Brief information about the project

Name of the project	AP09258679 "Molecular-biochemical characteristics of the created mutant germplasm of spring wheat, resistance to leaf and yellow rust, morphometry and grain quality" (0121PK0037)
Relevance	The project includes the creation and molecular-biochemical characterization of new mutant resources of spring wheat for resistance to leaf and yellow rust, morphometry and grain quality, including the content of important micronutrients and their bioavailability. The integrated approach is based on mutation selection through the application of various doses of gamma irradiation and the genetic basis of the rust-resistant variety Kazakhstanskaya-19, includes phenotyping using hyper-spectral imaging, screening for grain protein content, iron (Fe) and zinc Zn, and their bioavailability, visual assessment of the development of fungal structure and hyperspectral imaging, study of the expression of b-1,3.-glucanase and endochitinase genes and determination of their activity as a temporary response to infection by pathogenic fungi and the use of competitive allele-specific PCR (KASP) to determine discrimination of Lr gene alleles.
Purpose	The goal of the project is to study biosurfactant-producing microorganisms and their applicability for enhanced oil recovery.
Objectives	1. Most wheat varieties widely used in agricultural production suffer significantly from fungal diseases, and grain yield losses amount to 50–85%. In this regard, there is a need to create a new germplasm of spring wheat that is resistant to leaf and yellow rust. To expand the genetic variability of spring wheat based on the rust-resistant variety Kazakhstanskaya-19, and to use induced mutagenesis using different doses of gamma irradiation (300, 350 and 400 Gy), new mutant lines (M3–M4) were created generation) for phenotyping for resistance to rust fungal diseases. These mutant resources have been used to determine parameters associated with grain yield and morphometry, as well as to analyze rust resistance characteristics through visual assessment of fungal structure development (detecting hypersensitive response or chlorosis around sites of infection) and hyperspectral imaging (analysis of differences in values reflections in the range from 404 to 2511 nm and to identify candidate compounds that cause stability. 2. Mutant spring wheat resources were assessed based on grain quality characteristics, such as protein content and grain microelements (Fe and Zn). To determine the bioavailability of micronutrients, the mutant lines were screened for the content of phytic acid, which is the main "antinutrient" due to its strong metal chelating ability.

	Mutant lines have been identified that are resistant to leaf and yellow rust, biofortified with grain micronutrients and low phytic acid content, as well as improved bioavailability of microelements. 3. Plant b-1,3-glucanases themselves, or preferentially in combination with chitinase, being pathogenesis-related proteins (PBPs), are directly involved in defense mechanisms by hydrolysis of b-1,3-glucans and chitin, the main structural components of the cell wall fungi and inhibit the growth of pathogens. Their roles in resistance were studied by determining gene expression as transient responses against the phytopathogen and enzyme activity. Molecular marker technology as valuable tools have been applied to assess genetic diversity, identify and select desired genotypes. Competitive allele-specific PCR (KASP), one of the uniplex SNP genotyping platforms critical for the identification of Lr genes, was used to screen mutant wheat germplasm.
Expected and achieved results	In this project, the genetic variability of spring wheat based on the rust-resistant variety Kazakhstanskaya-19 is expanded using induced mutagenesis through three doses of gamma irradiation (300-, 350- and 400-Gy) from a ^{60}Co source in the laboratory Plant Breeding and Genetics IAEA, Seibersdorf, Austria. New M3 – M4 generation mutant lines have been created. These three radiation doses were selected based on a laboratory radiosensitivity test, which had an LD50 of 330 Gy for the Kazakhstanskaya-19 variety. The created 300-, 350- and 400-Gy mutant lines were phenotypically identified by the resistance of adult plants to leaf and yellow rust. 75 immune or resistant adult plant M3 mutant lines were selected along with the variety Kazakhstanskaya-19, which were characterized for the resistance of seedlings. The mutant germplasm included 42 lines of 300 Gy, 16 samples of 350 Gy lines and 17 lines of 400 Gy lines, which significantly exceeded the Kazakhstanskaya-19 variety in productivity parameters by 1.6-1.7 times (weight and number of grains in the main spike). New mutant lines, resistant in adult plants to leaf and yellow rust, were phenotyped for seedlings resistance after inoculation with rust in greenhouse experiments. The background of juvenile resistance was analyzed microscopically and using hyperspectral imaging to identify and/or structural changes in the leaf surface that cause resistance. Based on the resistance of mature plants to leaf and yellow rust, and microscopic studies of juvenile resistance, it was revealed that the majority of 75 M3 mutant lines, and generated by gamma radiation doses of 300-, 350- and 400-Gy (89.33%) combined juvenile resistance with that of an adult plant to both types of rust, leaf and yellow rust.

