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VIABILITY AND VIRULENCE OF WHEAT LEAF RUST AGENT (*Puccinia triticina* Eriks.) ISOLATES AFTER LONG TERM PRESERVATION

N.S. ZHEMCHUZHINA, M.I. KISELEVA, A.I. ZHEMCHUZHINA, S.A. ELIZAROVA

All-Russian Research Institute of Phytopathology, 5, ul. Institute, pos. Bol'shie Vyazemy, Odintsovskii Region, Moscow Province, 143050 Russia, e-mail zhemch@mail.ru (✉ corresponding author), kiseleva@vniif.ru, zhemchuzhina@vniif.ru, elizarova@vniif.ru
ORCID:
Zhemchuzhina N.S. orcid.org/0000-0001-6374-403X
Kiseleva M.I. orcid.org/0000-0001-7813-3266
Zhemchuzhina A.I. orcid.org/0000-0002-2060-3306
Elizarova S.A. orcid.org/0000-0001-9224-8430
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Abstract

The State Collection of phytopathogenic microorganisms (ARRIP) accumulates a great number of wheat leaf rust agent (*Puccinia triticina* Eriks.) isolates, an extremely harmful and epiphytotic pathogen. Annually the collection is replenished with new leaf rust isolates from different populations. Annual estimation of the virulence genes' frequency in isolates makes it possible to track the dynamics of the fungal populations. One of the main tasks of the State collection is to preserve the isolates of the fungus without losing their biological properties to involve these isolates in further laboratory and field experiments. For this purpose, the viability and virulence of *P. triticina* collection isolates was evaluated during 10-year preservation in a household refrigerator (+4 °C) and in a REVCO freezer (–80 °C, Revco, USA). We used 124 *P. triticina* isolates collected in 2005, 2006, 2008, 2009, 2010 and 2012 from damaged wheat (*Triticum aestivum* L.) samples in the Central, North Caucasus and West Siberian regions of the Russian Federation. The isolates differed in virulence and were assigned to 74 phenotypes. The viability of the isolates after storage was determined by spore germination on 2 % water agar plates and by inoculation of susceptible wheat cultivars. Storing of the leaf rust uredospores at low positive temperatures quickly led to a weakening of the viability of the fungus, up to complete destruction. After 1-2 months at +4 °C, the isolates had a high germination capacity, from 48 to 95 %, which decreased in 6 months to 3.0-22.7 %. The correlation between the number of germinated spores on 2 % agar-agar and the duration of storage at a low positive temperature was 0.79. Leaf rust isolates remained viable during storage for 3-10 years under low negative temperatures (–80 °C). The number of germinated spores of different isolates regardless of the preservation period was 25-79 %, and the disease intensity reached 25-100 %. Many factors influence viability of isolates. These mostly are improper sample processing prior to putting into storage and during reviving from an anabiotic state, or disturbance of storage of technical character. However, storage of *P. triticina* isolates at low negative temperatures ensured a rather high survival rate for 10 years. Checking the virulence of the isolates after 7-year storage with the use of susceptible wheat cultivar and *Lr9* and *Lr19* lines showed identical indicators before and after the storage. The method of storing spores at –80 °C allows rather high rate of preservation without changing fungal viability and virulence.

Keywords: collection of microorganisms, leaf rust, isolate, population, virulence, preservation

The State Collection of Phytopathogenic Microorganisms of the All-Russian Research Institute of Phytopathology is intended for the long-term storage of pathogens of agricultural crops in a viable state [1-3]. The Collection fund contains about 5,500 strains of the causative agents of plant diseases (fungi, bacteria and viruses). For the recent 10 years, more than 1,000 isolates of *Puccinia triticina* Eriks. from different wheat cultivation regions of the Russian Federation have been collected. The virulence spectrum of the collected samples of this fungus makes it possible to preserve the diversity of the natural populations of *P. triticina*, to use them for predicting the dynamics of the resistance gene frequency and tests during wheat breeding [4, 5].

Monitoring of *P. triticina* population virulence makes it possible to reveal new resistance genes. In the nature, mutations and genetic recombination, as well as spore migration, significantly influence virulence of the fungus populations [6-8]. Due to joint evolution in the "host-pathogen" system, the permanent selection of virulent clones of leaf rust goes on in the resistant wheat varieties. Virulent clones accumulate in *P. triticina* populations; the avirulent clones become displaced and eventually either eliminate or remain in small amounts. The annual variations in the frequency of leaf rust races are influenced by weather conditions and by the set of cultivated wheat varieties [5, 9]. The annual sampling of *P. triticina* isolates to the collection makes it possible to compare the genetic material of different years and thus to trace the population changes in the frequency of virulence genes, as well as to determine the influence of the cultivated wheat varieties on the appearance and spread of the pathogen's races [10, 11]. As the result of monitoring the virulence of *P. triticina* populations, new potentially dangerous races are detected. Basing on the study of the dynamics and frequency of virulence genes, the effectiveness of wheat *Lr* genes in Russian regions is determined to predict their inclusion in breeding programs. Information about the gene pool of the pathogen populations makes it possible to use proper pathogen compositions for artificial inoculants and to evaluate the wheat genotype resistance to the leaf rust pathogen [12, 13].

