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THE MASS SPECTRAL ANALYSIS OF SOME CHEMICAL ELEMENTS' CONTENT IN THE FLAG LEAVES OF WHEAT (*Triticum aestivum* L.) ISOGENIC LINES WITH DIFFERENT RESISTANCE TO BROWN RUST

L.E. KOLESNIKOV[1], O.I. BUROVA[2], Yu.R. KOLESNIKOVA[3], A.V. LAVRISHCHEV[1], M.N. PAVLOVA[1]

[1]*Saint-Petersburg State Agrarian University,* 2, Peterburgskoe sh., St. Petersburg—Pushkin, 196601 Russia, e-mail kleon9@yandex.ru (✉ corresponding author), avlavr@rambler.ru, mn_pavlova@mail.ru;
[2]*Institute of Hygiene, Occupational Diseases and Human Ecology*, Federal Medical and Biological Agency of Russia, 93, st. Kapitolovo, Kuzmolovskii, Leningrad Province, 188663 Russia, e-mail burova.olga.spb@mail.ru;
[3]*Federal Research Center the Vavilov All-Russian Institute of Plant Genetic Resources,* Federal Agency for Scientific Organizations, 42-44, ul. Bol'shaya Morskaya, St. Petersburg, 190000 Russia
ORCID:
Kolesnikov L.E. orcid.org/0000-0003-3765-1192
Burova O.I. orcid.org/0000-0001-9436-2085
Kolesnikova Yu.R. orcid.org/0000-0002-4002-220X
Lavrishchev A.V. orcid.org/0000-0003-3086-2608
Pavlova M.N. orcid.org/0000-0002-4826-3593
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Abstract

The wheat resistance to diseases, including the leaf rust pathogen, is the essential factor contributing to yield preservation. The Thatcher lines with *Lr*-genes are widely used in the assessment of differential interaction between resistance and virulence genes of a host plant and the pathogen. The aim of this work was a quantitative analysis of chemical element composition of flag leaves in 29 Thatcher isogenic lines with various genes for resistance to wheat leaf rust, Tc*Lr28*, Tc*Lr29*, Tc*Lr24*, Tc*Lr47*, Tc*Lr18*, Tc*Lr19*, Tc*Lr36*, Tc*Lr3ka*, Tc*Lr3bg*, Tc*Lr16*, Tc*Lr17*, Tc*Lr44*, Tc*Lr1*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr3a*, Tc*Lr10*, Tc*Lr11*, Tc*Lr14a*, Tc*Lr20*, Tc*Lr33*, Tc*Lr26*, Tc*LrB* (juvenile resistance); Tc*Lr35*, Tc*Lr12*, Tc*Lr21*, Tc*Lr48* (age-related resistance); Tc*Lr46*, Tc*Lr34* (partial resistance genes). The content of twenty-one chemical elements, including heavy, light metals, and metalloids (Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Cu, Ni, Zn, Se, Mo, Ba, Pb, Sb, As, Cd, Be, Ag) in leaves of the isogenic lines was identified by mass spectrometry analysis. For quantitative analysis, leaves without visual symptoms of wheat leaf rust were collected. The disease development according to the R.F. Peterson's scale, pustule number on flag leaves, pustule area according to the ellipse area formula, and reaction type according to the scale of E.B. Mains and H.C. Jackson have been considered as parameters of the pathogenesis caused by the wheat leaf rust agent. The wide range of the parameters enabled us to use various statistical methods and to improve the accuracy of the differences identified. On the highly resistant lines protected by the *Lr*-genes of juvenile resistance, *Lr24, Lr28, Lr29, Lr47*, the signs of brown rust have not been revealed. The lines Tc*Lr18*, Tc*Lr19* and Tc*Lr36* were moderately resistant to the disease whereas the lines Tc*Lr3ka*, Tc*Lr3bg*, Tc*Lr12*, Tc*Lr16*, Tc*Lr17*, Tc*Lr44*, and Tc*Lr46* were moderately susceptible. The high susceptibility to the wheat brown rust was found for Tc*Lr1*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr3a*, Tc*Lr10*, Tc*Lr11*, Tc*Lr14a*, Tc*Lr20*, Tc*Lr33*, Tc*Lr26*, and Tc*LrB*. In the leaves of highly resistant isogenic lines with juvenile resistance and no symptoms, there was significantly less amount of heavy metals (Ni, Ag, Cr, Fe, Co, and Cd) and also K as compared to that in the lines with high susceptibility to the disease. The brown rust intensity decreased with the increase of the selenium content in the flag leaves. The lines bearing juvenile resistance *Lr*-genes showed a fewer reliable correlations between the chemical elements accumulation in the flag leaves and the greater affection by the leaf rust pathogen compared to the lines with genes of age-related resistance. It was found that the flag leaf levels of Al, Cr, Co, Sb, K in the lines with *Lr*-genes of juvenile resistance and Al, Fe, Ni, Zn in the lines with genes *Lr1, Lr10, Lr21, Lr3a, Lr24* significantly correlate with leaf rust manifestations. The leaf rust severity intensified significantly as the coefficients of Al, K, CR, Fe, Co, Ni, Sb, and Cd biological accumulation in the flag leaves increased. The line with *Lr34* gene, encoding a wheat protein similar to ABC transporters, differs from the other lines in lower biological accumulation coefficients for some toxic elements when compared to the lines which express plant NBS-LRR proteins. These findings can be helpful in spring soft wheat screening when breeding cultivars adapted to the environment conditions of the north-western regions of Russia.

