serum albumin, water-soluble components of yolk and lipoproteins, extract of crude sunflower oil, proline, trimethylglycine (betaine) and nanomaterials. **Results.** The results of experimental studies on the interaction between cryopreserved ejaculated sperm cells of boars with nanoparticles of ultra fine silica (UFS) and saccharose were presented. It is noteworthy that the studies are related both to the technology of gamete cryopreservation and its de-preservation. Nanomaterial A-300 with S_{spec}= 285 sq.m./g (Kalush, Ukraine) was used in the studies after previous heating for 2 h at 200°C and surface modification with the carbohydrate – saccharose. UFS/saccharose was added to the cultivation medium for defrosted sperm cells (2.9 % sodium citrate solution) as well as prior to freezing into lactose-yolk-glycerin cryomedium in the concentrations of 0.1; 0.01 and 0.001 %. The analysis of obtained results demonstrated different impact of nanomaterial, used by us as an additive to media. It was established that after defrosting, boar sperm cells with UFS/saccharose in 0.001 % concentration demonstrated the activity at the level of 14.2 % and the total time of their survival was 4.0 h. UFS/saccharose concentration of 0.001 %, applied during the cryopreservation of ejaculated boar sperm, ensured 25.0 % activity after defrosting with the total survival time of 5.5 h. **Conclusions.** The article reflects the promising nature of conducting further biotechnological studies with the application of nanomaterials of different origin in the system of preservation and rational use of genetic resources of farm livestock.

Keywords: ejaculated sperm cells, boar, nanomaterial, ultra fine silica, cryopreservation, gene fund preservation.

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INTRODUCTION

In modern conditions the priority task of preserving biological diversity is increasing the role of agriculture in its maintenance. Within the system of long-term preservation of genetic resources of farm livestock, urgent improvement is needed in the field of media for dilution, cryopreservation and storage of genetic material of animals; technological elements of cryopreservation of genetic material of animals and biotechnological means of obtaining embryos from cryopreserved genetic material outside of the organism [1].

The fertilizing capacity of cryopreserved sperm cells depends on numerous factors, in particular, the composition of diluents and technological processes of preparing ejaculates to freezing and defrosting of sperm doses. Diluent components mitigate the negative effect of cold shock and ensure the integrity of acrosome and plasmatic membranes of sperm cells. It is known that

the medium for bull sperm cryopreservation is added the following components: glutathione, soy lecithin instead of chicken egg yolk, complex of biologically active substances (oestrophanum, eosin, unitiol, glutathione, l-cysteine) [2]. The medium for boar sperm cryopreservation is added the following components: hydrophilic extract of oaken silkworm pupa [3, 4], aqueous extract of propolis [5], bovine serum albumin, water-soluble components of yolk and lipoproteins, extract of crude sunflower oil, proline, trimethylglycine (betaine) and nanomaterials [6].

According to approximate estimations, at present there are over 800 different products, made via nanotechnologies. In 2007 the global sale of nanomaterials was estimated at 147 billion US dollars, and in 2015 this index increased up to 3.1 trillion US dollars. Nanoproducts have already been used in energetics, chemical and construction industry, cosmetics production. The application of nanotechnologies and nanomaterials in food industry and environment protection is also a promising approach. Positive results have already been obtained from the application of nanotechnological preparations in modern agriculture and veterinary practice. The specialists of the US state program "National Nanotechnology Initiative", established in 2000, determine nanotechnology as "science, engineering and technology conducted at the nanoscale which is about 1 to 100 nanometers with the purpose of obtaining fundamental knowledge about the nature of phenomena and properties of different materials in the nanometer scale and of creating and using structures, devices and systems, which acquire new properties due to their nanosizes" [7].

After adopting the Law of Ukraine "On Ratification of the Convention on Biological Diversity", ratifying the Interlaken Convention in 2007 and the Global Plan of Action for Animal Genetic Resources, signing the Nagoya Protocol on Access to Genetic Resources, Ukraine started the work in optimizing and applying nanomaterials in the technology of preservation and rational use of genetic resources of domestic breeds of swine to improve the intensity of applying the reproductive potential of breeders using cryopreservation of ejaculated sperm cells.

To substantiate the most efficient directions in applying nanomaterials in animal breeding, it is reasonable to improve the methods of obtaining embryos *in vitro*, cryopreserving gametes and embryos with the use of nanomaterials. Due to this fact, the aspects of ultra fine silica (UFS) – one of modern and promising nanomaterials – is considered. It was demonstrated that the addition of UFS in some concentrations to the standard cryomedium may stimulate the viability of bull sperm cells. UFS is pyrogenous; its surface layer consists of a large number of hydroxylic groups and has a high sorption capability regarding different molecules. In biological media, silica nanoparticles demonstrate their ability to bind the cells via intermolecular interactions; in this case a cell is still alive, but it may lose its activity, and, as a result, have deceleration of metabolic processes.

UFS is widely used as a supplementary treatment means in medical practice. The modification of its surface with some biomolecules, for instance, mono- and oligosaccharides, which promote better motility and survival of sperm cells of bulls, rams and humans, when added to the cryomedium, allowed creating promising nanomaterials on their basis [8–11].

It was demonstrated that in case of adding 0.001 % concentration of UFS/saccharose to lactose-yolk-glycerin cryomedium, the de-preserved epididymal sperm cells of boars had 10.0 % higher activity compared to the control (cryopreservation without the addition of nanomaterials, the activity of sperm cells after defrosting of 10.0 %) with the preservation of this level for 2 h. It should be noted that prior to freezing the activity of these gametes was at the level of 50 % [12].

Thus, our studies were aimed at estimating the biological activity of nanomaterials (on the basis of UFS, whose surface had previously been modified with carbohydrate – saccharose) under conditions of adding it to boar sperm cells after defrosting.

MATERIALS AND METHODS

The study was conducted in the Laboratory for Reproduction Biotechnology of the Institute of Animal Breeding and Genetics n.a. M. V. Zubets, NAAS.

The experiments studied the impact of nanomaterial UFS/saccharose on the viability of boar sperm cells of Myrhorod breed (Dnipro 641, Komysh 853, Kokhany 289), preserved in the bank of genetic resources of the animals at the Institute of Animal Breeding and Gene-tics n.a. M.V. Zubets, NAAS. Nanomaterial UFS/saccharose is a ultra fine silica (UFS), chemical formula SiO_2 of brand A-300 with S_{spec} = 285 sq.m./g (Kalush, Ukraine), whose surface was modified with saccharose at the O.O. Chuiko Institute of Surface Chemistry, NAAS of Ukraine. UFS/saccharose was added to the cultivation medium for defrosted sperm cells (2.9 % sodium citrate solution) as well as prior to

freezing into lactose-yolk-glycerin cryomedium in the concentrations of 0.1; 0.01 and 0.001 %. The impact of UFS/saccharose on the viability of boar sperm cells (each one separately and using the average indices) was analyzed in terms of their activity in per cent and the survival index in hours of cultivation in the thermostate at +37 °C.

Six ejaculates were used to conduct the studies and transported at 7 °C to the laboratory, where the main qualitative indices of sperm were determined. The duration of transporting ejaculates to the laboratory did not exceed six hours.

The estimation of the study results for quantitative and qualitative indices was conducted via the analysis of tables and charts. The obtained results of studies were processed using descriptive statistics method based on the estimated arithmetic mean (M), deviation from the indices of arithmetic mean error (m) (software package ×7, version 2.0.0.9).