The main factor influencing the emergence and frequency of new *P. triticina* races is natural selection which occurs in fungal populations under the influence of the varieties with the race-specific resistance [14]. In this regard, the information about finding out of the clones of wheat leaf rust pathogen which overcomes the resistance of host varieties having the effective resistance genes becomes relevant for the selection of samples which can be used in the breeding for immunity to the disease.

It is known that more than 90% of microorganisms, including rust fungi, cannot be cultured on an artificial medium. The *P. triticina* fungus is an economically important fungus, the storage of isolates of which often causes certain difficulties. The studies related to the necessity to preserve such microorganisms imply the choice of the conditions of conservation and reactivation under which the restoration of the pathogen viability is possible. The maintaining of the isolates in working state and preserving their valuable properties are of importance for practical use [15-17]).

The creators of the collection were faced with the task not only to maintain the *P. triticina* isolates in a viable state, but also to optimize the conditions for maintaining physiological properties of the fungus [18, 19]. It is known that traditional methods of storage of rust fungi (drying and sealing of uredospores in ampoules) do not guarantee the maintenance of high viability for a long time.

Storing in liquid nitrogen requires significant costs. Storing the rust spores in household refrigerators at low positive temperatures allows them to remain viable for several months, and in the form of herbarium material — up to a year. In a routine daily work which consists of continuously repeating cycles requiring the short-term maintenance of the fungus in a viable state, storing the spores at low positive temperatures is sufficient. However, the long-term storing of the fungus requires different conditions.

One of the most widely used contemporary methods of preserving biomaterial without changing its viability is storage in freezers at ultra-low temperatures [20, 21].

It is recommended for many mycological objects to place them in freezing chambers at –80 °C. Low negative temperatures stops biochemical processes in cells including the metabolic process. Using the freezers with ultra-low tem-

peratures ensures the preservation of rust fungi spores without changing their biological properties for 7 years or more [22-24].

In this work, for the first time, the correlation relationship between the viability of leaf rust isolates and the duration of storage at low positive and ultra-low temperatures has been established. As the time of spores storing at a low positive temperature extended, the viability of the isolates decreased that had been expressed in the reduction of spore germination on 2% starvation agar and in the decrease of the intensity of wheat seedlings infecting. Under the conditions of ultra-low temperatures, regardless of the storing period, the isolates had been retaining the ability to germinate on the starvation agar and to infect the plants. The duration of storage at ultra-low temperatures had not influenced the changing of the virulence sign.

The goal of our researches was the comparative assessment of the viability and virulence of the collected isolates of *Puccinia triticina* at the long-term storage under conditions of low positive (+4 °C) and low negative temperatures (−80 °C).

Techniques. The material for the 10-year study was 124 isolates of *P. triticina* taken from the infected wheat samples (*Triticum aestivum* L.) in 2005, 2006, 2008, 2009, 2010 and 2012 in the Central, North Caucasus and West Siberian regions of the Russian Federation.

Isolation, reproduction and identification of virulence in single-pustule isolates of *P. triticina* were performed under optimal conditions for the development of plants and pathogen, the relative average daily air temperature +20 °C, relative air humidity 60% (daytime) and 70% (in night), light intensity 10-15 thousand lux, and 16-hour photoperiod.

The plants of the susceptible Khakasskaya line and the wheat lines with the single resistance genes were grown according to the standard technique in hydroponic culture [12]. The virulence genes in the *P. triticina* isolates were determined with Thatcher *Lr* lines having juvenile resistance genes. The set with juvenile resistance genes contained 42 *Lr* lines, i.e. *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr19*, *Lr20*, *Lr21*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr27+Lr31*, *Lr28*, *Lr29*, *Lr30*, *Lr32*, *Lr33*, *Lr36*, *Lr38*, *Lr39*, *Lr40*, *Lr 41*, *Lr 42*, *Lr44*, *Lr45*, *Lr46*, *Lr47*, *Lr51*, *Lr53*, and *LrB* [25].