Keywords: common spring wheat, isogenic Thatcher lines, *Lr* genes, elemental composition, pathogenesis, wheat brown rust

For successful crop production, conditions for growing plants, including spring soft wheat, must be optimal [1, 2]. Wheat yields are limited by abiotic and biotic stresses associated with both the phytosanitary state of agrocenoses and chemical pollution of the environment [3-6]. In farms with an unfavorable phytosanitary condition of spring wheat or in the presence of unfavorable lands in their surroundings, it is almost impossible to fully realize the achievements of plant breeding, seed production and advanced technologies [1]. Human activity affects composition and structure of species included in the natural and artificial biocenoses. Giant agrocenoses result in a sharp decrease in biodiversity, stimulate rapid evolution of pathogenic microorganisms, and lead to regular outbreaks of diseases [7, 8]. Chemical pollution of the environment affects yield and mechanisms of crop adaptation to environmental factors. Harmful chemical compounds may decrease yields of grain crops by 25-35 %, of fruit crops by 35-40 %, and of fodder plants by 35-50 % [9].

In recent years, considerable attention has been paid to absorption and accumulation of chemical elements and compounds by agricultural crops (10, 11). Although many elements (Zn, Cu, Mn, Mo, Co, Cr, Sn, V, Ni, etc.) in microdoses are essential, in high concentrations they become toxic. Some elements (Sb, As, Cd, Pb, Hg, Ag) are highly toxic in small quantities [12-14]. Wheat plants are particularly sensitive to the content of Mg, Cu, Mn, Zn, Mo, the lack of which disrupts the carbohydrate and nitrogen metabolism, as well as protein synthesis [15].

Brown rust caused by *Puccinia triticina* Erikss. is among the most dangerous diseases of wheat. Under favorable weather conditions and in a short period of time, the diseases can affect crops in vast areas, causing great damage to the grain yield [16, 17]. During evolution, plants developed protective mechanisms that ensure resistance to biotic and abiotic stresses [18]. Wheat resistance to diseases, including the causative agent of leaf rust, is the most important factor contributing to crop preservation [19]. At present, 77 *Lr* genes have been identified, of which 67 genes are mapped on chromosomes [7], and products of a number of *Lr* genes are also known. It has been found that the *Lr1*, *Lr10*, *Lr21*, *Lr3a*, and *Lr24* genes encode NBS-LRR proteins responsible for the recognition of *Avr* genes of phytopathogens [20-22], *Lr34* encodes a protein similar to the ABC transporters involved in the removal of toxic compounds from cells [23]. Despite the fact that plant resistance to pathogens is a genetically controlled trait, its manifestation is subjected to environmental influences and may be due to the heterogeneous structure of phytopathogen populations [24], deficiency or excess of macro- and microelements, and also depends on intake of phytotoxic elements into plants [2, 25-27].

It is impossible to assert with complete certainty that a particular nutrition can largely adapt a plant to various environmental conditions, weaken the disease or reduce its spread [27]. Thus, the diverse genotypes of wheat differ in their ability to efficiently absorb nutrients [28, 29]. The degree of development of pathogens from different groups (facultative and obligate parasites) depends on a certain ratio of chemical elements in plants [6]. With the development of pathogenesis in plants, basic physiological functions are disturbed, especially the movement of nutrients upward from the roots, the redistribution of chemical elements in organs, the utilization of elements. Moreover, in some organs there may be a lack of chemical elements, and in others — an overabundance up to toxic concentrations. In particular, cotton plants with symptoms of *Fusarium* *oxysporum* f. *vasifectum* infection show an increase in amount of P in the leaves and a decrease in N, K, Ca, and Mg amounts [30].

This paper is the first report worldwide which identifies differences in the elemental composition of the Thatcher isogenic lines, varying in resistance to the brown rust pathogen. Our findings revealed the relationship between the leaf levels of a number of chemical elements and indicators of pathogenesis (intensity of the disease, the number of pustules, the area of pustules, and the type of response).

Our goal was a quantitative analysis of the elemental composition of the flag leaves of the Thatcher isogenic lines with different resistance to the wheat brown rust.

Techniques. In the test we used 29 isogenic Lr lines of spring soft wheat (*Triticum aestivum* L.) of Thatcher varieties with identified *Lr* genes: Tc*Lr28*, Tc*Lr29*, Tc*Lr24*, Tc*Lr47*, Tc*Lr18*, Tc*Lr19*, Tc*Lr36*, Tc*Lr3ka*, Tc*Lr3bg*, Tc*Lr16*, Tc*Lr17*, Tc*Lr44*, Tc*Lr1*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr3a*, Tc*Lr10*, Tc*Lr11*, Tc*Lr14a*, Tc*Lr20*, Tc*Lr33*, Tc*Lr26*, Tc*LrB* (juvenile); Tc*Lr35*, Tc*Lr12*, Tc*Lr21*, Tc*Lr48* (age-related); Tc*Lr46*, Tc*Lr34* (partial resistance genes) by courtesy of Research Institute of Plant Protection (St. Petersburg—Pushkin) and Federal Research Center the Vavilov All-Russian Institute of Plant Genetic Resources, (St. Petersburg). These lines are widely used in phytopathology to evaluate differential interaction of nonspecific plant resistance and the pathogen virulence [31]. Wheat varieties Leningradka (k-47882), Leningradskaya 97 (k-62935), Leningradskaya 6 (k-64900) were resistance standards.