RESULTS AND DISCUSSION

It was established that after defrosting sperm cells demonstrated the average activity at the level of 16.7 ± ± 3.33 %. This index in the control decreased only by 1.7 % within 30 min (15.0 ± 2.89 %). After sperm cells stayed in the medium, containing UFS/saccharose in the concentrations of 0.1; 0.01 and 0.001 %, for 30 min, there was a decrease in the activity of gametes by 7.5; 5.0 and 2.5 % compared to the initial activity (Fig. 1). It is noteworthy that after 1.5 h since the start of the study the activity of sperm cells in the control was 9.2 %, and similar activity was noted in the experimental groups within this time period, namely, 10.0 % for 0.001 % concentration of UFS/saccharose; 9.1 and 6.7 % for 0.01 and 0.1 % concentration of UFS/saccharose. The total survival time of sperm cells in the control was five hours, and in the experimental group with 0.001 % concentration of UFS/saccharose this index was 30 min higher.

It was demonstrated that the addition of UFS/saccharose in the concentration of 0.001 % to the medium (2.9 % sodium citrate solution) had positive impact on the viability of defrosted sperm cells, which is manifested with slow decrease in the activity and prolonged total survival period of gametes. Taking this fact into consideration, we estimated the biological activity of UFS/saccharose during cryopreservation of boar sperm cells. UFS/saccharose was added to lactose-yolk-glycerine cryomedium in three concentrations directly prior to freezing sperm cells.

It was established that sperm cells, frozen with UFS/saccharose in the concentration of 0.001 %, demonstrated the highest activity on average after defrosting, which was 25.0 ± 1.44 % (Dnipro 641 – 25.0 %; Komysh 853 – 27.5 %; Kokhany 289 – 22.5 %, respectively) which was 5.0 % higher compared to 0.01 % concentration and 7.5 % higher compared to 0.1 % concentration of UFS/saccharose (Fig. 2).

After 30 min since the start of the experiment, there was a decrease in the activity of gametes in all the groups, for instance, this index (without adding UFS/saccharose) decreased only by 1.7 % in the control (15.0 ± 2.89 %; Dnipro 641 – 20.0 %; Komysh 853 – 15.0 %; Kokhany 289 – 10.0 %, respectively) in 0.001 % concentration – by 2.5 % and by 5.8 and 5.0 % in the concentrations of 0.01 and 0.1 %. Within the following 30 min (one hour since the start of the experiment), the activity in some groups was almost at the same level, as this index in the control was 10.8 ± ± 3.82 % (Dnipro 641 – 15.0 %; Komysh 853 – 10.0 %; Kokhany 289 – 7.5 %, respectively), in the concentration 0.01 % 10.8 ± 2.21 % (Dnipro 641 – 12.5 %; Komysh 853 – 10.0 %; Kokhany 289 – 10.0 %, respectively), in 0.1 % concentration 10.0 % (Dnipro 641 – 12.5 %; Komysh 853 – 10.0 %; Kokhany 289 – 7.5 %, respectively). In case of freezing sperm cells with UFS/saccharose in 0.001 % concentration, the activity within this time period was 19.2 % (Dnipro 641 – 17.5 %; Komysh 853 – 22.5 %; Kokhany 289 – 17.5 %, respectively).

The total period of survival for sperm cells in the control and in case of freezing sperm cells with 0.1 % concentration of UFS/saccharose was 3.2 h (Dnipro 641 – 4.0; Komysh 853 – 3.0; Kokhany 289 – 2.5 h, respectively,), and in experimental groups where sperm cells were frozen with 0.001 % concentration, this index did not exceed 4.8 h (Dnipro 641 – 5.5; Komysh 853 – 4.5; Kokhany 289 – 4.5 h, respectively), with 0.01 % concentration the survival was 3.7 h (Dnipro 641 – 4.5; Komysh 853 – 3.5; Kokhany 289 – 3.0 h, respectively).

It should be noted that the cryocollection of ejaculated sperm cells of Myrhorod breed boars was first created in Ukraine (750 doses). The efficient application of the mentioned and new cryocollections will ensure the implementation of a complex of measures at the state level regarding the functioning of "virtual gene fund cryo-animal stocks" which is economically more profitable compared to keeping stocks of farm animals.

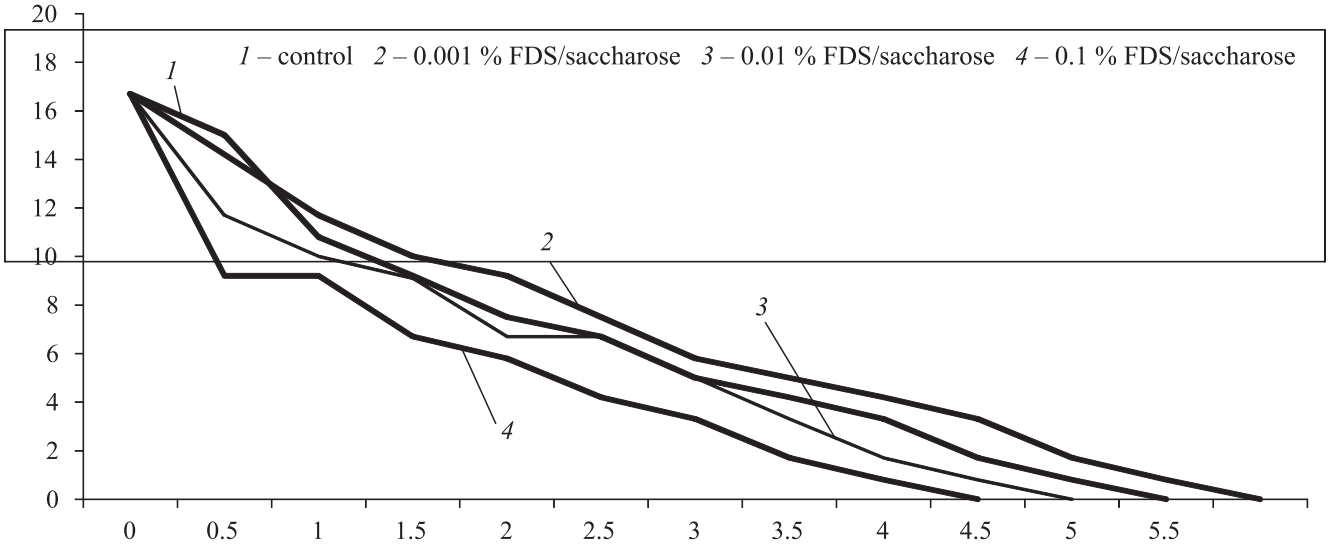

Fig. 1. The impact of UFS/saccharose on the viability of defrosted ejaculated sperm cells of boars