In 25 5-6-day old seedlings lower side of the leaf was inoculated with aqueous suspension of uredospores (0.5 mg spores per 1 ml of water). For better adhesion, one or two drops of Tween^{R} 20 were added to the water. The spores were applied using the scalpel after the wax removal from the leaves with fingers. The infected wheat plants were placed into the humid chamber at +18-20 °C for 16-20 hours, and then into the artificial climate chamber with controlled conditions of temperature, humidity and lighting. In 5-7 days after the inoculation, the plants were examined to detect the disease signs which were evaluated according to the common international practice [26-28]. After the differentiation by virulence, each isolate of *P. triticina* was propagated on the susceptible Khakasskaya line until 4-5 mg biomass accumulation and placed for storage to the State collection of the All-Russian Research Institute of Phytopathology.

Spores were stored for 1-10 months at +4 °C in a household refrigerator in test tubes and for 3, 4, 5, 6, 7, and 10 years at –80 °C in the REVCO freezing chamber (Revco, USA) in plastic containers. Before each inspection of the material, the uredospores stored in the freezers were recovered from the anabiosis by heating at +45 °C for 5 minutes [29].

The viability of the isolates was determined by germination on plates with 2% starvation agar and by inoculation of the seedlings of susceptible wheat

variety with spore suspension [30]. In the first case, the molten 2% starvation agar was poured onto the glass slides laid out in the sterile Petri dishes. The spores were plated using a preparation needle. The dissemination evenness was achieved by slight knocking with the needle on the Petri dish's edge. Then the dishes were closed and kept at room temperature. After 6 hours, the glasses were examined under the microscope at high magnification and the number of germinated spores in 100 examined samples (triple replication) was counted out. The viability of the spores of each isolate was expressed in percentage value.

In order to test the ability to infect the 5-6-day-old wheat seedlings of the universally susceptible Khakasskaya line, the plants were inoculated with spores taken from storage and recovered from anabiosis. The formation of pustules on the leaf surface served as the indicator of the spore viability.

In the statistical processing of the results, the coefficients of correlation (*r*) and coefficients covariance were calculated using the Microsoft Excel program [2, 3].

Results. All *P. triticina* isolates used in this work have been marked with the stating of their origin and virulence. The differentiation of the *P. triticina* isolates with the wheat lines having the juvenile resistance genes has revealed significant differences in virulence. The isolates contained different number of virulence genes and were classified into 74 phenotypes. Although the isolates derived from the *P. triticina* populations in different years, all of them were characterized by the presence of the same 12 virulence genes: *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p17*, *p18*, *p21*, *p30*, *p33*, and *pB*. The *P. triticina* isolates differed among themselves by the presence of genes *p1*, *p2a*, *p2b*, *p2c*, *p9*, *p11*, *p15*, *p16*, *p19*, *p20*, *p23*, *p25*, *p26*, *p27 + p31*, *p28*, *p32*, *p36*, *p38*, *p39*, *p40*, *p44*, and *p46*. No *p24*, *p29*, *p41*, *p42*, *p45*, *p47*, *p51*, and *p53* genes have been detected in isolates from the Collection.

While storing the uredospores of the leaf rust pathogen under low positive temperatures, their viability weakened rapidly (Table 1). Thus, after the storing at +4 °C for 1-2 months, the isolates demonstrated a high (from 48 to 95%) germinability of spores, and after 6 months this value decreased to 3.0-22.7%. After 10 months of storing, the spores turned to be completely non-germinable. A 0.79 correlation was found between the number of spores germinated on 2% starvation agar and the duration of storage in the household refrigerator. The similar results have been also obtained when infection of the seedlings of the susceptible Khakasskaya variety with the spore suspensions: as the duration of storing extended, the intensity of affection of the wheat seedlings decreased.

1. Viability of *Puccinia triticina* Eriks. uredospores on 2% starvation agar and on seedlings of susceptible Khakasskaya wheat variety after 1-10 month storing at +4 °C

Storing, months	Isolates	Germinated spores (min-max), %	Affection of the wheat seedlings (min-max), %
1	8	55,9-89	60-100
1,5	5	69,4-84,6	60-100
2	32	48,0-95,0	60-100
3,5	12	23,0-76,0	25-60
6	23	3,0-22,7	1-20
8	5	2,3-7,3	1-5
8,5	3	0-2,3	0-1
9	4	0,7-1,3	0-1
10	5	0	0
Correlation coefficient		–0,79	–0,65
Covariance coefficient		0,62	0,42

At –80 °C the isolates remained viable for 3-10 years. Regardless of the storing duration, in different isolates the percentage of germinated spore was 25-

79% and the plants affection reached 25-100%.