The isogenic wheat lines of the Thatcher series were sown in 2014 (experimental field of VIR Pushkin Laboratory, Leningrad region). The area of the experimental plot for each sample was 1 m^2, the total number of plants on five rows of the experimental plot was 300 pcs. The elemental composition of flag leaves was assayed at the beginning of flowering, when the infectious process was in progress and it was still possible to find leaves without symptoms of pathogenesis on both resistant and susceptible varieties.

Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Cu, Ni, Zn, Se, Mo, Ba, Pb, Sb, As, Cd, Be, and Ag levels in wheat leaves were evaluated by inductively coupled plasma mass spectrometry (ICP-MS, an ICP-MS 7700x mass spectrometer, Agilent Tech-Technologies, USA). The concentration of each element was determined by the average value of five repeated measurements with ICP-MS MassHunter software (Agilent Technologies, USA). Samples were mineralized in a Start D microwave digestion system in Teflon autoclaves (CEM, United States). Nitric acid was purified using DuoPUR system with sub-boiling distillation (Milestone, Italy). Deionized water with conductivity not less than 18.2 M was a solvent in all experiments. For measurement, samples (0.10 g) were exactly weighted using an analytical balance and placed in a Teflon autoclave with 5 ml of concentrated nitric acid added. The samples were subjected to decomposition in a microwave oven with temperature rise to 200 °C for 15 minutes, incubation at 200 °C for 15 minutes, and cooling to 45 °C. The dissolved sample was transferred to a 15 ml tube and diluted to 10 ml with deionized water. Aliquots of 1 ml were adjusted to 10 ml with 0.5 % nitric acid and used for analysis.

Soil elemental composition was assessed in samples from 10 randomized plots. Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, and Zn concentrations were estimated by mass spectrometry method as described hereinabove. Coefficients of biological accumulation, characterizing the degree of selective absorption of the element by plants, were calculated from the ratio of the element in plants to the soil.

Plant resistance to leaf rust was assessed by generally accepted phytopathological indicators (the lesion intensity according to the Peterson scale and the type of reaction of Mains and Jackson) [32] and by additional parameters (the number of pustules and the area of a pustule calculated by the formula for the ellipse area) [33].

A complex of indicators of pathogenesis was determined for each line using 20 flag leaves collected at the beginning of flowering, at full flowering, and at the beginning of the grain milky ripeness. Phytopathological examination was performed by microscopy technique (MBS-10 stereo and monocular Mikromed R-1 microscopes, OOO Optical Devices, Russia).

The relationship between the content of chemical elements in wheat leaves and indicators of pathogenesis was estimated by parametric methods based on 95 % confidence intervals for mean values (*M*), standard errors of means (±SEM) with Student's t-test, and by non-parametric statistics method (Mann-Whitney test) as well as cluster analysis (*k*-means method) [34]. Statistical analysis was performed with the software packages SPSS 21.0, Statistica 6.0, and Excel 2013 [35, 36].

Results. Brown rust epiphytoty was recorded in 2014 in the spring soft wheat collection. The estimates of the damage to samples which were used as resistant standards were as follows: R_d (development of the disease) = 80±16 %, N_p (number of pustules) = 1594±824, S_p (area of pustules) = 0.096±0.014 mm^2, T (type of reaction) = 4 for Leningradka variety, R_d = 6±2 %, N_p = 54±19, S_p = 0.103±0.030 mm^2, T = 3 for Leningradskaya 97 variety, and R_d = 14±6 %, N_p = 255±183, S_p = 0.171±0.062 mm^2, T = 4 for Leningradskaya 6 variety. The Thatcher isogenic Lr lines with the *Lr24*, *Lr28*, *Lr29*, *Lr47* genes were high resistant (with reaction type 0) to the local population of wheat brown rust causative agent (Table 1).

1. Damage to wheat (*Triticum aestivum* L.) Thatcher isogenic lines with *Lr* genes caused by *Puccinia triticina* Erikss. under natural infection (St. Petersburg—Pushkin, 2014)

