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ANALYSIS OF THE *Triticum aestivum* L. GENETIC DIVERSITY INDUCED BY THE CHEMICAL MUTAGEN PHOSPHEMIDE

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Abstract

Currently, climate change and growing demand for food necessitate effective methods for crop improvement. Induced mutagenesis is a promising tool to create breeding material. This work, for the first time, established the potential of the chemical mutagen phosphemide on spring soft wheat. Particularly, it was revealed that seed treatment with an aqueous solution of the mutagen in optimal concentrations effectively increases genetic variability to select economically valuable forms. Our goal was to increase the genetic diversity of spring bread wheat (*Triticum aestivum* L.) using the chemical mutagen phosphemide and to determine the biological potential of mutants (M_5, M_6) based on the variability of morphological and productive traits under the conditions of the Northern Trans-Urals. A total of 29 spring soft wheat samples selected from mutant populations of two cultivars, Cara and Skant 3, from a hybrid (Cara × Skant 3), as well as three control cultivars, the Tyumenskaya 25, Tyumenskaya 29, Novosibirskaya 31 were involved in the study. Mutant samples were generated using the chemical mutagen phosphemide. The seeds were treated with a 0.002 and 0.01 % phosphemide aqueous solution for 3 hours. Identification of mutations and testing their stability were carried out in the second (M_2) and third (M_3) generations. Here, we submit data for M_5 and M_6 mutants grown under contrasting meteorological conditions in 2021-2022. Sowing, observations, records, description of morphological characteristics and biological properties of plants were carried out at the experimental site of the biological station of the Tyumen State University Lake Kuchak (Nizhnetavdinsky District of Tyumen Province). Electrophoretic analysis of gliadins was carried out in caryopsis of the 2021 harvest (M_5). Based on grain electrophoretic analysis of the original and mutant plants, genetic formulas of gliadin were compiled, and the frequency of gliadin coding loci alleles (*Gli*) was determined. In field tests, significant differences occurred between genotypes in quantitative traits, e.g., plant height, linear dimensions and area of the flag leaf, number of productive stem per 1 m^2, ear length, grain number and weight per ear. Correlation analysis revealed that the strength of the relationship between yield and other traits depends on the meteorological conditions of the growing season. The dependence of grain yield on the number of plants ($r = 0.71$, $p < 0.05$) and productive stems ($r = 0.71$, $p < 0.05$) per 1 m^2, on plant height ($r = 0.82$, $p < 0.05$), chlorophyll content in flag leaf cells ($r = 0.28$, $p > 0.05$), and the number of grains per ear ($r = 0.73$, $p < 0.05$) was stronger under water and heat stress. Five mutants of spring soft wheat with a relatively high biological potential compared to other samples and standard cultivars were selected for a set of valuable traits. These mutants had the same allelic composition for gliadins. The yield was higher in 2022 and amounted to 396.1-518.2 g/m^2 for the best mutants, and 355.0-424.5 g/m^2 for the standard cultivars. Thus, the adaptation potential of spring soft wheat in the Northern Trans-Urals extreme conditions can be increased due to genetic variability of mutant populations. The biological effect of the chemical mutagen phosphemide has been proven to induce beneficial mutations in *T. aestivum*. Therefore, combination of mutational and recombination variability is effective in increasing genetic diversity.

Keywords: spring soft wheat, genotype, mutant forms, gliadin-coding loci, stress, resistance, quantitative characters, correlation

Climate change, leading to droughts, soil salinity, high temperatures, and the emergence of new diseases and pests, is a serious threat to global crop yields [1]. The growing demand to increase crop production to meet food demand necessitates effective methods for plant improvement.

Currently, plant improvement involves the integration of traditional and molecular methods. Among the available selection and genetic tools that allow the creation of donor plants with economically valuable traits, the induced (artificial) mutagenesis remains promising. Among varieties registered in the International Atomic Energy Agency database (FAO/IAEA, http://mvgs.iaea.org), there are 3402 mutant varieties of various crops, including 265 wheat varieties [2].

The effectiveness of molecular genetic analysis depends on the properties of the mutant population which determine the frequency of mutations, their diversity and quality. Considering that recombination variability can increase under environmental changes which is typical for the sharply continental climate of Western Siberia, we should expect an increase in the emergence of plant forms with transgressive expression of traits over generations.

To involve these genetic resources in breeding, it is necessary to assess their genetic and phenotypic variability [3], which will improve the breeding efficiency [4]. Morphological assessment of plants remains an important tool, despite the fact that morphological traits are controlled by different genes [5] and are influenced by environmental factors [6].

The search for chemicals that have mutagenic properties and can effectively change the hereditary nature of cultivated plants continues [7, 8].

This paper is the first to report the biological potential of the chemical mutagen phosphemide on spring soft wheat. We revealed that seed treatment with an aqueous solution of mutagen in optimal concentrations is effective for increasing genetic variability and selecting valuable forms.