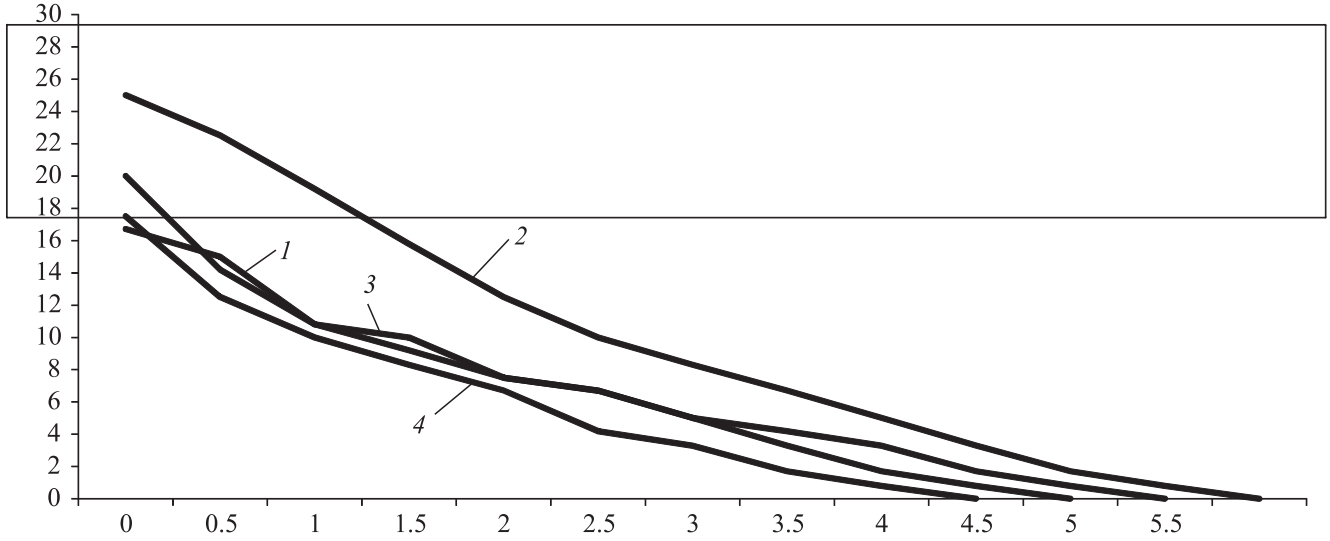

Fig. 2. The viability of cryopreserved ejaculated sperm cells of boars at the addition of UFS/saccharose

On condition of using cryopreserved ejaculated sperm cells for fertilization of porcine oocytes, which have matured *in vitro*, the level of embryo development will ensure the additional use of genetic potential of animals and improvement of complex biotechnological methods for implementation of tasks of preserving the gene fund of farm animals in Ukraine.

CONCLUSIONS

It was established that after defrosting, boar sperm cells with UFS/saccharose in 0.001 % concentration demonstrated the activity at the level of 14.2 % and the total time of their survival was 4.0 h.

It was demonstrated that lactose-glycerin-yolk-medium may be effectively used in case of cryopreservation of ejaculated sperm cells of boars. The application of such sperm cells may ensure a sufficient level of the effective formation of embryos both *in vitro* and *in vivo*.

UFS/saccharose concentration of 0.001 %, applied during the cryopreservation of ejaculated boar sperm, ensured 25.0 % activity after defrosting with the total survival time of 5.5 h.

Therefore, during the cryopreservation of biological objects the threshold concentrations of nanomaterials in cryomedia should be noted in its final definition with the consideration of the activity of specific types of cells. It will allow enhancing the efficiency of biomaterial cryopreservation.

The technology of cryopreserving genetic resources of farm animals, including "virtual gene fund cryoanimal stocks", will be implemented in close cooperation with the breeding farm, keeping domestic breeds of swine (State enterprise "Experimental farm named after Decemberists at the Institute of Swine Production and Agroindustrial Production, NAAS of Ukraine"). This will ensure efficient implementation of tasks in terms of preserving biodiversity and estimating agroecosystem balance, obtaining scientific research.

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Життєздатність сперматозоїдів кнурів за додавання високодисперсного кремнезему до складу середовищ для кріоконсервації та розморожування

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Ціль. В сучасних умовах пріоритетним завданням збереження біологічного різноманіття є підвищення ролі сільського господарства в підтримці біорізноманіття. В системі довготривалого збереження генетичних ресурсів сільськогосподарських тварин наразі потребують удосконалення середовища для розрідження, кріоконсервації та зберігання генетичного матеріалу тварин; технологічні елементи кріоконсервації генетичного матеріалу тварин та біотехнологічні прийоми отримання поза організмом ембріонів із кріоконсервованого генетичного матеріалу. **Методи.** Відомо, що до середовища для кріоконсервації сперми кнурів додають: гідрофільний екстракт лялечок дубового шовкопряда, водний екстракт прополісу, альбумін сироватки крові великої рогатої худоби, водорозчинні компоненти жовтка та ліпопротеїди, екстракт нерафінованої соняшникової олії, пролін, триметилгліцин (бетаїн), а також наноматеріали. Представлено результати експериментальних досліджень щодо взаємодії кріоконсервованих еякульованих сперматозоїдів кнурів з наночастинками високодисперсного кремнезему (ВДК) та сахарози. Слід зазначити, що дослідження стосуються не тільки технології кріоконсервації гамет, але й деконсервації. В дослідженнях використано наноматеріал марки А-300 із $S_{пит}$= 285 м^2/г (м. Калуш, Україна), який попередньо прожарювали 2 год за температури 200 °С, поверхню якого модифікували вуглеводом – сахароза. ВДК/сахарозу додавали в середовище культивування розморожених сперматозоїдів (2,9%-вий розчин цитрату натрію), а також перед заморожуванням у лактозо-жовтково-гліцеринове кріосередовище у концентраціях 0,1; 0,01 та 0,001%. **Результати.** Аналіз отриманих результатів показав різний вплив використаного нами наноматеріалу в якості добавки до середовищ. Встановлено, що сперматозоїди кнурів з ВДК/сахароза у 0,001%-й концентрації після розморожування проявили активність на рівні 14,2 %, а загальний час їх виживаності становив 4,0 год. Застосована 0,001%-ова концентрація ВДК/сахарози під час кріоконсервації еякульованих сперматозоїдів кнурів забезпечила 25,0%-ову активність після розморожування із загальним часом виживаності 5,5 годин. **Висновки.** В статті відображена перспективність проведення подальших біотехнологічних досліджень з використанням наноматеріалів різного походження у системі збереження та раціонального використання генетичних ресурсів сільськогосподарських тварин.

Ключові слова: еякульовані сперматозоїди, кнур, наноматеріал, високодисперсний кремнезем, кріоконсервація, збереження генофонду.

Жизнеспособность сперматозоидов хряков при добавлении высокодисперсного кремнезема к составу сред для криоконсервации и размораживания

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Цель. В современных условиях приоритетной задачей сохранения биологического разнообразия является повышение роли сельского хозяйства в поддержке биоразнообразия. В системе длительного сохранения генетических ресурсов сельскохозяйственных животных необходимо усовершенствовать среды для разбавления, криоконсервации и хранения генетического материала животных; технологические элементы криоконсервации генетического материала животных и биотехнологические приемы получения вне организма эмбрионов из криоконсервированного генетического материала. **Методы.** Известно, что к среде для криоконсервации спермы хряков добавляют: гидрофильный экстракт куколок дубового шелкопряда, водный экстракт прополиса, альбумин сыворотки крови крупного рогатого скота, водорастворимые компоненты желтка и липопротеиды, экстракт нерафинированного подсолнечного масла, пролин, триметилглицин (бетаин), а также наноматериалы. В исследованиях использован наномате-

риал марки А-300 с Sудел = 285 м²/г (г. Калуш, Украина), который предварительно прожаривали два часа при температуре 200 °С, поверхность которого модифицировали углеводом – сахароза. ВДК/сахарозу добавляли в среду культивирования размороженных сперматозоидов (2,9%-ный раствор цитрата натрия), а также перед замораживанием в лактозо-желтково-глицериновую криосреду в концентрациях 0,1; 0,01 и 0,001%. **Результаты.** Представлены результаты экспериментальных исследований по взаимодействию криоконсервированных эякулированных сперматозоидов хряков с наночастицами высокодисперсного кремнезема (ВДК) и сахарозы. Следует отметить, что исследование касаются не только технологии криоконсервации гамет, но и деконсервации. Анализ полученных результатов показал различное влияние использованного нами наноматериала в качестве добавки к средам. Установлено, что сперматозоиды хряков с ВДК/сахароза в 0,001%-ной концентрации после размораживания проявили активность на уровне 14,2 %, а общее время их выживаемости составило 4,0 ч. Примененная 0,001%-ная концентрация ВДК/сахарозы при криоконсервации эякулированных сперматозоидов хряков обеспечила 25,0%-ную активность после размораживания с выживаемостью 5,5 ч. **Выводы.** В статье отображена перспективность проведения дальнейших биотехнологических исследований с использованием наноматериалов различного происхождения в системе сохранения и рационального использования генетических ресурсов сельскохозяйствен-ных животных.

