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Introducing useful genetic variation in Bulgarian hexaploid wheat (*cv. Fermer*) through radiation mutagenesis approach

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Abstract

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Mutation is an effective strategy not only for creating novel variation into crop genome, but also for direct releasing adapted and high-yielding genotypes. In this study, radiation induced mutagenesis (gamma rays of 200 and 250 Gy) was applied, to develop new mutant lines of Bulgarian wheat *cv. Fermer* with improved agronomic characteristics, focused on higher biotic and abiotic stress tolerance, and bread making quality traits. The selection was carried out in the second season (M2-M4), according to morpho-physiological and yield characteristics: plant height; spike length; grain yield per spike and disease tolerance. Backcross of highly susceptible to *P. graminis* mutant plants (M2 generation) with parental genotype – *cv. Fermer* was performed for purifying selection of the mutant phenotype. In M 5, for the first year, the yield is monitored in a control experiment (10 m^2 in 1 replication). By monitoring the traits of plant height, resistance to some economically important diseases and bread making quality traits. The reported studies are carried out in M5, when it is expected that we have conducted sufficiently long selection on the traits. The selected mutant lines (M5 generation) showed clear phenotype variations, including low and high plant height. For the complete evaluation, a physiological assessment was carried out, including chlorophyll content index (CCI) and photosynthesis activity measurements. Technological assessment for grain (sedimentation value; grain vitreousness; fermentation number; crude protein; wet gluten content; gluten release ; dry luten) and bread, and making strong index. Phytopathological evaluation was performed to establish the resistance of the mutant lines to economically important diseases (leaf rust (*Puccinia recondite* f.sp. tritici), yellow rust (*Puccinia striiformis* f.sp. tritici), powdery mildew (*Blumeria graminis* f. sp. tritici), septoria (*Septoria tritici*). The study confirms the usefulness of radiation induced mutagenesis as a promising tool to improve important agronomic traits in wheat. The obtained results highlighted the importance of these doses of applied mutagens to induce useful genetic variability in bread wheat, for improving grain yield and contributing.

The molecular characterization of M5 lines using SSR and other DNA markers identified mutant alleles in some of the selected lines, thus confirming that the observed phenotype differences reflect the changes at DNA level. Taken together, the presented results demonstrate that the applied doses of Gamma ray induced useful variation in wheat *cv. Fermer* at both phenotype and DNA level. Some of the mutant M5 lines showed improved agronomic and technological traits.

Keywords: hexaploid wheat (*T. aestivum* L.); radiation; mutagenesis; agronomic traits; molecular markers

Introduction

Bread wheat (*Triticum aestivum* L.), a major source of daily caloric intake, is grown in more than 60 countries on 221.86 million ha, with an annual production of 775.8 million metric tons (https://apps.fas.usda.gov/psdonline/circulars/production.pdf) during 2020 – 2021. The most cultivated wheat is allohexaploid (2*n* = 6x, AABBDD) that harbors a large genome of the size ~17 Gb (Arumuganathan and Earle, 1991), and was originated recently through polyploidization and hybridization of *Triticum turgidum* (tetraploid, AABB genome) and *Aegilops tauschii* (diploid, DD genome) (Marcussen et al., 2014). Wheat production plays an important role in food supply both today and in the future, but this sector is highly sensitive to climate and environmental changes (Semenov and Stratonovich, 2013; Figueroa et al., 2018).

Extreme weather is more common due to the climate changes in many parts of the world, including changes in precipitation patterns. Decreased rainfall in combination with elevated air temperatures is the most important factor in limiting yields, and they threaten food security worldwide (Daryanto et al., 2017). Mutation breeding and plant mutagenesis play a significant role in increasing the genetic variability for desired traits in various food crops (Adamu and Aliyu, 2007; Mostafa, 2011; Kozgar et al., 2012). Mutation breeding is one of the breeding tools based on artificially inducing hereditary changes in plants, using either physical or chemical mutagens. This approach has been successfully used to develop diverse and valuable breeding materials in several crops with agronomical important traits (Thapa, 2004; Borzouei et al., 2010; Shah et al., 2012). Induced mutagenesis is one of the most efficient tools used for the identification of key regulatory genes and molecular mechanisms. It is a promising approach to develop new varieties with improved agronomic characteristics, such as higher stress tolerance potential (biotic and abiotic stress) and bio-fortification. Additionally, various mutagenesis approaches have been used to study the evolutionary relationship as well as for the genetic improvement of many organisms, including microbes, animals, and plants (Sikora et al., 2011; Kodym and Afza, 2003). Technological advances in molecular biology have re-augmented the mutation breeding, making it more efficient than ever thought before. Therefore, mutagenesis is frequently applied to fix few blemishes in a cultivar, that has several agronomic traits preferable by farmer. In wheat breeding, Sakin et al. (2004; 2005) obtained superior mutant types having better agronomic values in term of yield and yield components. Gamma rays in particular, is well known physical mutagen that is often used in mutation breeding programmes, to induce desirable mutations in various crops (Konzak, 1987; Knott, 1991).