Our goal was to expand the genetic diversity of spring bread wheat (*Triticum aestivum* L.) using the chemical mutagen phosphemide and to assess the biological potential of mutant samples (M_5, M_6) based on the variability of various traits in the conditions of the Northern Trans-Urals.

Materials and methods. Spring soft wheat *Triticum aestivum* L. accessions from the world collection of the Vavilov All-Russian Institute of Plant Genetic Resources (VIR) were selected based on a preliminary study in the Tyumen Province in 2006-2010.

Variety Skant 3 (var. *lutescens*; originated by Research Institute of Agriculture of the Northern Trans-Urals and Kazakh Research Institute of Agriculture and Breeding) was created by individual selection from the F_3 population [F_1 (Shtorm × Saratovskaya 29) × Saratovskaya 29], registered in the Tyumen Province since 2003. Variety Cara from the world VIR collection (k-64381, var. *eritrospermum*, Mexico) is a carrier of the *Lr13* resistance gene according to GRIS (Genetic Resources Information System for Wheat and Triticale, http://wheatpedigree.net). Hybrid F_4 (Cara × Skant 3) was selected by a diallelic analysis of 5 parental and 10 hybrid forms produced at the Institute of Biology of Tyumen State University.

Spring wheat seeds were treated with a 0.002% and 0.01% aqueous solution of phosphemide for 3 h; control seeds were kept in distilled water. Phosphemidum, or di-(ethylenimide)-pyrimidyl-2-amidophosphoric acid, a white or yellowish crystalline powder soluble in water and alcohol, was synthesized at Lomonosov Moscow State University.

Phenotypic changes were assessed by the morphological characteristics of the ear, stem, leaves (color, pubescence, shape, size) and biological properties (late-ripening, early-ripening, winter-type plants, dwarfs). Selection in the second mutant generation (M_2) and testing for stability in the third (M_3) and subsequent generations were carried out for large, pyramidal, speltoid ear; bright yellow and anthocyanin colored straw; strong straw; wide flag leaf; tall plants; late ripening, early ripening.

Native electrophoresis of the storage protein gliadin was performed using grains of the 2021 harvest (M_5) according to common method [9] at the Analytical Center for Determining the Quality of Soil and Crop Products (LLP Baraev SRC of Grain Production). Vertical chambers for electrophoresis VE-20 (Helikon, Russia) and chemical reagents of the extra pure category (Sigma-Aldrich, USA) were used. Gliadins were identified according to a catalog of gliadin-coding loci alleles [10], the loci were *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2*, and *Gli-D2* [11].

In 2021-2022, 29 mutant samples M_5 and M_6 stored at the Institute of Biology of Tyumen State University were compared in field trials with the original varieties and the hybrid, and with varieties Tyumenskaya 25, Tyumenskaya 29 and Novosibirskaya 31 grown in the Tyumen Province.

Sowing, observations, records, description of morphological parametes and biological properties were performed at the experimental site of the Tyumen State University biological station Lake Kuchak (Tyumen Province, Nizhnetavdinsky District, 57°20′57.3″N 66°03′21.8″E) according to methodological guidelines [12, 13]. The soil of the site is soddy-podzolic sandy loam with 3.67% humus, pH 6.6. The experiment was designed in 4 repetitions with randomized 1 m^2 plots. Sowing density was 650 seeds per 1 m^2, or 6.5 million viable seeds/ha, with 20 cm row spacing. Sowing was carried out in the second decade of May, the crop was manually harvested at the stage of complete grain ripeness.

Plant height was measured from the soil surface, including the top leaf or ear depending on the phenological phase. The chlorophyll content in flag leaves of 10 plants was measured in sunny weather, between 11.00 and 14.00 with an optical counter SPAD 502 (Minolta Camera Co., Ltd., Japan). The area of the leaf blade was calculated by the formula [14]:

$$A = LWb_i,$$

where L is the length of the leaf blade, cm, W is the maximum width of the leaf blade, cm; $b_i = 0.835$.

After harvesting, plants and productive ears per 1 m^2 were counted, the grain yield, grain number and grain weight per ear for 10 plants in each replication were determined.

Environmental conditions were monitored at the experimental site (a professional local weather station IMetos IMT300, Pessl Instruments, Austria), information was also used on average daily air temperature and precipitation from the Weather and Climate reference and information portal (http://www.pogodaiklimat.ru/).

Statistical processing of experimental data was performed according to proven methods [12, 15] using the Microsoft Excel spreadsheet processor and STATISTICA 6.0 software (StatSoft, Inc., USA). The mean values (*M*), standard errors of the means (±SEM), coefficients of variation (*Cv*, %) were calculated, correlation analysis was performed. The significance of the differences between the mean values was assessed by Student's *t*-test.

Results. Preliminary cytogenetic studies on the phosphemide effects on plants were carried out on the model plant *Crepis capillaris* L. which has three pairs of clearly distinguishable chromosomes. Dry seeds were treated with a phosphemide solution, and the types and number of chromosome rearrangements were analyzed on seedlings [16, 17]. It was important to determine how long the mutagenic effect could last. We found that with a single application of the phosphemide to the seeds of *C. capillaris*, chromosome rearrangements and the frequency of seedlings with mitoses remained detectable within 3 months. Therefore, it can be assumed that when storing treated seeds, the mutagen phosphemide does not decompose and its effect is practically not reduced [16, 17].