Ключевые слова: эякулированные сперматозоиды, хряк, наноматериал, высокодисперсный кремнезем, криоконсервация, сохранения генофонда

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STRATEGIES OF DECREASING HARMFULNESS OF FUSARIOSIS AGENTS IN AGROPHYTOCENOSES

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Infections of cultivated plants, transmitted by fusariosis agents, are among the most harmful factors for humans in grain production. Thus, there is an obvious need for effective control over harmfulness of fusariosis agents in agrophytocenoses. Summarizing scientific data on the issues of forming the strategy of decreasing harmfulness of fusariosis agents in agrocenoses. The major factors of decreasing the level of infecting cereal crops with fusarioses are genetic improvement of plants via selection of species and hybrids, resistant to infections, agrotechnical means and chemical control using modern fungicides with a high level of inhibiting the development of the agent during the whole growing season. The main attention should be paid to controlling the presence and prevalence of infections of plants by such species as *F. graminearum*, *F. pseudograminearum*, *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. avenaceum* and *F. verticillioides*, producing deoxynivalenol, nivalenol, T2- and HT2-toxins, moniliformin, and fumonisins, dangerous for vertebrates. Effective control over fusariosis agents in agrophytocenoses may be achieved via the introduction of resistant varieties and hybrids, restoration of crop rotations, required agrotechnical means and application of efficient fungicides. Summarizing the works in investigating fundamental and applied problems of fusarioses of cultivated crops is important for the organization of the effective system of mycotoxicological monitoring of cereals in Ukraine.

Keywords: fusariosis, *Fusarium*, mycotoxins, fungicides, agrophytocenoses.

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In recent years the problems of excessive field infections due to fusariosis agents have risen to a dangerously high level. Every 2–4 years, up to 5–15 % of winter cereal fields perish due to root rot infections, primarily, fusarioses. Practically each year a considerable share of Ukrainian grain is ranked lower since the fields are infected with fusariosis and cereals are damaged by mycotoxins.

Agrophytocenoses with *Fusarium* inoculum are contaminated at a global level. Rather low levels of controlling the disease using current agrotechnical and chemical protection means urge geneticists and breeders to create varieties and hybrids of cultivated plants, resistant to *Fusarium* species. However, the results of industrial experiments in all soil-climatic regions of Ukraine have demonstrated that novel genetic and biotechnological achievements and introduction of varieties/hybrids of cereals, resistant to fusariosis, cannot ensure a proper level of controlling the disease and a possibility of obtaining high quality grain.

Thus, it is important to pay attention to all the constituents of the technologies of cultivating plants while elaborating the means of effective control over fusarioses in cereals, which has also been noted by prominent phytopathologists in their works – from classic (Bilay) to modern ones (Gagkaeva, Retman, McMullen) [1–12]. Some means of controlling fusarioses are not efficient enough and thus cannot ensure a proper level of controlling the disease. Therefore, it is possible to achieve high and quality yields of cereals via complex application of different strategies of disease control: breeding resistant species/hybrids, agrotechnical means, first and foremost, returning the plant production of the country to biologically substantiated crop rotations and applying highly efficient fungicides, ensuring a better way of maintaining crop productiv-

ity, decreasing the risk of mycotoxin accumulation, ensuring high quality of grain and economic viability of grain production.

Fusarium head blight of cereals and kernel rot are highly harmful diseases, annually decreasing the level of productivity of cereals in Ukraine and causing the contamination of yield with mycotoxins, dangerous for humans and animals. Root rot, caused by the agents of *Fusarium* species, is highly harmful in Ukraine. Taking into consideration economic losses due to diseases and danger for health of the warm-blooded, most countries regulate the set levels of mycotoxins in grain at rather a low level, ppb and less.

It is often believed that plant diseases are caused by one species of the agent or even a specific strain. However, in nature microbes primarily exist in the composition of complex groups, which was noted as far as in the times of van Leeuwenhoek in the 16th century. It is noteworthy that most laboratory studies are based on specific strains of microorganisms, grown in a pure culture. Therefore, at present little is actually known about possible interspecies interactions and/or interactions between different taxons of pathogenic microbes in nature. Numerous infections and many diseases of humans and animals are results of multispecies synergetic interactions. It complicates the disease and should be considered while elaborating efficient control measures. On the other hand, there are scarce data about synergetic pathogen-pathogen interactions in case of diseases of plants, and the mechanisms of interactions are yet unknown. For instance, severe infections of root rot of wheat are caused by *F. graminearum*, *F. culmorum*, *F. poae* and *F. sporotrichioides*, and head blight – by a complex of *Fusarium graminearum* species. Root rot of corn is caused by *F. meridionale* and *F. boothii*, and both root rot and kernel rot – by *Trichoderma* sp., *Penicillium* sp., *Pyrenochaeta indica*, *F. moniliforme*, *F. graminearum* and *oxysporum*. These examples of synergetic interactions between the agents of plant diseases, causing the diseases of whole complexes, may be found to have achieved higher prevalence than expected, and the understanding of the main mechanisms may have important consequences in the field of plant disease epidemiology and fighting diseases [13].

SPECIFICITIES OF GENETIC DETERMINATION OF THE RESISTANCE OF CEREALS TO FUSARIOSES

Resistance to fusarioses is a multigenic feature of cereal crops. The differences between varieties and hybrids in terms of resistance may vary among different countries according to the changes in soil-climatic conditions and specificities of farming. The distinguished types of resistance are as follows: type I – resistance to the primary infection, type II – resistance to the spreading of a disease agent along the plant, type III – resistance to head damage, and type IV – resistance to head blight and trichothecenes. Type V is defined as resistance to the accumulation of trichothecenes. Type V resistance may be formed both via blocking of the accumulation of trichothecenes by inducing the metabolism of toxicants and via inhibiting the biosynthesis of mycotoxins.

250 QTL, present in all 21 chromosomes, have been identified so far. There is a known multigenic resistance: Fhb1 from Sumai 3 = 3B; Fhb2 from Chinese wheat = 6B. Others are QTLs from all the chromosomes of wheat, except for 7D. The most stable ones are on: 1B, 1D, 2B, 2D, 3A, 3B, 5A, 6B. DON resistance is related to 2D, 3B and 5A. The decrease in the damage levels is related to 2D, 3A and 5A [14].

THE ROLE OF CROP ROTATIONS IN INFECTING CEREAL CROP PLANTS WITH FUSARIOSES

Taking into consideration the role of harvest residues, infected with fusariosis agents, in ensuring a high level of inoculum harmfulness in agrophytocenosis, many authors note an important role of crop rotations in decreasing the damage of corn and grain crops by fusarioses [15–20].

Fusariosis agent, *Fusarium graminearum,* usually over-winters on plant residues. Some part of the agent may over-winter on seeds [20, 21]. The degree of the agent over-wintering is higher on plant residues, not infected with rot, for instance, on internodes of grain cereals [22, 23].