Line Thatcher with *Lr* gnes	Intensity of diseases, %	Number of pustules per leaf	Area of apustule, mm^2	Type of reaction, points
Tc*Lr28*	0.0	0.0	0.000	0
Tc*Lr29*	0.0	0.0	0.000	0
Tc*Lr24*	0.0	0.0	0.000	0
Tc*Lr47*	0.0	0.0	0.000	0
Tc*Lr19*	1.0±0.0	1.3±0.1	0.079±0.015	1
Tc*Lr18*	5.0±0.0	56.7±2.1	0.020±0.005	3
Tc*Lr35*	6.0±1.2	52.0±12.1	0.034±0.008	3
Tc*Lr48*	3.0±0.3	47.0±12.0	0.033±0.005	3
Tc*Lr17*	10.0±2.8	245.8±68.3	0.095±0.014	4
Tc*Lr44*	5.0±1.0	65.0±12.1	0.034±0.004	3
Tc*Lr46*	12.5±2.5	289.7±73.4	0.106±0.014	4
Tc*Lr3bg*	17.5±7.5	284.2±89.0	0.021±0.002	4
Tc*Lr3ka*	20.0±5.0	522.9±191.9	0.035±0.005	3
Tc*Lr16*	21.7±8.2	602.6±121.4	0.030±0.008	4
Tc*Lr12*	30.0±4.5	930.7±230.2	0.035±0.009	3
Tc*Lr21*	50.0±23.2	1198.7±334.2	0.038±0.008	3
Tc*Lr33*	50.0±12.1	629.2±41.2	0.056±0.016	4
Tc*Lr34*	52.5±12.5	922.5±231.2	0.085±0.014	4
Tc*Lr20*	64.0±12.3	1464.4±434.2	0.145±0.023	4
Tc*Lr2c*	70.0±5.0	928.8±119.6	0.035±0.009	4
Tc*Lr26*	75.0±25.0	1301.2±139.4	0.114±0.039	4
Tc*Lr1*	87.5±12.5	1345.4±91.0	0.028±0.007	4
Tc*Lr3a*	87.5±12.5	2388.4±271.6	0.069±0.011	4
Tc*Lr2b*	91.7±8.3	1043.7±329.8	0.079±0.029	4
Tc*Lr11*	100.0±0.0	2684.6±171.4	0.040±0.006	4
Tc*Lr10*	100.0±0.0	1259.4±194.9	0.056±0.006	4
Tc*Lr14a*	100.0±0.0	3247.3±91.7	0.061±0.007	4
Tc*LrB*	100.0±0.0	1337.0±182.7	0.053±0.014	4

On plants of the Thatcher line carrying the *Lr19* gene, isolated pustules were observed, and the disease progression of did not exceed 1 %. In plants with genes *Lr18*, *Lr35*, Lr36, *Lr48*, the disease progression was from 1 to 3 % with the number of pustules from 1 to 57. The lines with genes *Lr17*, *Lr44*, *Lr46* (disease progression from 5 *to 13 %) were relatively resistant. In plants with genes Lr3ka, Lr3bg, Lr12, Lr16, the progression of the disease was 18-30 %. The lines with Lr1, Lr2c, Lr2b, Lr3a*, *Lr10*, *Lr11*, *Lr14a*, *Lr20*, *Lr21*, *Lr26*, *Lr33*, *Lr34*, *LrB* showed high lesion (of 50 to 100 %).

It should be noted that in recent years, the frequency of brown rust isolates virulent to the Tc*Lr3a*, Tc*Lr3bg*, Tc*Lr3ka*, Tc*Lr11*, Tc*Lr12b*, Tc*Lr16*, Tc*Lr17*, Tc*Lr18* lines in the North-West Russia was high and reached 80-100 %. The Tc*Lr1*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr15* and Tc*Lr26* lines showed high virulence polymorphism from 38 to 100 %. That is, the trend towards an increase in the frequency of isolates virulent to Tc*Lr1* observed since the beginning of the 2000s, continues [24].