Based on these results, a single seed treatment was used in a study on varieties and hybrids of *T. aestivum*, followed by lab and field trials. When selecting a research object, we proceeded from the fact that the specified mutagen had not been used previously, and one of the tasks was to determine its effectiveness based on the frequency and spectrum of mutations. In this regard, we selected samples that differed in botanical and geographical origin and could presumably differ in their response to the action of phosphemide. In the M_2 generation, a wide range of mutations with modified plants (12 types) were identified with a frequency of 30.3% in the hybrid and 15.3-28.5% in the original varieties. In terms of the number of mutations that stably manifested the trait in the offspring, the phosphemide solution concentration of 0.01% had an advantage.

Table 1 describes spring soft wheat mutants we studied.

1. Phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants (experimental site of the Tyumen State University biological station Lake Kuchak, Tyumen Province, Nizhnetavdinsky District, 2021-2022)

Nos.	Designation	Nos.	Designation
1	P_{1K} (Cara), contro;	17	P_2 (0.002 %) Skant 3
2	$F_{4к}$ (Cara × Skant 3), contro	18	P_2 (0.002 %) Skant 3
3	P_{2K} (Skant 3), contro	19	P_2 (0.002 %) Skant 3
4	F_4 (0,01 %) Cara × Skant 3	20	P_2 (0.002 %) Skant 3
5	F_4 (0,01 %) Cara × Skant 3	21	F_4 (0.002 %) Cara × Скэнт 3
6	P_1 (0,002 %) Cara	22	P_2 (0.002 %) Skant 3
7	P_1 (0,002 %) Cara	23	P_2 (0.002 %) Skant 3
8	P_1 (0,002 %) Cara	24	P_2 (0.002 %) Skant 3
9	P_1 (0,002 %) Cara	25	P_2 (0.002 %) Skant 3
10	F_4 (0,01 %) Cara × Skant 3	26	P_1 (0.01 %) Cara
11	F_4 (0,01 %) Cara × Skant 3	27	P_1 (0.01 %) Cara
12	F_4 (0,01 %) Cara × Skant 3	28	P_1 (0.01 %) Cara
13	F_4 (0,01 %) Cara × Skant 3	29	P_1 (0.01 %) Cara
14	P_2 (0,002 %) Skant 3	30	P_2 (0.01 %) Skant 3
15	P_2 (0,002 %) Skant 3	31	P_2 (0.01 %) Skant 3
16	P_2 (0,002 %) Skant 3	32	F_4 (0.002 %) Cara × Skant 3

Note. F_4 is a fourth generation hybrid, P_1 is the original variety Cara, P_2 is the original variety Skant 3. The phosphemide concentration is indicated in parentheses.

2. Gliadin allele formulas of phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants

Nos	Gliadin coding loci (*Gli*)					
	A1	*B1*	*D1*	*A2*	*B2*	*D2*
1, 2 (control)	*c*	*l*	*d*	*n*	*p*	*b*
3 (control)	*a*	*e*	*b*	*f*	*t*	*a*
8, 10, 11, 12, 13	*c*	*l*	*d*	*n*	*p*	*b*
6, 7	*h*	*l*	*b*	*m*	*f*	*q*
17, 18, 19, 20	*o*	*f*	*a*	*l*	*p*	*n*
23, 24, 25, 26, 27, 28, 29, 16	*f*	*b*	*a*	*l*	*b*	*i*
5, 21, 22, 32	*c*	*e*	*b*	*n*	*p*	*q*
14, 30	*g*	*e*	*f*	*c*	*n*	*b*
4	*o*	*e*	*b*	*n*	*i*	*e*
9	*f*	*e*	*a*	*b*	*b*	*b*
15	*c*	*e*	*a*	*l*	*n*	*i*
31	*k*	*e*	*a*	*k*	*u*	*q*

Notиe. The numbers correspond to the names of the samples given in Table 1.

Based on electrophoretic analysis of grains samples form the original and mutant, genetic formulas of soft wheat gliadin were compiled. It was found that the mutants often had identical spectra and, therefore, the gliadin formula (Table 2).

For all loci, there were alleles with the maximum frequency of occurrence. In the 1st homeological group (loci *Gli-A1*, *Gli-B1*, *Gli-D1*), the alleles *Gli-A1c* and *Gli-B1e* (34.5%), *Gli-D1a* (51.7%) were more common. In the 6th homeological group (loci *Gli-A2*, *Gli-B2*, *Gli-D2*), the *Gli-A2l* and *Gli-B2p* alleles (44.8%) and the *Gli-D2i* allele (31.0%) predominated. In total, gliadin electrophoresis revealed 4 alleles at the *Gli-B1* and *Gli-D1* loci, 5 alleles at the *Gli-D2* locus and 6 alleles at the *Gli-A1*, *Gli-A2* and *Gli-B2* loci (Fig. 1).