Corn fields are dangerous as they promote the development of fusarioses agents, therefore, the recommendations of scientific literature state the need to ensure at least a one-year-period between cereal crops or two years – between crops, sensitive to fusarioses, to decrease harmfulness of the agent [24]. After sowing corn and wheat for three years during the experiments in determining harmfulness of fusariosis agents in the crop rotations, E.B. Khonga and J.C. Sutton found perithecia and ascospores of *Gibberella zeae* – ascigerous stage of the agent of *F. graminearum* in the field, which was mostly found in the course of the first and second year.

S. Inch and J. Gilbert established that *F. graminearum* may be preserved on the infected seed for up to two years, regardless of the location of the seeds – on the soil surface or at the depth of 10 cm in soil [25]. These studies focus the attention on increasing harmfulness of fusarioses in recent years, which is obviously caused by enlarging the area of corn fields in Ukraine. It should be noted that corn fields are enlarged in some regions, first and foremost, in the "grain belt" of Ukraine and mostly in the fields of agroholdings. Due to economic reasons within the recent decade agroholdings and farms have introduced shorter crop rotations, where a high degree of damaging cultivated crops with fusarioses is observed. The agroholdings with large land banks proper define a great export potential of Ukraine in grain production.

A situation, similar to the current one for Ukraine, was observed almost 115 years ago in the eastern and central districts of the "corn belt" in the USA. For instance, D.E. Mathre reported the results of the profitability analysis of cultivating barley and the increase in the level of head blight infection spreading in early 1900s after the corresponding enlargement in the area of corn fields. Fusariosis damage to barley was so extensive that the production of this crop was almost terminated [18].

THE TURNOVER OF A SOIL LAYER: TILLAGE, STUBBLE PLOUGHING – THE FIRST ELEMENT IN CONTROLLING *FUSARIUM* AGENTS

Each methodological recommendation, issued since 1960s up till now to lay out the fundamentals of control fusariosis agents, starts with the thesis about the need of immediate processing of residues (stubble ploughing/tillage) after gathering the harvest. Scientific literature on the depth of tillage after gathering the harvest, deep soil tillage at the depth of 20–30 cm or the surface layer from 10 to 20 cm does not distinguish the impact on the decrease of the development of fusariosis agents, but the predominant majority of decades-long data about the efficiency of *no-till* demonstrate the danger of increased grain damage by mycotoxins. For instance, it was shown that, compared to ploughing, minimal tillage on corn fields resulted in the increase in the level of DON accumulation in the subsequent crop in the crop rotation – wheat – dozens of times [26].

Ploughing/turnover of the soil layer is the first constituent in the strategy of fighting fusarioses of cultivated plants. It should be noted that there is a preserving element of ploughing in terms of keeping harmfulness of fusariosis agents in soil. As a rule, the preservation of fusariosis agents in soil requires plant residues. Here, in case of deficient free oxygen for aerobic processes, the turnover of a heavy soil layer poses a threat of preserving plant residues and keeping harmfulness of the agent in soil. Despite a considerable amount of data about controlling fusariosis in the world literature, starting with Andersen [27] and finishing with modern publications, this specificity of decreasing the efficiency of controlling the disease is almost not considered in the publications. This problem is considered only in the works of D.W. Parry et al., M. McMullen et al., and R.W. Stack [10, 17, 28]. The system of soil tillage affects the prevalence of head blight in the field and the accumulation of mycotoxins [29].

Plant residues, parts of vegetative and generative organs, are the main sources of inoculum in case of infecting with *Fusarium* species [22]. According to the data of different authors, the distances, onto which ascospores are transferred, are in the range from several centimeters to dozens and even hundreds of kilometers. Recent publications have demonstrated that up to 90 % of inoculum comes from rather short distances – up to 6 m [30, 31]. The transfer of ascospores on long distances – dozens or hundreds of kilometers – decreases their harmfulness considerably because of UV-radiation [32–34].

It is noteworthy that economic conditions of grain production form a constant tendency of reducing the elements of cultivation technologies in soil tillage which requires efficient decisions in controlling fusarioses via the introduction of resistant species and application of effective agrochemicals.

THE IMPACT OF NUTRITIOUS BACKGROUND ON THE LEVEL OF CONTROLLING THE AGENTS OF *FUSARIUM* SPECIES

As early as in 1969, P.E. Onuorah demonstrated that the differences in the reaction of wheat varieties to the agents of *Fusarium* depend on the balance of nutrients and the phase of plant development. According to the mentioned author, manual treatment using high doses of nitrogen and potassium on the background of a low level of phosphorus in vegetative experiments decreased the damage of wheat plants by the fusariosis agent [35].

Numerous classic studies of the second half of the previous century demonstrated the efficiency of the main introduction of phosphorus (in the form of orthophosphate), potassium, sulfur, magnesium in terms of

decreasing the prevalence of field infection by fusariosis agents.

Also, the introduction of microelements, which are components of redox-systems of plants, may promote the increase in plant resistance to damage from disease agents. Copper, iron, manganese and zinc are relevant elements in this regard. Within recent 10 years, during the experiments in achieving high productivity of winter wheat, the authors studied this dependence and it was not each year that they received statistically reliable yield gains in case of specific application of microelements. It is reasonable to conduct industrial trials of the application of modern complex fertilizers, containing the components of redox-systems of plants along with fungicides.

As for the role of nitrogen nutrition in the level of infecting plants with fusariosis agents, there is no agreement between our own information and the literature data. It is known that cereal crops evidently prefer nitrogen and neither medium nor high productivity level may be achieved without the nitrogen fertilizers. On the other hand, the main nitrogen nutrition for wheat, if introduced within the vegetation period, promotes powerful development of plant mass which may create conditions for increased risk of field damage with the agents of head blight. Also, foliar introduction of nitrogen in different forms, ammonium first and foremost, may cause the damage of a leaf apparatus and stalks of plants with subsequent infecting by disease agents.

Noteworthy is the fact that foliar introduction of both complex and monoform fertilizers containing organic acids, for instance, citrate, etc., cause the dissolution of cuticular waxes and the increased damage of plants with diseases. Further prevalence of diseases among plants also occurs in case of foliar introduction of complex and monoform fertilizers with a high level of ashen index (in particular, ammonium sulfate) or in high physiologically unsubstantiated doses (for instance, 20–25 kg/ha carbamide in the spraying solution with fungicides in the form of emulsion concentrate, *etc.*). In the experiments of 2004–2006, M. Yoshida et al. established that the application of nitrogen in the phase of blossoming increased the content of wheat protein considerably and did not promote the increased damage of plants with head blight and the accumulation of DON (deoxynivalenol) and NIV (nivalenol). These results demonstrate that nitrogen nutrition for wheat may be conducted closer to the phase of blossoming without any limitations in terms of increasing the accumulation of mycotoxins in grain in case of head blight [36]. It was demonstrated [37] that the increase in the background of nitrogen nutrition from 0 to 160 kg/ha caused the relevant increase in the level of infecting wheat heads with head blight – from 2.2 % at 0 N to 6.6 % at the introduction of 160 kg N per hectare. The form of the introduced nitrogen had no reliable impact on the prevalence of fusariosis. In the second series of experiments, after artificial inoculation with strains of *F. graminearum* and *F. culmorum*, the increase in DON accumulation was observed in case of higher nitrogen nutrition from 0 to 80 kg/ha. The level of DON accumulation remained unchanged with further increase in the nitrogen dose. It was also established [38] that the additional introduction of nitrogen and growth regulator Etefon promoted the increase in infecting wheat and triticale with *Fusarium* agents. It was determined [39] that the genotype and the level of zinc supply are factors, affecting the tolerance of wheat to root rot. Zinc deficiency mostly decreased the accumulation of dry substance mass of wheat seedlings. Infecting by *F. solani* decreased the mass of the seedling considerably only in one variety out of the investigated ones. However, infecting with the agent caused the decrease in the level of SH-groups in the roots. The processing with zinc prior to infecting with *Fusarium* increased the resistance of wheat plants to the agent [39–43].