The viability values of isolates at the same storing duration had been influenced by many factors, the main of which could be faults when the preparation of the material before putting for storage and technological violations of the storage conditions. Nevertheless, the storage of the Collection samples of *P. triticina* at low negative temperatures ensured a rather high survival rate for 10 years (Table 2).

2. Viability of *Puccinia triticina* Eriks. isolates on 2% starvation agar and on seedlings of susceptible Khakasskaya wheat variety depending on duration of storing in the freezer

Years	Storing, years	Number of isolates	Spores germinated on the agar (min-max), %	Affection of the wheat seedlings (min-max), %
2005-2015	10	15	34-69	25-80
2008-2015	7	8	34-56	25-60
2006-2012	6	6	45-48	40
2006-2011	5	10	25-60	25-80
2006-2010	4	10	45-68	40-80
2009-2012	3	11	50-67	40-80
2010-2012	3	16	35-79	25-100
2012-2015	3	9	53-78	60-100
Correlation coefficient			–0,22	–0,41
Covariance coefficient			0,05	0,17

The storing of the *P. triticina* isolates at low negative temperatures does not affect their virulence. This has been confirmed by the results of inoculation of the susceptible Khakasskaya line and two monogenic lines of the Thatcher variety (*Lr9* and *Lr19*) with isolates taken from the storage (Table 3). The *Lr9* and *Lr19* lines were used as the indicators of virulence of the isolates with the *p9* and *p19* genes. The isolates of *P. triticina* (34 ones in total) collected in the Krasnodar Territory, Omsk and Moscow regions demonstrated the same response on the tester lines as when putting for storage (see Table 3).

Thus, the comparison of isolates 729-5 (from the Omskaya 32 variety), 730-1, 730-2, 730-4, 730-6, 730-11 (from the Chernyava variety), 733-3 (from the Chernyava 13 variety) and 732-1 (from the Omskaya 29 variety) collected in the West Siberian region did not reveal differences in the virulence to *Lr9* and *Lr19* lines before and after the storage. The uredospores collected in 2010 in Moscow region from the Pamyati Fedina, Moskovskaya 39, Mironovskaya 808 and Moskovskaya 39 winter wheat varieties and in 2008 in the Krasnodar Territory from Batko, Valeria, Krasnodar 99, Kupava, Delta, Michigan Amber varieties, in 2015 year, as when putting for storage, were avirulent to *Lr9* line. In the virulence formulas of 648-4, 648-11, 649-2 and 676-14 isolates, the *p19* gene has been noted. Testing of the virulence of these isolates after 7 years storage showed the identical reactions on the tester lines.

Despite the significant progress in genetics, biochemistry, physiology, and ecology of microorganisms, the mechanisms responsible for the reversible transition of cells to the anabiotic state are still studied insufficiently. For the many years of creating the collections of bacteria and fungi, general but not always clear concepts about managing the processes of conservation and restoration of the viability of each specific organism have accumulated. The interest to the works on investigation of the structural and functional cell transformations of microorganisms under the influence of conservation-reactivation factors appeared after the finding of alive microbes in the Arctic ice [31]. The experience of working with collections testifies that many contemporary methods of conservation turn to be relatively effective in maintaining laboratory cultures of microorganisms. However, the conservation at ultralow temperatures with the complete preservation of populations and genomes is the most effective especially if

3. Virulence of the *Puccinia triticina* Eriks. isolates before and after the storing at low negative temperatures (–80 °C)