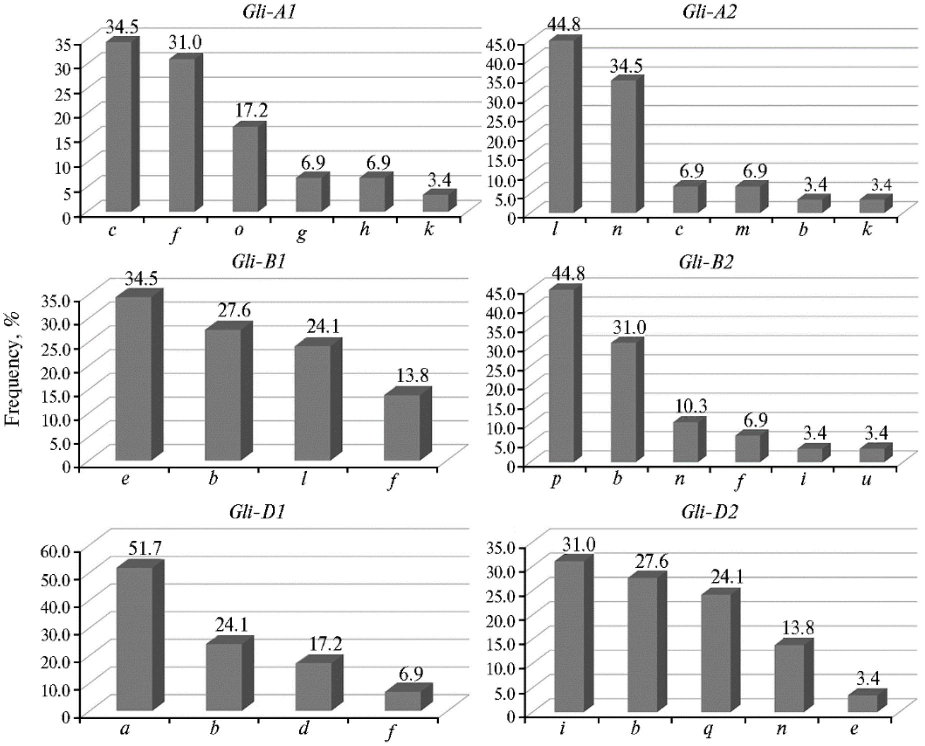

Fig. 1. Frequency of alleles of gliadin-coding loci (%) in phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants.

Analysis of gliadin formulas showed a high frequency of occurrence of the *Gli-A1c* allele (10 out of 29 samples). It is worth noting that this allele encodes the synthesis of a gliadin block, which is very close in number and mobility of components to the gliadins controlled by the *Gli-A1a* allele (Skant 3) [10], the difference lies only in the mobility of one protein band in the γ-zone of the electrophoretic spectrum. Rearrangements of the genetic apparatus under the influence of mutagen can occur that are expressed in the disappearance or appearance of one or two protein components in the gliadin spectrum [18]. It is likely that changes occurred in the genome due to the action of the mutagen modify the gliadin spectrum.

At the *Gli-B1* locus, the *Gli-B1e* allele had the maximum frequency of occurrence (see Fig. 1). It is worth noting that this allele is widespread in many Russian [19, 20] and Kazakh [21] wheat varieties and is probably associated with economically valuable traits.

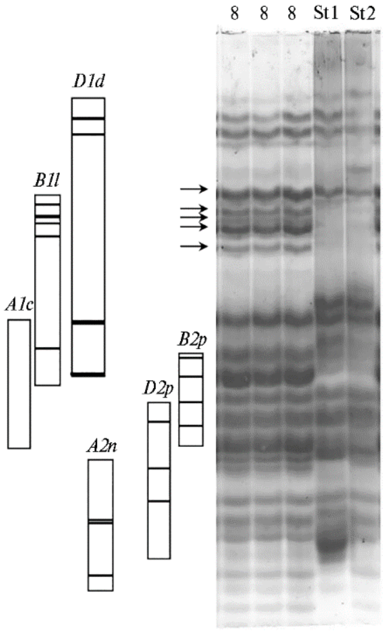

Fig. 2. Electropherogram and identified gliadin blocks in a phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutant: 8 — sample No. 8 P1 (0.002%) Cara, St1 — standard variety Bezostaya 1, St2 — standard variety Mironovskaya 808. Arrows indicate the main components marking the wheat-rye translocation.

The *Gli-B1l* allele is of interest, which controls a block of components, serves as a marker for the wheat-rye translocation 1RS.1BL and occurs with a frequency of 24.1% (Fig. 2). This allele is associated with plant resistance to a number of diseases, namely powdery mildew (*Pm8*), yellow rust (*Yr9*), stem rust (*Sr31*), and leaf rust (*Lr26*) [22, 23].