	Hyperspectral imaging analysis showed that infected leaves of wheat genotypes have increased relative reflectance in visible and near-infrared light compared to uninfected genotypes, with peak mean values at 462 and 644 nm, and 1936 and 2392 nm, respectively. Five spectral indices including Red Normalized Vegetation Index (RNDVI), Structure Insensitive Pigment Index (SIPI), Ratio Vegetation Index (RVSI), Water Index (WI) and Normalized Difference Water Index (NDWI), demonstrated significant potential for determining the degree of resistance of seedlings. The most significant differences in reflectivity between the sensitive and resistant mutant lines appeared at wavelengths of 694.57 and 987.51 nm. The created lines were assessed according to the morphometric parameters of the grain (area, length, width and thickness of the grain). The mutant lines generated with doses of 350 and 400 Gy, with numbers 8/2 and 25/3, respectively, had the largest grain area (16.2 and 16.5 mm2). The grain length variation ranged from 6.43 to 7.29 mm with an average value of 6.75 ± 0.18 mm in the entire mutant population. Nine M4 mutant lines (28.0%) had significantly longer grains, which is like the grain area parameter found in the 350- and 400-Gy mutant germplasm. Compared to the morphometric parameters of the grain, such as grain area and grain length, the smallest ranges of variability were observed for grain thickness with intervals from 2.6 to 3.34 mm with an average value of 3.15 ± 0.13 mm in all irradiated lines. 6 genotypes with statistically significant higher grain thickness values than the Kazakhstanskaya-19 variety were identified. Based on an important characteristic such as grain quality, grain protein content (GPC), it was found that the ranges for mutant wheat germplasms were 9.6–15.6% with an average value of 13.99 ± 1.16, 12.63 ± 1.49 and 12.43 ± 1.54% in 300-, 350- and 400 Gy lines, respectively (n = 138). 17 mutant lines (37.0%) were identified, of which 8, 6 and 3 samples represented by 300-, 350- and 400 Gy lines had GPC that was statistically significantly higher than the Kazakhstanskaya-19 variety (13.95 ± 0.12%) by 1.08-1.12 times. ANOVA analysis for GPC revealed significant differences between the Kazakhstanskaya-19 variety and the 350- and 400-Gy lines, which indicates the effectiveness of high levels of gamma irradiation for generating genetic variability of GPC. Thus, the mutant lines created by Kazakhstan-19 are new valuable genetic sources, combining plant resistance to leaf and yellow rust with a high GPC. Of the 18 screened 300 Gy mutant lines (89%), the majority (16 samples) statistically significantly exceeded the Kazakh-19 variety by 1.82–3.19 times in terms of iron