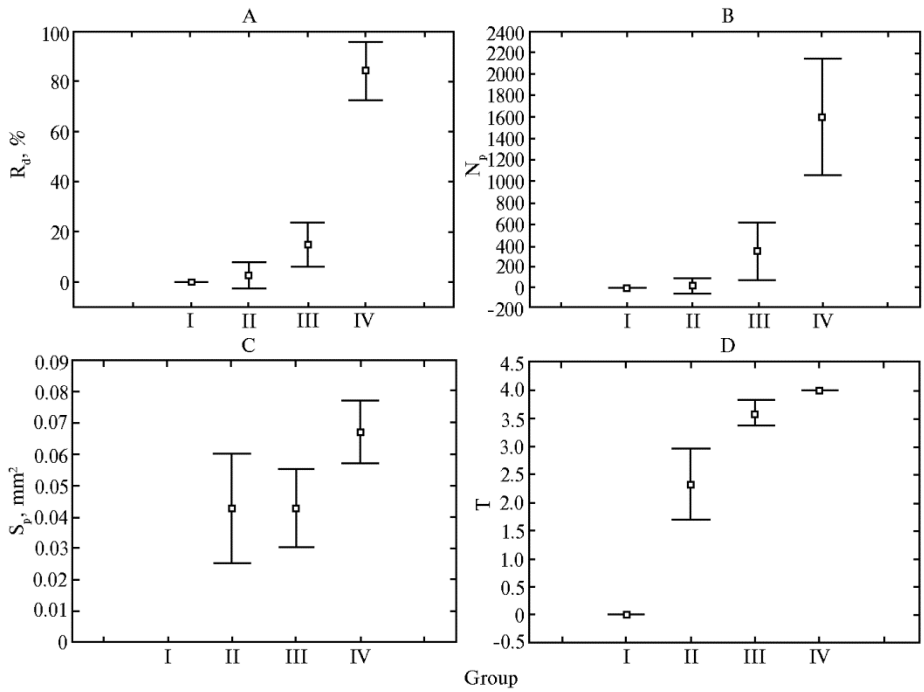

Fig. 1. Damage caused by *Puccinia triticina* Erikss. to wheat (*Triticum aestivum* L.) Thatcher isogenic lines with juvenile *Lr* genes: A — disease development (group I of high resistance, the lines with no symptoms; group II of moderate resistance at R_d = 2.5±1.2 %; group III of moderate sensitivity at R_d = 14.8±3.2 %; group IV of high sensitivity at R_d = 84.2±5.2 %); B — number of pustules (group I with no signs of the diseases; group II at N_p = 22.0±7.5; group III at N_p = 344.1±97.5; group IV at N_p = 1602.7±244.4); C — pustule area (group I with no signs of the diseases; group II at S_p = 0.043±0.018 mm^2; group III at S_p = 0.043±0.013 mm^2; group IV at S_p = 0.067±0.011 mm^2); D — type of the reaction (group I with no signs of the diseases; group II at T = 2.3±0.7; group III at T = 3.6±0.2; group IV at T = 4.0±0.0). The graphs show average values and 95 % confidence intervals (St. Petersburg—Pushkin, 2014).

Clustering (*k*-means method) divides the carriers of *Lr* genes of juvenile resistance to brown rust pathogen into four groups (Fig. 1). The highly resistant Thatcher lines (Tc*Lr28*, Tc*Lr29*, Tc*Lr24*, Tc*Lr47*) without symptoms of brown rust (R_d = 0 %) during the vegetative period are assigned to group I. The moderately resistant Thatcher lines (Tc*Lr18*, Tc*Lr19*, Tc*Lr36*) forme group II. In group II, the damage from the causative agent of brown rust is significantly lower during all phases of wheat growth compared to moderately susceptible lines Tc*Lr3ka*, Tc*Lr3bg*, Tc*Lr16*, Tc*Lr17* of group III and highly susceptible lines

Tc*Lr1*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr3a*, Tc*Lr10*, Tc*Lr11*, Tc*Lr14a*, Tc*Lr20*, Tc*Lr33*, Tc*Lr26*, Tc*LrB* of group IV.

As the resistance of isogenic lines to brown rust is commonly evaluated by lesions caused by the pathogen on wheat flag leaves, we determined the elemental composition of flag leaves. The use of a set of indicators increased the number of statistical data analysis methods and improved the accuracy of identifying differences between the leaves in elemental composition.

Comparison of the average amounts of the elements by Student's *t*-test showed that the Thatcher group of highly resistant lines with no signs of brown rust (Tc*Lr24*, Tc*Lr28*, Tc*Lr29*, Tc*Lr47*) differ from the combined group of moderately resistant, moderately susceptible and highly susceptible lines in a number of indicators. The high resistant lines have significantly lower levels of K (by 21.41 %), Ni (by 56.54 %) (at $P < 0.05$). A nonsignificant decrease occurs in the amounts of Ca (by 10.64 %), Mg (by 10.06 %), Al (by 14.18 %), Cr (by 44.24 %), Mn (by 1/41 %), Fe (by 9.03 %), Co (by 11.34 %), Pb (by 0.25 %), Sb (by 23.40 %), Cd (by 18.23 %), Be (by 28.03 %), and Ag (by 35.97 %). The differences are not significant ($P > 0,05$) for microelements Na (by 33.04 %), Cu (by 6.73 %), Zn (by 4.65 %), Se (by 15.16 %), Mo (by 3.07 %), Ba (by 3.31 %), and As (by 5.56 %) most of which are essential for plant growth and development.

Calculation of 95 % confidence intervals for means *M* (Fig. 2) revealed that highly resistant Thatcher lines (group I) contain significantly lower K, Cr, Fe, Co, Ni, Cd, and Ag levels compared to highly susceptible (group IV). Differences appear between moderately resistant group II and moderately susceptible group III in Ni concentration, and between groups III and IV in Cr, Fe, Co, Ni, and Ag concentrations.

Parametric statistics methods showed that plants of group I have significantly lower Co content (Mann-Whitney test $U = 7.0$ at $P = 0.04$) and Ni ($U = 5.0$ at $P = 0.03$) than iof group IV. Moderately resistant isogenic lines (group II) had significantly lower Ni level as compared to highly susceptible lines of group IV ($U = 3.0$ and $P = 0.04$). Lines with moderate susceptibility to brown rust (group III) show significantly lower levels of Sb ($U = 7.0$ at $P = 0.02$), Ca ($U = 10.0$ at $P = 0.02$), Co ($U = 9.0$ at $P = 0.03$), and Pb ($U = 10.0$ at $P = 0.03$) compared to those in group IV.

The non-parametric analysis of Spearman correlations (at $P < 0.05$) indicates that the brown rust damage to isogenic Thatcher lines with juvenile *Lr* genes increases significantly with a rise in concentrations of Al ($r = 0.6$ for R_d), Cr ($r = 0.5$ for R_d and $r = 0.5$ for N_p), Co ($r = 0.6$ for R_d and $r = 0.5$ for N_p), Ni ($r = 0.7$ for R_d, $r = 0.6$ for N_p and $r = 0.5$ for T), Sb ($r = 0.5$ and $r = 0.4$ for N_p), and K ($r = 0.6$ for T).

The lesion intensity in Thatcher isogenic lines with *Lr* genes for age-related resistance significantly decreases with an increase in leaf Se ($r = –0.91$ for R_d and $r = –0.89$ for N_p), in contrast to higher damage in lines with juvenile *Lr* genes ($r = –0.11$ for R_d, $r = –0.13$ for N_p and $r = 0.34$ for S_p).