The predominant allele at the *Gli-D1* locus was *Gli-D1a* with a frequency of 51.7%. At the *Gli-A2* locus, alleles *l* and *n* were identified in mutants with a frequency of 44.8 and 34.5%, respectively. The distribution of the *n* allele among mutants is probably associated with the Cara variety which carries it in the genotype.

Therefore, treatment of parent varieties and hybrids with a chemical mutagen increased the genetic diversity in gliadin loci. This can later be used to create varieties with high grain productivity and quality, resistant to biotic and abiotic environmental factors.

Field testing of spring soft wheat mutants of the fifth and sixth generations (M_5, M_6), isolated by screening and further selection, was carried out in contrasting conditions of the growing seasons of 2021-2022 (Table 3).

3. Average daily air temperature and precipitations during the growing seasons 2021-2022 (experimental site of the Tyumen State University biological station “Lake Kuchak”, Tyumen Province, Nizhnetavdinsky District)

Month	Average daily air temperature, °C			Total precipitation, mm		
	n	2021 год	2022 год	*n*	2021 год	2022 год
May	11.3	17.6	12.1	45.3	4.6	93.9
June	17.1	18.0	15.8	58.5	22.9	59.4
July	18.8	18.6	19.7	86.0	49.6	65.5
August	15.8	19.5	18.1	60.0	20.0	56.0
M, °C	15.8	18.4	16.4			
∑, mm				249.8	97.1	274.8

Notee. *n* — long-term averages (1968-2021), conditional norm.

The weather conditions of the growing season in 2021 provided selection of mutants that can withstand water and heat stress and form fully fledged grain. A record anomalous excess of the average daily air temperature vs. the norm (+6.3 °C) occurred in May under atmospheric and soil drought, when the amount of precipitation did not exceed 10.2% compared to the norm. With relatively favorable temperature conditions in June and July, the precipitation amounted to 39.1% and 57.6% of the norm, respectively. In August, grain ripened at elevated average daily air temperatures and deficit of precipitation (33.3% of the norm).

In 2022, the limiting factor for plant growth was the lack of moisture, but the harmfulness of dry periods was reduced due to average daily air temperatures slightly different from normal. Analysis of the average daily air temperature during the growing season revealed deviations from the norm in June (1.3 °C lower), July (0.9 °C higher), and August (2.3 °C higher). The amount of precipitation only in May significantly exceeded the long-term average. In other months, the indicator varied from 101.5 (June) to 76.2% (August) compared to the norm.

Environmental factors significantly influenced quantitative traits (Table 4).

4. Morphophysiological traits of phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants under contrasting growing conditions (experimental site of the Tyumen State University biological station “Lake Kuchak”, Tyumen Province, Nizhnetavdinsky District)

Trait	2021		2022		Comparison index, %
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	
Plant height, cm	55.3±2.13	22.87	76.4±2.36*	18.38	38.2
Flag-leaf length, cm	10.8±0.21	11.20	17.4±0.28*	9.38	61.1
Flag-leaf width, mm	8.5±0.30	20.69	12.1±0.23*	11.18	42.4
Flag-leaf area, cm^2	6.4±0.30	28.06	14.3±0.38*	15.92	23.4
Chlorophyll content, Spad units	49.2±2.02	6.71	45.1±0.64	8.38	9.1
Productive stem number per 1 m^2	249.0±8.27	23.79	358.0±10.93*	20.89	43.8
Ear length, cm	6.1±0.46	16.64	8.1±0.17*	12.50	32.8
Ear grain number	15.0±3.00	41.83	30.2±1.18*	22.92	101.3
Ear grain weigtt, g	0.42±0.08	49.23	1.0±0.04*	28.88	147.6
Yield, g/m^2	138.1±4.88	20.96	279.4±7.75*	25.04	102.3

Note. For sample sizes, see Materials and methods section.

* Differences compared by year are statistically significant at $p < 0.01$.

The growing season of 2022 was more favorable compared to 2021 for the a number of traits. During the heading stage, the plants had an advantage in height and development of the flag leaf length, width, and area. More plants and productive stems were obtained for harvesting, which indicated increased plant survival and provided an increase in yield.

The drought influenced the physiological development of spring wheat plants. Plants responded to water and heat stress in 2021 by increasing the amount of chlorophyll in flag leaf cells. M. Yildirim et al. [24] reported that measuring chlorophyll content in leaves at the milky ripeness of the grain could be used in the selection of wheat plants with high yield potential both under relatively optimal conditions and under heat stress.

The variability of traits in most cases increased under the influence of stress factors, which was confirmed by the coefficient of variation. Differences across the years of the study were most significant in grain weight and the number of grains per ear, as well as in yield. A minimal decrease under stress occurred in the chlorophyll content in flag leaf and its area.