THE IMPACT OF NANOCOMPOUNDS ON THE LEVEL OF CONTROLLING *FUSARIUM* AGENTS

A noteworthy recent work was the search for antifungal preparations among silver compounds. For instance, silver nanoparticles were investigated with the purpose of decreasing the prevalence of infecting rice *Oryza sativa* with the agent *Gibberella fujikuroi* (conidial stage of *Fusarium moniliforme*). It was established that silver nanoparticles decreased the level of harmfulness of *Gibberella fujikuroi* and did not affect seed germination and the development of seedlings [44]. Both silver nanoparticles and, probably, nanoparticles of microelements as components of redox-systems of plants may be promising constituents of the compositions of known fungicides.

THE BIOLOGICAL CONTROL OF DAMAGING CULTIVATED PLANTS BY *FUSARIUM* AGENTS

Constant increase in the application of chemical means to control diseases triggers the occurrence of resistant strains which, along with the increased contamination of agrophytocenoses with xenobiotics, promotes the search for the means of controlling diseases among

biological agents. Large-scale studies identified a great number of species which have fine potential in terms of controlling phytopathogenic organisms. About 150 species of plants from 30 families and about 50 compounds were found to have potential antifungal activity. These substances may be used to control the species of *Fusarium* [45, 46]. Numerous means of biological control of fusariosis agents are available at the Ukrainian market of agrochemicals. The authors do not have any statistically reliable confirmations of the efficiency of these means in industrial trials. There were no positive reproducible results, obtained in industrial conditions for the administration of preparations of biological control of *Fusarium* species. This problem is discussed in the works of M. McMullen et al. [11, 12].

The drawback of suggestions on the application of some preparations for biological control is separating them from specific agrophytocenoses, wherefrom they have been isolated. In our opinion, a promising way of decreasing the harmfulness of *Fusarium* species is not just restoring crop rotations in the understanding of "classic" specialists but also introducing/forming biologically substantiated complex agrophytocenoses with a proper number of crops. It is known that the highest current result in the productivity of winter wheat – 15.015 t/ha (2003), 15.636 t/ha (2010), 16.791 t/ha (2017) was obtained by the scientists from New Zealand at simultaneous cultivation of two technical crops. It is evident that both different layering of crops and differences in quality indices can form the cenosis with high productivity. It would be reasonable to consider the possibility of simultaneous cultivation of species/hybrids, different in their level of resistance to diseases. For instance, the drawbacks of species with highly productive plasma in terms of the level of resistance to many diseases may be compensated with an underlaying crop (esparcet for wheat, vetch-oats, etc.). This approach may serve as a factor of decreasing the total level of damaging agrophytocenoses by the agents of *Fusarium* species and stable and commercially viable plant cultivation.

It should be noted that in recent years the margin between the chemical and biological methods has been vanishing quickly. For instance, a well-known fludioxonil is a synthetic analogue of pyrrolnitrine, which is a natural antifungal antibiotic of *Pseudomonas pyrrocinia.*

THE DECREASE IN THE LEVEL OF WEEDINESS OF FIELDS IN CONTROLLING THE AGENTS OF *FUSARIUM* SPECIES

A high level of field weediness is related to a relevant increased level of infecting with head blight [47]. The agents of fusarioses of cultivated plants were found on many grass weeds [48, 49] as well as on dicotyledon weeds [50].

There are scarce data in the scientific literature about the impact of herbicides on the level of infecting cereal crops with fusariosis agents. Perennial studies demonstrated the increase in the level of infecting spring wheat plants with fusariosis agents via spring application of glyphosate [51–53]. C.A. Levesque et al. [54–56] demonstrated that the application of glyphosate increased the level of colonization of six weed species with *Fusarium* species and led to a higher level of inoculum/mycelium density in the arable layer of soil. After the introduction of glyphosate and lactophyte, there was a decrease in the germination of conidia, the growth of mycelium and sporulation of *Fusarium solani* f. sp. *glycines* [57].

Outside of immediate control over weeds, herbicides may change the course of development of diseases which usually occurs via direct impact on the agent or indirect effect via the response of the plant to a phytotoxicant. In laboratory experiments herbicides MCPA and flumetsulam did not affect the growth of fungi. 2.4-DB inhibited the growth of fungus by 16–35 %. Such a herbicide as bentazon had a strong inhibiting effect on the development of *F. oxysporum*. Haloxyfop-methyl stimulated the growth of fungus by 29 %. Therefore, the application of some herbicides may affect the development of soil pathogens such as *Fusarium oxysporum*, stimulating or inhibiting their development [58]. The review of D. Sanyal et al. [59] states that the program of complex fighting with pests, pathogens and weeds requires deep studies on the interaction of agrophytocenosis constituents. Vegetative pathogenic organisms get infected from other pests and systems of applying agrochemicals.

The dependence between the manifestation of phytotoxicity of herbicides and changes in soil microflora was first described in 1945 [60, cit. in 59]. For instance, trifluraline may induce the overgrowth and shatter of soy hypocotyl, which creates conditions for the penetration of *Fusarium oxysporum* and complicates the course of foot rot [61]. The literature decribes numerous facts of inhibiting the development of pathogenic microorganisms, for instance, *Fusarium solani* f. sp. *pisi* with glyphosate on field pea *Pisum sativum* L. [62]. S. Sanofo et al. established that glyphosate decreased the level of germination for conidia, the growth of mycelium and sporulation of *Fusarium solani* [63]. Kidney bean plants (*Phaseolus vulgaris* L.) are exposed to

more infecting with *Fusarium* according to the increase in glyphosate concentration [63].

THE IMPACT OF DAMAGE FROM INSECTS ON THE LEVEL OF INFECTING PLANTS WITH *FUSARIUM* SPECIES

Effective control of harmfulness of insects in the fields decreases the level of damage to seedlings and adult plants, and thus the level of field damage by root rot and head blight/kernel rot.

As for direct impact of transferring the agents of *Fusarium* species to plants by insects, it may be foreseen but hard to determine correctly in field and industrial conditions. It should be noted that the presence of many *Fusarium* species in the organisms of numerous species of insects is well-known. As for references, it was written in the review of G.H. Teetor-Barsch and D.W. Roberts in 1983 for 50 years of studies [64].

The works of G.P. Munkvold et al. [65, 66] established that effective control over insects in corn fields with genetically modified insect-resistant corn lines decreased the level of infecting with root rot and the level of fumonisin accumulation in corn along with the decrease in the level of infecting the crop with insects. The differences in the main ways of infecting the plants may also determine the level of the effect of introducing the means of controlling pests on the decrease in infecting the cultivated plants by fusariosis agents. For instance, the level of controlling root rot from pathogen *F. verticillioides* due to the damage of plants by insects will be sufficiently high [67]. This effect of controlling the damage by insects will be considerably lower or will not be detected at all for *F. graminearum*, which infects plants via generative organs of plants [64].

THE IMPACT OF FUNGICIDES ON THE LEVEL OF CONTROLLING F*USARIUM* AGENTS

Modern strategies of controlling the disease involve the use of fungicides, introduction of resistant species/hybrids and ensuring the relevant crop rotation.