	content in grain (CFe3). In 350- and 400-Gy mutant germplasms for CFe3, 15 and 13 lines were screened, respectively. Of which, 14 and 10 samples were characterized by statistically significant higher CFe3 compared to the original variety by 1.43–3.13 times and 1.43–2.42 times. Thus, based on the biofortified ability of Fe in three dosed mutant germplasms, 40 promising samples were identified that are resistant to leaf and yellow rust (LR and YR) and have high indicators of productivity elements. The ranges of variation in zinc content in grain at 300-, 350- and 400 Gy were, respectively, 42.7-97.3, 72.0-119.5 and 100.2-105.8 mg/kg, with mean values for each dosed germopalasma, 64.43 ± 19.18, 98.3 ± 10.78 mg/kg, and 102.05 ± 1.43 mg/kg. Identified biofortified by Zn from three mutant germplasms (39 genotypes in total) statistically significantly exceeded the parent variety, respectively, in 300 Gy lines by 1.47–2.77 times, in 350 Gy lines by 2.05–3.40 times and 400 Gy lines by 3.0 times. The expression of *b*-1,3-glucanase encoding pathogenesis related proteins was studied in the spring wheat variety Erythrospermum-35 (juvenile-sensitive) and 100 Gy- and 200 Gy-created mutant lines and 4 juvenile-sensitive and 4 juvenile-resistant lines, as a temporary reaction to leaf rust. After 24 hours of infection, its level was significantly higher in sensitive genotypes compared to resistant ones. The greatest differences between genotypes appeared at 48 and 72 hours of infection with the ratio, respectively, 32.67 and 2.78 times higher in resistant lines. The expression profile of endochitinase genes, also encoding pathogenesis related proteins, in the spring wheat variety Erythrospermum-35 and juvenile-sensitive and resistant lines, as a temporary response to leaf rust, shows its reduced level in most sensitive genotypes. Similar to the reaction of *b*-1,3-glucanase gene expression, that of endochitinase between both genotypes was detected with a long-term effect of leaf rust. The expression level of endochitinase genes was 2.60 times higher in resistant lines, which indicates the manifestation of its protective role during prolonged exposure to the pathogen. Determination of endochitinase activity in the flag leaves of Erythrospermum-35 mutant lines, differing in resistance to leaf and yellow rust at 2 stages of development, reveals that in 2 resistant mutant lines it was 9.73 and 4.30 times higher than that of the Erythrospermum-35 variety. 35. A KASP marker was developed and evaluated for 6 Lr genes: *Lr1*, *Lr2a*, *Lr3*, *Lr9*, *Lr10* and *Lr17*. The created mutant lines of the Kazakhstanskaya-19 variety had high frequencies of the “a” resistance allele (0.88) in all six *Lr* genes, which were significantly associated with the resistance of seedlings to leaf rust (LR) and suggest the

	possibility of favorable introgression of the haplotype through functional markers. 9 mutant lines were characterized by the presence of the “b” allele in the *Lr9* and *Lr10* genes, with the exception of 1 line with the “a” allele in *Lr9* and 3 lines with the “a” allele in L*r10*, which were sensitive to the progressive development of a number of fungal haustorium cells early stage of inoculation. In general, priority fundamental and practical results were obtained related to the expansion of the genetic diversity of spring wheat, the creation and molecular-biochemical characterization of new mutant resources for resistance to leaf and yellow rust, morphometry and grain quality, including the content of important micronutrients and their bioavailability. The results of the project contribute new knowledge and expand the understanding of the mechanisms of resistance to rust diseases. The project has high potential for commercialization. Thus, the project will make a significant contribution to reducing the negative effects of rust, which is the most economically important disease of wheat, causing significant crop losses. The lack of predictability of the emergence of new rust races with high epidemic potential highlights the need for additional research efforts to assess the disease vulnerability of global food crops before their introduction on a large scale.
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles	1. Kenzhebaeva Saule Sagindykovna, Doctor of Biological Sciences, Hirsch Index – 5, Researcher ID Q-4381-2016, ORCID: 0000-0003-0238-2607, Scopus author ID: 56230125100. 2. Atabaeva Saule Dzhumagalievna, Doctor of Biological Sciences, Hirsch Index – 4, Researcher ID N-9656-2014, ORCID: 0000-0002-4704-6909, Scopus author ID: 562295975000. 3. Shoinbekova Sabina Alimzhanova, Doctor of Chemical Sciences, Hirsch Index – 2, Researcher ID P-2773-2015, ORCID: 0000-0003-0238-2607, Scopus author ID: 8370741600.
List of publications with links to them	S.S. Kenzhebayeva, S.A. Shoinbekova, D. Zharassova3, K.D. Miatzhanova1, A. Abekova, S.Sh. Asrandina,Moahid Ahmal Javid. New spring wheat mutant resources with yellow rust resistance, improved grain morphometric parameter, and high grain protein content. Bulletin of KazNU named after. Al-Farabi, Ecological Series, 2021, No. 3 (68), 55-63. https://doi.org/10.26577/EJE.2021.v68.3.06. Kenzhebayeva, S.S., Atabayeva, S.D., Sarsu, F. Iron-deficiency response and differential expression of iron homeostasis related genes in spring wheat (*Triticum aestivum*) mutant lines with increased grain iron content *Crop and Pasture Science* 2022, 73(2), pp. 127–137 Web of Science Q-2, Scopus Q-2, percentile – 75. Scopus

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Patents	-