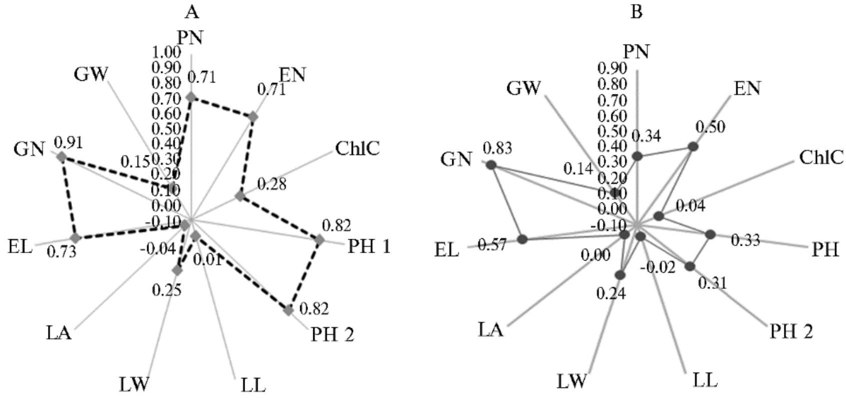

Fig. 3. Correlation of yield values with quantitative traits in 2021 (A) **and 2022** (B) **in phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants:** PN — plant number per 1 m^2, EN — ear number per 1 m^2, ChlC — chlorophyll content, Spad units, PH-1 — plant height, cm (records of 06/20/2021, 06/29/2022), PH-2 — plant height, cm (records of 07/10/2021, 07/17/2022), LL — leaf length, cm, LW — leaf width, cm, LA — leaf area, cm^2, EL — ear length, cm, GN — number of grains per ear, M3 — grain weight per ear, g (experimental site of the Tyumen State University biological station Lake Kuchak, Tyumen Province, Nizhnetavdinsky District).

The correlation between the yield of the mutant accessions studied and important quantitative traits depended on the genotype and environmental factors (Fig. 3). In the dry year of 2021, a significant correlation occurred between the yield values and plant height at booting and heading ($r = 0.82$, $p < 0.05$), plant number per 1 m^2 ($r = 0.71$, $p < 0, 05$), productive ear number per 1 m^2 ($r = 0.71$, $p < 0.05$), grain number per ear ($r = 0.73$, $p < 0.05$), and grain weight per ear ($r = 0.91$, $p < 0.05$). There was a direct relationship between yield and chlorophyll content in the flag leaf ($r = 0.28$, $p > 0.05$) and its width ($r = 0.25$, $p > 0.05$).

In 2022, the correlation between yield and grain weight per ear (r = 0.83, $p < 0.05$) remained high; a medium-strong relationship was found with the number of productive ears per 1 m2 (r = 0.50, $p < 0.05$) and the number of grains in the ear (r = 0.57, $p < 0.05$). The influence of other traits (except for the width and area of the flag leaf) on grain productivity decreased.

Based on a complex of valuable traits, five samples were selected from mutant populations that were significantly superior to the original forms and corresponded to the level of varieties grown in the Tyumen Province (Tyumenskaya 25, Tyumenskaya 29, Novosibirskaya 31) (Fig. 4, Table 5).

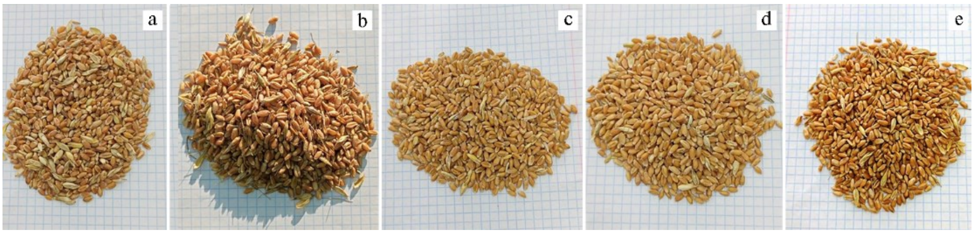

Fig. 4. Grain of phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants: a — sample No. 4 F_4 (0.01%) Cara × Skant 3, b — sample No. 5 F_4 (0.01%) Cara × Skant 3, c — sample No. 17 P_2 (0.002%) Skant 3, d — sample No. 20 P_2 (0.002%) Skant 3, e — sample No. 32 F_4 (0.002%) Cara × Skant 3 (experimental site of the Tyumen State Biological Station University "Lake Kuchak", Tyumen region, Nizhnetavdinsky district, 2022).

Three samples outstanded from the population of the hybrid Cara × Skant 3 after treating the seeds with phosphemide in two concentrations (0.01% and 0.002%), two samples were created on the basis of the Scant 3 variety (concentration 0.002%). The potential of grain productivity was more pronounced in more favorable growing conditions in 2022 (396.1-518.2 g/m^2); for standard varieties, the yield was 355.0-424.5 g/m^2.