In their work M.P. McMullen et al. studied a wide range of active substances of fungicides and made the following conclusions [12]:

- *Fusarium* head blight is a severe disease of cultivated plants, which is hard to control.
- Simultaneous treatment with fungicides of the class of triazoles may lead to a considerable decrease in the content of mycotoxins (DON) and the increased yield.
- The highest efficiency was manifested by prothioconazole, metconazole, tebuconazole + prothioconazole at the anthesis. The application of fungicides at earlier stages decreased the level of controlling head blight.
- It is impossible to achieve the level of controlling head blight of 50–55% and to decrease DON content by 40–45% via the introduction of resistant species. The conclusion is – it is possible to control fusariosis via the introduction of complex systems of protection.
- The application of fungicides of the strobilurin class to control head blight should be avoided due to its inefficiency [12, 68–71].

The results of extensive studies of Folicur (tebuconazol, 38.7 %) in 1998–2003 demonstrated the decrease in head blight infection only by 39.4 % and the content of mycotoxin DON – by 27.4 % [72]. Other fungicides were considerably less effective in controlling the disease.

The studies in Asia also established that tebuconazol was the most efficient in controlling wheat and barley head blight and decreasing the content of DON. Repeated treatment with tebuconazol did not result in statistically reliable relevant decrease in DON content. The efficiency at the level of tebuconazol was ensured by the introduction of Captain (thiophanate-methyl) and copper in the form of Cu-8-quinolate [73]. The limitation of the number of active substances, efficient against head blight, may create a threat of resistance in the strains of agents. It was demonstrated on the isolates of *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*, where the efficiency of tebuconazol decreased after many applications [74, 75].

However, it should be noted that large-scale application of fungicides in the plant cultivation of Ukraine does not take place due to economic reasons. Therefore, the problems regarding the occurrence of resistant species of head blight strains may be postponed for some time. The application of specific active substances of fungicides should be considered not as a factor of controlling a wide range of disease agents but rather as a factor of changing microflora balance in agrophytocenosis. Therefore, the efficient control of fusariosis agents should also be accompanied with proper control over the agents of other diseases, dangerous for the region, which may be achieved by the introduction of complexes of fungicides.

For instance, the specificities of sensitivity of *Fusarium* species and saprophytic fungi, which damage wheat head and are antagonists to *Fusarium* species, were investigated. The investigation was carried out

on isolates from winter wheat heads of *Alternaria alternata*, *Arthrinium* sp., *Aspergillus niger*, *Epicoccum* spp., *Microdochium* spp., *Rhizopus oryzae* and *Trichoderma* spp. In a polycomponent culture, *A. niger*, *R. oryzae* and *Trichoderma hamatum* were more efficient in inhibiting the growth of mycelium of *Fusarium* species compared to *Microdochium majus*. The species *A. alternate* and *Epicoccum* spp. were less efficient due to slow growth of mycelium. Saprophytic species were sensitive to triazoles. Prothioconazole and tebuconazole inhibited the growth of *Fusarium* species. Due to differences in the sensitivity to fungicides, remarkable for *Fusarium* species and their antagonists – saprophytic species, colonizing winter wheat heads, the application of fungicides modifies the balance of microflora of wheat head, which may impact the contamination of grain with mycotoxins [76].

It was established that the decrease in the level of infecting plants with head blight after the application of fungicides did not necessarily cause a relevant decrease in the accumulation of mycotoxins in grain.

A considerable amount of fungicides in sublethal concentrations stimulates the accumulation of mycotoxins *in vitro* [77, 78]. This fact testifies to the inadmissibility of decreasing the set doses of fungicides and using preparations, non-selective to disease agents.

It is important to use modern fungicides, highly active to disease agents, from the class of inhibitors of succinate dehydrogenase of generation II, first and foremost. For instance, this is Adepidyn (active substance – pidiflumetofen), which enhances the efficiency of known triazoles in controlling *Fusarium* agents, for instance, tebuconazole, considerably. Pidiflumetofen in compositions with fungicides of the group of triazoles enhances the efficiency of the composition, prolonging the terms of effective controlling of the agents and efficiently fighting the formation of resistance in the agents of harmful diseases, including fusariosis, Septoria blight, mildew *etc*.

There was also an investigation of the impact of infecting with the agents of *Fusarium* spp. and *Microdochium nivale* on quality indices of the grain of winter wheat, spring wheat, and oats in Sweden after previous treatment with such fungicides as Celest Extra, Formula M (CEFM, difenoconazole + fludioxonil) and Celest, Formula M (CFM, fludioxonil). During field experiments, the treatment of spring wheat seeds with CEFM did not have a considerable impact on most agronomic indices, including harvest. The treatment of the grain of winter wheat and oats with CFM led to the increase in the yield by 7–11 % and the density of plant stand by 33 % without any considerable impact on other indices [78].

The estimation of the term of applying fungicides against the fusariosis agents established that the efficiency of preparations, used 7 days after infecting, was much lower in case of introducing fungicides one day prior to infecting [79].

In the studies of C. Rodriguez-Brljevich, when corn damage started immediately after sowing, the dominating species was *F. graminearum*, and during the vegetative season the colonies of *F. subglutinans* and *F. verticillioides* were the most frequent in the plants of the crop. *Fusarium graminearum* was the most competitive species among *Fusarium* spp. in the colonization of corn rhizosphere; this specificity may have ensured its domination in the cenosis up to the phase of the second corn leaf [80].

Therefore, infecting the cultivated plants with fusariosis agents is one of the main harmful factors for humans in grain production although the agents of *Fusarium* species are saprophytes for a greater part of their life. The active development of plant cultivation in Ukraine highlighted many problems which only get more complicated with time. These super-complicated issues involve the need of efficient control over harmfulness of fusariosis agents in agrophytocenoses. First, this approach is of exclusive relevance for the application, and grain damage by the agents of different *Fusarium* species and mycotoxins is regulated by Ukrainian legislation and normative documents of the leading countries. Therefore, the need to solve this issue has powerful economic substantiation.

The major factors of decreasing the level of infecting cereal crops and other relevant agricultural crops with fusarioses are genetic improvement of plants via selection of species and hybrids resistant to infections, and chemical control using modern fungicides with a high level of inhibiting the development of the agent for a long time, actually – the whole growing season of the crop. Due to the threat of grain contamination with mycotoxins, the main attention should be paid to controlling the presence and infection with the species of *F. graminearum*, *F. pseudograminearum*, *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. avenaceum* and *F. verticillioides*. The main mycotoxins, forming the most widespread species of fungi of *Fusarium* species, – deoxynivalenol, nivalenol, T2- and HT2-toxins, moniliformin, fumonisins – are exclusively dangerous for vertebrates. Therefore, there is an urgent need of creating a reliable

system of measures in preventing mycotoxicoses of humans and animals. The use of PCR and ELISA allows to rapid and inexpensive control the presence of pathogens and mycotoxins. This relevant task requires uniting the efforts of specialists, which would allow summarizing extensive studies of fundamental and applied problems of fusarioses of cultivated plants with the purpose of increasing the efficiency of controlling *Fusarium* agents in agrophytocenoses of Ukraine.

Стратегії зменшення шкодочинності збудників фузаріозу в агрофітоценозах

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Інфікування культурних рослин збудниками фузаріозів є одним із головних шкодочинних факторів для людини у зерновиробництві. Тому, очевидна необхідність ефективного контролю шкодочинності збудників фузаріозу в агрофітоценозах. Узагальнено наукові дані з питань формування стратегій зменшення шкодочинності збудників фузаріозу в агроценозах. Головними факторами зниження рівня інфікування зернових культур фузаріозами є генетичне поліпшення рослин шляхом створення резистентних до інфікування сортів і гібридів, агротехнічні заходи та хімічний контроль з використанням сучасних фунгіцидів з високим рівнем інгібування розвитку збудника протягом усього вегетаційного сезону. Основна увага повинна приділятися контролю присутності та інфікування рослин видами *F. graminearum*, *F. pseudograminearum*, *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. avenaceum* та *F. verticillioides*, які продукують небезпечні для хребетних тварин дезоксиніваленол, ніваленол, Т2- і НТ2-токсини, моніліформін та фумонізини. Ефективний контроль збудників фузаріозів в агрофітоценозах може бути досягнуто за впровадження резистентних сортів та гібридів, відновлення сівозмін, необхідних агротехнічних заходів, а також застосування ефективних фунгіцидів. Узагальнення розробок з дослідження фундаментальних та прикладних проблем фузаріозів культурних рослин важливо для організації ефективної системи мікотоксикологічного моніторингу збіжжя по Україні.