In isogenic lines with juvenile *Lr* genes of resistance ($R_d = 43.80 \pm 8.67$ %; $N_p = 844.17 \pm 194.90$; $S_p = 0.046 \pm 0.007$ mm^2), there are significantly lower ($P < 0.05$) leaf levels of Be (by 21.41 %; Student's *t*-test $T_{05} = –2.17$; Mann-Whitney test $U = 11$) compared to the lines carrying *Lr* genes of age-related resistance ($R_d = 22.25 \pm 9.05$ %; $N_p = 557.10 \pm 200.12$; $S_p = 0.035 \pm 0.001$ mm^2). Insignificantly lower ($P > 0.05$) amounts are detected for Cd (by 22.12 %), Ag (by 21.66 %), Cr (by 16.86 %), Pb (by 13.67 %), Co (by 2.67 %), Mo (by 2.45 %), Na (by 1.85 %), and Al (by 1.7 %). And finally, unreliably higher ($P > 0.05$) concentrations are found for Fe (by 1.51 %), Sb (by 1.73 %), Ca (by 1.74 %), Mn (by 4.33 %) Ba (by 4.46 %), As (by 4.72 %), Se (by 5.91 %), Zn (by 7.64 %), Mg (by 19.58 %), K (by 21.31 %), Ni (by 35.58 %), and Cu (by 54.03 %).