Morphophysiological comparison of mutant samples with standard varieties revealed some features in the response to environmental stress factors. In height, the mutant plants obtained from the hybrid were significantly inferior in both years to the mutants selected from the Scant 3 variety and to the standards. The length of the flag leaf in 2021 was maximum in sample No. 5 F_4 (0.01%) Cara × Skant 3, in 2022 in No. 32 F_4 (0.002%) Cara × Skant 3. Among the standards, Tyumenskaya 25 (2021) and Novosibirskaya 31 (2022) stood out in turms of flag leaf length and width. The pattern revealed throughout the entire studied breeding material and manifested itself in an increase in the chlorophyll content in the flag leaf was confirmed by the data for the best mutant samples.

Lack of moisture and elevated air temperatures decreased plant viability, the number of productive stems per 1 m^2, the grains number and grains weight per ear. The maximum and minimum number of productive ears of mutants and standard varieties in 2021 had relatively small differences, 229-278 and 229-259 per 1 m^2, respectively. In 2022, this trait changed in mutants within 322-436 per 1 m^2, in standards within 272-317 per 1 m^2. With a smaller number of plants and productive stems at harvesting, the zoned varieties had an advantage in the number of grains per ear and their weight compared to the new samples. .

5. The best of phosphemide-induced spring bread wheat (*Triticum aestivum* L.) mutants compared to standard varieties under contrasting conditions of two-year growing seasons (*M*±SEM, experimental site of the Tyumen State Biological Station University "Lake Kuchak", Tyumen region, Nizhnetavdinsky district)

T	No. 4 F_4 (0.01 %) Cara × Skant 3		No. 5 F_4 (0.01 %) Cara × Skant 3		No. 17 P_2 (0.002 %) Skant 3		No. 20 P_2 (0.002 %) Skant 3		No. 32 F_4 (0.002 %) Cara × Skant 3		Tyumenskaya 25		Tyumenskaya 29		Novosibirskaya 31	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
1	46.4±1.36a	62.2±2.24b	51.6±1.57a	66.5±1.80a	60.8±1.03a	91.7±1.86a	62.1±1.33b	81.9±1.60	53.3±0.64a	68.4±1.17	72.3+0.86	92.5±1.53	70.7+0.73	90.5±1.16	67.3+1.50	91.0±1.88
2	13.6±0.43	16.3±1.16a	12.2±0.54	17.8±0.51b	10.7±0.69	17.7±0.66b	10.9±0.62a	18.5±0.86	10.4±0.57a	18.9±1.08	13.2±0.53	17.4±0.87	12.3±0.56	19.5±1.02	13.0±0.56	19.4±0.68
3	6.4±0.32a	12.2±0.58	7.4±0.51b	11.8±0.40c	8.6±0.37b	14.4±0.68c	8.2±0.37b	14.6±0.80c	8.8±0.40b	13.6±0.93	11.2±0.51	12.8±0.51	10.3±0.32	11.6±0.40	10.4±0.45	11.2±0.45
4	7.27	16.51	7.53	17.54	7.68	21.28	7.46	22.55	7.64	21.46	12.34	18.60	10.58	18.89	11.29	18.14
5	46.8±0.71b	46.6±1.16c	47.2±0.78b	45.9±2.90c	49.1±1.18b	45.3±2.50c	49.5±0.83b	44.4±1.00c	54.8±0.67	45.6±1.53b	53.2±1.62	51.1±0.96	55.5±1.64	42.3±0.64	51.7±1.41	44.0±0.62
6	246±8.2	408±13.6b	229±7.6c	417±10.8a	242±7.5c	322±11.1c	236±6.7	386±8.1	278±9.7b	436±8.9a	259±12.0	306±9.3	246±5.5	272±9.8	229±7.8	317±10.1
7	8.3±0.47a	9.4±0.49	9.2±0.83a	9.6±0.82	5.7±0.36	8.3±0.21	5.6±0.37	8.8±0.77a	7.4±0.73a	9.1±0.54a	6.2±0.72	8.4±0.62	6.4±0.71	8.3±0.36	6.4±0.59	7.4±0.31
8	24.0±6.38c	39.0±3.45	21.0±2.97b	45.0±2.27c	19.0±4.04b	38.0±3.95	16.0±4.26b	30.0±2.74b	13.0±3.24a	26.0±3.03c	18.0±3.25	41.0±2.30	23.0±4.32	37.0±3.17	26.0±5.49	39.0±3.26
9	0.47±0.13a	1.3±0.15b	0.54±0.08a	1.02±0.10a	0.65±0.14b	1.23±0.17a	0.66±0.17b	1.05±0.09a	0.31±0.08a	0.95±0.12a	0.63±0.12	1.42+0.13	0.79±0.15	1.31+0.10	0.76±0.16	1.12+0.18
10	115.6±2.90a	518.2±3.66a	123.7±3.08a	425.3±4.85b	157.3±4.12a	396.1±5.63b	155.8±4.01c	405.3±5.70b	86.2±4.83a	414.2±6.05	163.3±4.56	424.5±4.07	194.3±5.63	356.3±3.68	174.0±6.71	355.0±4.86

Примечание. T — trait: 1 — plant height, cm, 2 — flag-leaf length, cm, 3 — flag-leaf wifth, mmм, 4 — flag-leaf area, cm^2, 5 — chlorophyll content, Spad units, 6 — productive stem number per m^2, 7 — ear length, cm, 8 — grain number per ear, 9 — grain weight per war, g, 10 — yield, g/m^2. For sample size, see the Materials and methods section.

a, b, c The differences are statistically significant ($p < 0.05$) when comparing with three standards, with two standards and with one standard, respectively.