Ключові слова: фузаріоз, *Fusarium*, мікотоксини, фунгіциди, агрофітоценози.

Стратегии снижения вредоносности возбудителей фузариоза в агрофитоценозах

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Инфицирование культурных растений возбудителями фузариоза является одним из главных вредоносных факторов для человека в зернопроизводстве. Поэтому очевидна необходимость эффективного контроля вредоносности возбудителей фузариоза в агрофитоценозах. Обобщены научные данные по вопросам формирования стратегий уменьшения вредоносности возбудителей фузариоза в агроценозах. Главными факторами снижения уровня инфицирования зерновых культур фузариозами являются генетическое улучшение растений путем создания резистентных к инфицированию сортов и гибридов, агротехнические мероприятия и химический контроль с использованием современных фунгицидов с высоким уровнем ингибирования развития возбудителя в течение всего вегетационного сезона. Основное внимание должно уделяться контролю присутствия и инфицирования растений видами *F. graminearum*, *F. pseudograminearum*, *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. avenaceum* и *F. verticillioides*, продуцирующих опасные для позвоночных животных дезоксиниваленол, ниваленол, Т2- и НТ 2-токсины, монилиформин и фуминозин. Эффективный контроль возбудителей фузариоза в агрофитоценозах может быть достигнут при внедрении резистентных сортов и гибридов, восстановлении севооборотов, необходимых агротехнических мероприятий, а также применении эффективных фунгицидов. Обобщение разработок по исследованию фундаментальных и прикладных проблем фузариоза культурных растений важно для организации эффективной системы микотоксикологического мониторинга зерна в Украине.

Ключевые слова: фузариоз, *Fusarium*, микотоксины, фунгициды, агрофитоценозы.

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ПРАВИЛА ПРАВИЛА ДЛЯ АВТОРІВ

У журналі «Agricultural Science and Practice» публікуються результати фундаментальних і прикладних досліджень з питань грунтознавства, землеробства, рослинництва, ветеринарії, тваринництва, кормовиробництва, генетики, селекції та біотехнології, механізації, агроекології, радіології, меліорації, переробки та зберігання сільськогосподарської продукції, економіки, інноваційної діяльності.

Друкуються статті, які раніше не видавалися, огляди літератури, короткі повідомлення. Статті обов'язково рецензуються на конфіденційній основі.

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Таблиці повинні мати заголовок і порядковий номер. Примітки до таблиць розміщують безпосередньо під ними.

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Перелік літератури складається винятково англійською мовою (назви статей з періодичних видань повинні відповідати таким з англомовних резюме, розміщених у зазначених виданнях; заголовки монографій або статей з них також мають бути перекладені англійською мовою, транслітерація допускається лише у разі назв україно(російсько)мовних періодичних видань (Agrarna nauka i osvita), місць видання і видавництв (Kharkiv, NNC «IGA im. O. N. Sokolovsky»)) у порядку цитування, оформлення джерел слід здійснювати за прийнятим в журналі стандартом (див. приклади).

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2**.** *Oka Y, Chet I, Spiegel Y.* An immunoreactive protein to wheat-germ agglutinin antibody is induced in oat roots following invasion of the cereal cyst nematode Heterodera avenae, and by jasmonate. *Mol. Plant Microbe Interact*. 1997;**10**(8):961−9.

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3. *Pivovarov S.* Physico-chemical modeling of heavy metals (Cd, Zn, Cu) in natural environment. *Encyclopedia of Surface and Colloid Science*. Boca Raton, CRC Press. 2004;Vol. 6:468–93.

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- A Microsoft Word file with a typescript identical to the paperback copy. (The file should be named with the surname of the first author, e.g., orlyk.doc).
- All tables, charts, drawing and pictures, either full color or black and white, should be submitted in any of standard graphical formats: .ppt, .xls, .psd (e.g., ris1_ orlyk.ppt, ris2_ orlyk.xls).
- The Authors' Credits (file name – e.g., auth_ orlyk.doc) including surnames, first and middle names of all authors, at that it is necessary to appoint one of them responsible contact person for communication with the Editorial Board; e-mail and phone number (including area code); also official titles and mailing addresses of the institutions, where the research has been conducted. All data should be presented both in Russian and English.

The articles submitted either in Russian or Ukrainian are required to be accompanied with the Ukrainian/Russian-English glossary including at least 30 terms particularly involved in the text of the article (file name – e.g., voc_ orlyk.doc).

Typescript Requirements

The text for publication should be submitted in any of the file formats supported by Microsoft Word, paper format: A4, page orientation: portrait, font: Times New Roman, size: 14, line spacing: 2.

The complete size of any article dedicated to research results is limited to 27,000 characters including spaces (~13 pages), a review of literature – up to 50,000 characters including spaces (~24 pages), a brief note – up to 12,000 characters including spaces (~6 pages) including the text itself, all accompanying tables, charts, drawing and pictures with captions, abstracts in three languages, keywords and references.

The typescript should contain:

UDC identifier.
Title of the article.
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Title(s) and mailing address(es) of the institution(s) where the author(s) work(s).
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The text of the article describing research results should consist of "Introduction", "Research Procedure", "Main Points", "Conclusion" "Findings".
The tables should be entitled and enumerated. Any notes to them should be given exactly below.
The total quantity of figures should be not more than 4 for reviews of literature, 6 in articles on research results, and 2 for brief notes. All lengthy letterings should be replaced with either numbers or letters and explain them in legend.
When referred to any grants, point out the foundation, name and/or number of grant in section "Acknowledgements".

References should be compiled in English exactly and in order of citation. The list of sources must meet the requirements of the bibliographic standard accepted in the journal (see an example). The references should be enumerated in order of their quoting in the text, where they are marked with the correspondent number in square brackets.
Both hyperlinks and references to websites are strictly prohibited! The sources of citation should be generally available!

Well-defined abstracts in English, Russian and Ukrainian, identical to each other as concerning to content (up to 1,500 characters including spaces in each language). The suggested structure of its text: Objective, Methods, Results, Conclusions, Keywords (up to 10).

Examples of References:

Book citation –

1. Author's surname, title of the book (if there is no published translation into English, should be transliterated with roman letters). Kyiv: Naukova dumka. 2006;256 p.

Article citation –

2. Author's surname, title of the article (if there is no published translation into English, should be transliterated with roman letters). *Agroekologia*. 2005;**12**(10):923–30.

Attention!

The articles structured against the present requirements will be declined and sent back to the authors neither registered, nor reviewed.
The accepted articles are subject to review by independent experts appointed by the Editorial Board, thereafter the author will receive the article's copy accompanied with the reviewers' remarks. The version of the article amended by the authors and agreed with the reviewers is taken as a final one. It should be undersigned both by the authors and reviewers as "Gone To Bed". Thereupon no changes or amendments of text, tables, charts, drawing and pictures are available. The Editorial Board takes into account the acceptance date of the final version of the article in course of publication.
If the article is declined by the reviewers, the Editorial Board will send the written notice to the author via e-mail.