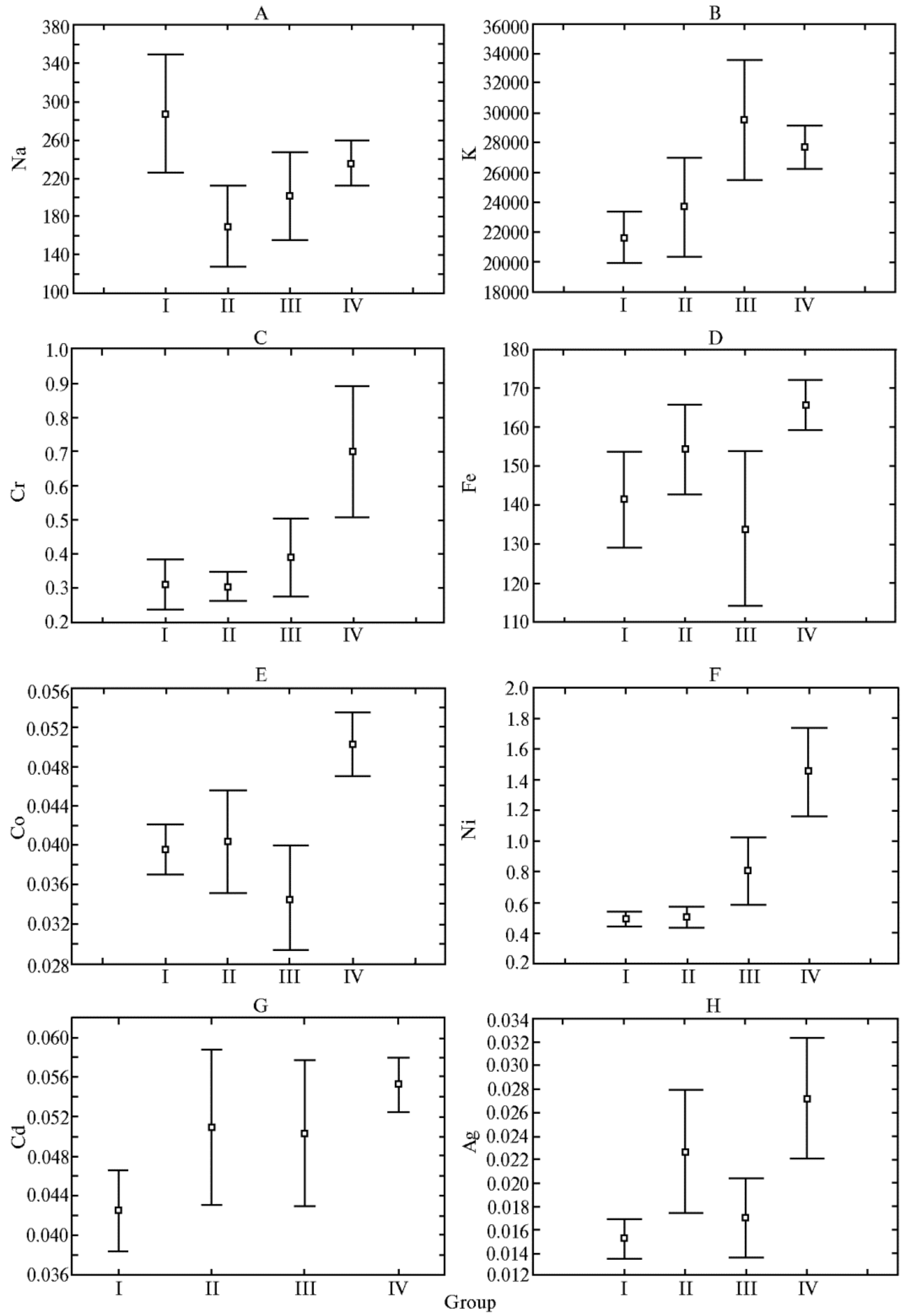

Fig. 2. Content of microelements in leaves of wheat (*Triticum aestivum* L.) isogenic lines Thatcher with juvenile *Lr* genes: A — Na, B — K, C — Cr, D — Fe, E — Co, F — Ni, G — Cd, H — Ag (group I of high resistance, the lines with no symptoms; group II of moderate resistance at R_d = 2.5±1.2 %; N_p = 22.0±7.5; S_p = 0.043±0.018 mm^2; T = 2.3±0.7; group III of moderate sensitivity at R_d = 14.8±3.2 %; N_p = 344.1±97.5; S_p = 0.043±0.013 mm^2; T = 3.6±0.2; group IV of of high sensitivity at R_d = 84.2±5.2 %; N_p = 1602.7±244.4; S_p = 0.067±0.011 mm^2; T = 4.0±0.0). The graphs show average values, μg/g, and 95 % confidence intervals (St. Petersburg—Pushkin, 2014).

Analysis of the matrix of mutual correlations of the wheat leaf elemental composition shows that a group of Thatcher isogenic lines with juvenile *Lr* genes of resistance have less reliable Spearman and Pearson correlation coefficients (by 65.34 and 74.22 %, respectively) compared to the lines with genes of age-related resistance.

In the isogenic lines with juvenile *Lr* genes, when compared to the lines with *Lr* genes of partial field resistance (R_d = 32.50±10.05 %; N_p = 606.06±316.40; S_p = 0.095±0.010 mm^2), the leaf concentrations are significantly higher ($P < 0.05$) for Ca (by 53.47 %), Al (by 55.38 %), Mg (by 85.76 %), though increased values are unreliable ($P > 0.05$) for Pb (by 6.03%), Zn (by 6.78 %), Mn (by 7.68 %), K (by 9.67 %), Be (by 10.14 %), As (by 15.40 %), Co (by 16.46 %), Fe (by 17.89 %), Cd (by 29.04 %), Sb (by 51.60 %), Mo (by 56, 90 %), and Ni (by 107.56 %). A decrease in values is unreliable ($P > 0.05$) for Cu (by 49.84 %), Ag (by 25.58 %), Cr (by 19.82 %), Ba (by 13.19 %), Na (by 3.93 %), and Se (by 3.14 %).

In the isogenic lines with *Lr* genes of age-related resistance, when compared to the lines with *Lr* genes of partial field resistance, there is an unreliably lower ($P > 0.05$) content of Cu (by 67.44 %), Ba (by 16.19 %), K (by 11.07 %), Se (by 8.55 %), Ag (by 5.0 %), Cr (by 3.55 %), Na (by 2.12 %), Zn (by 0.80 %) and unreliable higher ($P > 0.05$) content of Mn (by 3.21 %), As (by 10.20 %), Fe (by 16.13 %), Co (by 19.66 %), Pb (by 22.83 %), Sb (by 49.01 %), Ca (by 50.85 %), Ni (by 53.09 %), Mg (by 55.3 %), Al (by 58.07 %), Mo (60.12 %), Cd (65.7 %), and Be (147.22 %).

Nonparametric correlation analysis determined the relationship between the leaf elemental composition of the Thatcher isogenic lines with *Lr1*, *Lr10*, *Lr21*, *Lr3a*, *Lr24* genes encoding plant NBS-LRR proteins (20-22) and the indicators of wheat rust development (Table 2). It was established that higher levels of Al, Fe, Ni, and Zn are associated with more intensive development of wheat brown rust (the values of the Spearman correlation coefficient were reliable).

2. Correlation between the lesion of wheat (*Triticum aestivum* L.) isogenic lines Thatcher with *Lr1*, *Lr10*, *Lr21*, *Lr3a*, *Lr24* genes by brown rust pathogen (*Puccinia triticina* Erikss.) and the leaf elemental composition (St. Petersburg—Pushkin, 2014)

Element	R_d	N_p	S_p	T
Na	0.31	–0.10	–0.50	0.22
Mg	0.87	0.50	0.30	0.78
Al	0.95*	0.56	0.41	0.86
K	0.46	0.20	–0.30	0.45
Ca	0.87	0.60	0.60	0.78
Cr	–0.72	–0.60	–0.10	–0.78
Mn	0.41	0.00	–0.30	0.34
Fe	0.97*	0.60	0.50	0.89*
Co	0.46	0.50	0.60	0.45
Cu	0.67	0.20	0.70	0.45
Ni	0.82	0.70	0.30	0.89*
Zn	0.56	0.90*	0.50	0.78
Se	0.62	0.30	0.50	0.45
Mo	–0.56	–0.80	–0.20	–0.78
Ba	–0.21	–0.70	–0.70	–0.45
Pb	–0.41	0.00	0.30	–0.34
Sb	0.55	0.21	–0.10	0.52
As	–0.41	0.10	–0.60	–0.11
Cd	0.21	0.10	0.10	0.22
Be	0.16	–0.16	0.00	0.06
Ag	–0.36	0.20	0.20	–0.11

Note. R_d — disease development, %; N_p — number of pustules; S_p — the area of a pustule, mm^2; T — type of the response, points.
* Spearman correlation coefficients are statistically significant ($P < 0.05$).

We used the ratio of leaf level to soil level for each element to calculate the bioaccumulation coefficients which characterize selective absorption of the element by plants. It was turned out that the pathogen becomes significantly more aggressive as the coefficients of Al, K, Cr, Fe, Co, Ni, Sb and Cd accumulation in flag leaves grow (Table 3).