According to our data, the lack of moisture, combined with elevated air temperatures, had a limiting effect on the growth of plants in height and the development of the assimilation surface, which we observed in 2021. Other researchers have also reported growth limitation in wheat during drought, which affects plant height, leaf area, dry mass, and other growth functions [25, 26].

Drought-tolerant genotypes have been found to maintain high chlorophyll content in leaves, necessary for photosynthesis [27]. In addition, chlorophyll is an indicator of photosynthetic activity and biosynthesis of assimilates [28], which makes it possible to use its content for the selection of drought-resistant forms of wheat [29]. Our data indicate an increase in the chlorophyll content in flag leaf cells under the influence of water and temperature stress on average for the collection of mutants to 49.2±2.0 units Spad vs. 45.1±0.64 units Spad in relatively favorable weather conditions. Thus, the differences in the chlorophyll content in leaves in 2021 compared to 2022 were most pronounced in three samples promising for further use in breeding, the No. 17 P_2 (0.002%) Skant 3 with 8.3%; No. 20 P_2 (0.002%) Skant 3 with 11.5%; No. 32 F_4 (0.002%) Cara × Skant 3 with 20.2% (see Table 5). It can be assumed that sample No. 32 F_4 (0.002%) Cara × Skant 3 is a tolerant genotype, since it contains the largest amount of chlorophyll (54.8±0.67 units Spad) compared to other genotypes. In the leaves of sample No. 4 F_4 (0.01%) Cara × Skant 3, the amount of chlorophyll was almost the same over the years, 46.8±0.71 and 46.6±1.16 units Spad in 2021 and 2022, respectively.

The optimum temperature for photosynthesis in wheat plants is approximately 25 °C [30]. It has been shown that an increase in air temperature by 1 °C during the grain filling period reduces yield by 3-4% [31]. According to S.S. Bhullar et al. [32], if heat stress occurs during the post-flowering period (grain filling period), it negatively affects photosynthesis and inhibits starch synthesis, which leads to a decrease in grain weight and yield. According to our average data, in the studied samples under stress conditions, 2 times fewer grains were formed in the ear, their weight decreased by 2.5 times, and their yield decreased by 2 times. High air temperatures (30 °C or more) during the flowering and pollination period turned out to be critical for the grain formation in the ear. In some cases, only single grains were formed in the ear. Since selection of drought-resistant forms based only on yield is not always effective [33], it is recommended to use morphological and physiological traits that play an important role in plant adaptation for a relatively quick assessment of genotypes [34]. The integrated approach allowed us to select valuable spring bread wheat genotypes, starting from early mutant generations [35]. Therefore, theoretical foundations of chemical mutagenesis proposed by I.A. Rapoport [36], continue to be relevant for increasing the efficiency of mutation breeding of plants.

It should be noted that the mutants selected for a number of traits (M_5, M_6) had identical alleles. Thus, samples No. 4 F_4 (0.01%) Cara × Skant 3, No. 5 F_4 (0.01%) Cara × Skant 3, No. 32 F_4 (0.002%) Cara × Skant 3 carried the same alleles at the gliadin *B1*, *D1* and *A2* loci. The *Gli-A1o* allele was common for samples No. 4 F_4 (0.01%) Cara × Skant 3, No. 17 P_2 (0.002%) Skant 3, and No. 20 P_2 (0.002%) Skant 3, *Gli-B2p* for mutants No. 5 F_4 (0.01%) Cara × Skant 3, No. 32 F_4 (0.002%) Cara × Skant 3, No. 17 P_2 (0.002%) Skant 3, and No. 20 P_2 (0.002%) Skant 3. Note that for alleles *Gli-B1e* and *Gli-B1f*, found in the isolated mutants, the number and relative electrophoretic mobility of gliadins were similar, with the exception of one component. Therefore, it is acceptable to assume that the effect that the *Gli-B1e* and *Gli-B1f* alleles may have on morphological characters will be similar.

Thus, the adaptation potential of spring soft wheat in the extreme conditions of the Northern Trans-Urals can be increased by using the genetic variability of mutant populations. The chemical mutagen phosphemide has been proven to induce beneficial mutations in *Triticum aestivum*. The integrated use of mutational and recombination variability is effective to increase genetic diversity. Under the influence of water and temperature stress, a number of mutants showed an increase in chlorophyll content in flag leaf cells compared to that in favorable weather conditions. The identified differences can be used to select forms resistant to unfavorable environmental factors. Mutant accessions, characterized by diverse phenotypic and genotypic variations, are useful for improving economically valuable traits in wheat. Based on field trials of mutant generations M_5 and M_6, the best of them are of interest for breeding.

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