Years	Name code of the isolate	Virulence formula (presence of the virulence genes)	Virulence lines having the resistance gene	
			Lr9	*Lr19*
		Krasnodar Territory, North Caucasus		
2008-2015	670-2	*p1*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p25*, *p26*, *p27*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	670-6	*p1*, *p2c*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p21*, *p25*, *p26*, *p27* + *p31*, *p30*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	670-4	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p21*, *p23*, *p25*, *p26*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	S/S
2008-2015	670-7	*p1*, *p2b*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p25*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	670-8	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p21*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	S/S
		Omsk Region, West Siberia		
2008-2015	648-4	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p11*, *p14a*, *p14b*, *p15*, *p16*, *p17*, *p18*, *p19*, *p20*, *p21*, *p25*, *p26*, *p27* + *p31*, *p28*, *p30*, *p32*, *p33*, *pB*	R/R	S/S
2008-2015	648-9	*p1*, *p2c*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p21*, *p23*, *p25*, *p30*, *p33*, *pB*	R/R	R/R
2008-2015	648-11	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p19*, *p21*, *p23*, *p25*, *p28*, *p30*, *p33*, *p36*, *pB*	R/R	S/S
2008-2015	649-2	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p17*, *p18*, *p19*, *p20*, *p21*, *p25*, *p26*, *p27* + *p31*, *p30*, *p33*, *pB*	R/R	S/S
2008-2015	653-1	*p1*, *p2c*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p21*, *p25*, *p27* + *p31*, *p28*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	676-5	*p1*, *p2c*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p16*, *p17*, *p18*, *p20*, *p21*, *p26*, *p27* + *p31*, *p30*, *p32*, *p33*, *pB*	R/R	R/R
2008-2015	676-6	*p1*, *p2c*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p15*, *p16*, *p17*, *p18*, *p20*, *p21*, *p26*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	676-7	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p16*, *p17*, *p18*, *p20*, *p26*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *pB*	R/R	R/R
2008-2015	676-12	*p1*, *p3a*, *p3bg*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p20*, *p25*, *p30*, *p32*, *p33*, *pB*	R/R	R/R
2008-2015	676-14	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p20*, *p26*, *p30*, *p32*, *p33*, *pB*	R/R	S/S
2010-2012	729-1	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p20*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	R/R
2010-2012	729-5	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p19*, *p20*, *p21*, *p26*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	729-6	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p20*, *p21*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p44*, *pB*	R/R	R/R

Table 3 continued

2010-2012	730-1	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p20*, *p21*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	S/S	R/R
2010-2012	730-2	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p14a*, *p17*, *p18*, *p20*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	S/S	R/R
2010-2012	730-4	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p19*, *p20*, *p21*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *p46*, *pB*	S/S	S/S
2010-2012	730-6	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *p46*, *pB*	S/S	R/R
2010-2012	730-11	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p11*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p20*, *p21*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	S/S	R/R
2010-2012	733-3	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p20*, *p21*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	S/S	R/R
2010-2012	732-1	*p1*, *p2a*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p9*, *p10*, *p11*, *p14a*, *p14b*, *p15*, *p17*, *p18*, *p20*, *p21*, *p23*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*		
		Moscow Region, Central Russia		
2010-2012	728-3	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p17*, *p18*, *p19*, *p21*, *p23*, *p25*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	728-1	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	R/R
2010-2012	720-4	*p1*, *p2b*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p20*, *p21*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p44*, *pB*	R/R	S/S
2010-2012	728-4	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p17*, *p18*, *p19*, *p20*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	728-8	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p17*, *p18*, *p19*, *p20*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	718-3	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p15*, *p16*, *p17*, *p18*, *p19*, *p20*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	720-2	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *p39*, *p40*, *p44*, *pB*	R/R	S/S
2010-2012	724-1	*p1*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p16*, *p17*, *p18*, *p19*, *p21*, *p23*, *p25*, *p26*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *p39*, *p40*, *pB*	R/R	S/S
2010-2012	718-1	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p14a*, *p14b*, *p15*, *p16*, *p17*, *p18*, *p19*, *p20*, *p21*, *p23*, *p25*, *p30*, *p32*, *p33*, *p36*, *p39*, *p40*, *p46*, *pB*	R/R	S/S
2010-2012	720-5	*p1*, *p2b*, *p2c*, *p3a*, *p3bg*, *p3ka*, *p10*, *p11*, *p14a*, *p14b*, *p15*, *p16*, *p17*, *p18*, *p21*, *p23*, *p25*, *p27* + *p31*, *p30*, *p32*, *p33*, *p36*, *p39*, *p40*, *p44*, *p46*, *pB*	R/R	R/R

Note. R — resistance, S — susceptibility (types of plant responses to *P. triticina* isolates before/after the storage).

considering the phenomenal physiological diversity of microorganisms [19-22].

During the period of practical tests on the conservation of microorganisms, the techniques of turning vegetative cells into the anabiotic state have been developed [23, 24], however, the works on finding out the clearer criteria when managing the processes of conservation and restoration of the viability of certain microorganisms are still topical.

Thus, we have established that low temperatures (– 80 °C) is effective for the long-term (from 3 to 10 years) storing of the collection of wheat leaf rust isolates. Even while storing for 10 years, low temperatures had not been decreasing the pathogen viability and for 7 years had not been affecting its virulence. Low positive temperatures (+4 °C) are unsuitable for the long-term storing of the *Puccinia triticina* isolates and may be used for preserving only up to 2-3 months that makes it impossible to use these spores in the next growing season. The maintaining of *P. triticina* isolates in usable condition and preserving their valuable properties are important not only for population genetics, but also for selection studies.

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