In contrast to Thatcher lines with genes *Lr1*, *Lr10*, *Lr21*, *Lr3a*, *Lr24*, in the line with *Lr34* gene encoding a wheat protein similar to ABC transporters involved in detoxication processes [23], the bioaccumulation coefficients are reliably lower (P < 0,05) for Cr (by 75.1 %) and Ni (by 49.5 %), unreliably lower (P > 0.05) for Na (by 15.6 %), Mg (by 38.0 %), Al (by 41.2 %, K (by 22.9 %), Ca (by 45.1 %), Mn (by 8.4 %), Fe (by 17.5 %), Co (by 98.5%), Zn (by 12.7 %), Mo (by 47.9 %), Pb (by 13.3 %), and Cd (by 37.5 %) vs. a significant increase in Cu (by 50.3 %, P < 0.05). Additionally, in the line Tc*Lr34* compared to Tc*Lr1*, Tc*Lr10* and Tc*Lr3a*, the infection is less intensive and the number of pustules on flag leaves is smaller (by 66.7 % and 45.8 %; by 90.5 % and 36.5 %; and by 66.8 % and 158.9 %, respectively; P < 0.05).

3. Correlation between bioaccumulation coefficients of chemical elements and brown rust infection (*Puccinia triticina* Erikss.) intensity in wheat (*Triticum aestivum* L.) isogenic lines Thatcher (St. Petersburg—Pushkin, 2014)

Element	R_d	N_p	S_p	T
Na	0.11	0.07	–0.18	0.12
Mg	0.20	0.13	–0.14	0.11
Al	0.47*	0.43*	–0.04	0.30
K	0.42	0.38*	0.24	0.49*
Ca	0.27	0.21	–0.02	0.13
Cr	0.44*	0.43*	0.25	0.32
Mn	–0.02	–0.02	–0.05	0.00
Fe	0.39*	0.38*	0.07	0.27
Co	0.49*	0.46*	0.10	0.35
Cu	0.30	0.33	0.27	0.23
Ni	0.65	0.64*	0.14	0.51*
Zn	–0.02	–0.02	0.01	0.02
Se	–0.09	–0.19	–0.19	–0.04
Mo	0.21	0.19	0.08	0.05
Ba	0.23	0.13	0.14	0.18
Pb	0.03	0.08	–0.20	–0.05
Sb	0.43*	0.40*	–0.07	0.23
As	0.05	0.15	0.09	0.06
Cd	0.38*	0.41*	0.14	0.24
Be	–0.05	–0.03	–0.27	–0.13
Ag	0.16	0.19	0.29	0.27

N o t e. R_d — disease development, %; N_p — number of pustules; S_p — the area of a pustule, mm^2; T — type of the response, points.
* Spearman correlation coefficients are statistically significant (P < 0.05).

Interestingly, the Thatcher lines with juvenile resistance genes *Lr28*, *Lr29*, *Lr24* and *Lr47* without signs of brown rust during vegetation period have reliably lower accumulation of heavy metals Ni, Ag, Cr, Fe, Co, Ni, Cd, and also K, which is in line with our findings reported for other wheat varieties and lines [37]. Perhaps, this is due to probable phytotoxicity of heavy metals at higher leaf concentrations, which leads to phytoimmunity weakening [38] and more losses, especially in susceptible varieties [39]. Excessive amount of potassium in the leaves delays the sodium input into the plant, causing metabolic disturbances and growth retardation. In laboratory tests, it was shown [40] that application of potassium chloride in seedling growing significantly increases (4.9-fold) the efficiency of inoculum production of brown rust pathogen.

In our tests, the intensity of brown rust pathogen development decreased with an increase in the selenium content in flag leaves of Thatcher isogenic lines. As known, Se has a positive effect on wheat growth and photosynthesis indicators [41]. This tendency is most characteristic of lines with Lr genes of age-related resistance.

The Thatcher isogenic lines with *Lr* genes of juvenile resistance have fewer reliable correlations between the flag leaf levels of chemical elements and greater brown rust damage than the lines with genes for age-related resistance. This was confirmed by parametric and non-parametric Pearson and Spearman coefficients (at $P < 0.05$). The revealed peculiarities of correlations indicate a delicate tuning of biochemical processes in switching-on the host plant defense mechanisms against pathogens.

We found significant positive correlations between the damage to wheat plants caused by brown rust and the flag leaf levels of Al, Cr, Co, Sb, and K for the lines with juvenile resistance *Lr* genes, and of Al, Fe, Ni, Zn for the lines with *Lr1*, *Lr10*, *Lr21*, *Lr3a*, and *Lr24* genes. The intensity of the brown rust pathogen development significantly rises with an increase in the coefficients of Al, K, Cr, Fe, Co, Ni, Sb, and Cd biological accumulation in flag leaves.

Thus, as per our findings, the elemental profile of the Thatcher spring wheat soft wheat series, which is due to the genetically determined resistance and agrochemical conditions, can influence the intensity of brown rust pathogen development. The disease becomes more sever as the leaf amount of toxic elements increases, including heavy metals. These results can be used in breeding spring soft wheat varieties adapted to the environmental conditions of the North-West Russia.

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