Mutant screening is a process, involving selection of individuals from a large mutated population that meet specific selection criteria, e.g. early flowering, disease resistance as compared to the parent. However, these selections are often regarded as putative mutants or false mutants. Mutant confirmation, on the other hand, is the process of reevaluating the putative mutants under a controlled and replicated environment using large samples. Through this process, many putative mutants are revealed to be false mutants. In general, the mutations that are important in crop improvement, usually involve single bases and may, or may not affect protein synthesis (Mba, 2013).

Mutation breeding programmes have also been conducted in different European countries. For example, in Bulgaria, more than 76 new cultivars have been developed using induced mutagenesis (Tomlekova, 2010; Oladosu et al., 2016). Rachovska worked on this method at IPGR, and besides the well-known 2 released varieties (Fermer and Guiness) of common winter wheat, a collection of mutant lines with valuable agronomic traits is still maintained in the institute (Vitanova and Rachovska, 2009; Rachovska, 2010).

In this study, the beneficial genetic changes in Bulgarian hexaploid wheat (*cv. Fermer*) induced by gamma radiation mutagenesis, were investigated by phenotype assessment and molecular approaches.

Material and Methods

Plant material and mutants selection

To create novel genetic diversity, seeds of winter common wheat *cv. Fermer* were irradiated with two doses gamma rays (200 and 250 Gy, respectively). The M1 plants grown after mutagenic treatment, were multiplied based on the spike progeny method in the field of IPGR Sadovo. The M2 seeds obtained from each spike were sown in rows. Mutant selection was performed in generations M2 and M3. The differences of M2 plants from the control (the parental variety *Fermer* was also planted after every twenty rows for comparison with M2), were recorded, and plants with the desired phenotypes were collected individually. In the next season, the M3 progeny from selected M2 plants were grown according to the pedigree selection procedure (Morgun and Logvinenko, 1995). Mutants were identified by visual screening for phenotype differences and confirmed at M3 generation by measuring for one spike yield and grain yield from one plant in M2 and M3 generations. The selection of winter wheat mutants after the M4 generation was based on the following criteria: higher yield of 1 da (recalculated from 7 m^2), and low stem height. Two groups of mutant lines (M2-M5- the selection starts from M2 and continues to M5 on different traits) were selected: with low stem and disease resistance for further analyses. The mutant lines obtained were screened for improved agronomic traits focusing on high yield, higher tolerance to biotic and abiotic stress and quality traits for better bread production.

Physiological measurements

A non-invasive field screening of 20 promissing wheat mutant lines and the standard *cv. Sadovo 1* and parental *cv. Fermer* were performed by chlorophyll content index (CCI), and photosynthesis activity measurements with devices CCM200+ and LCpro T, manufactured by Opti science UK.

Technological assessment for grain and bread-making quality

Grain vitreousness (VG), particularly for grain quality in durum wheat was determined according to BSS 13378:1976, BSS EN 15 585:2008 (https://bds-bg.org/bg/project/show/bds:proj:17689).

The Sedimentation Value (SdV) (Iced Acetic Acid Test – 2%, (Pumpyanskiy, 1971) test was used to provide information on the protein quality and baking properties of the wheat (Angelova et al., 2020; Galushko and Sokolenko, 2021; Uhr et al., 2023).

The Fermentation Number (FN) – Pelschenke test was used to assess the fermentation capacity of the wheat, which is important for bread-making quality.The test is based on the retention of CO_2 gases released during dough fermentation. A 10 g sample of grain meal is mixed with a yeast solution (a biological product, representing a concentrated mass of yeast of the Saccharomyces cerevisiae species) in two replicates (Pelshenke et al., 1953). The experiment was carried out under controlled conditions (30°C – water thermostat). The longer the retention time of the sample on the water surface, the better the quality of the gluten. (Angelova et al., 2020; Galushko and Sokolenko, 2021; Uhr et al., 2023).

Gluten Content and Quality (WGC) (BSS EN ISO 21 415-2:2008, BSS EN ISO 21415-1:2007) test was applied to determine gluten characteristics, which are critical for the end-use quality of wheat, gluten relaxation, mm (BDS 13375:1990/Amendment 1: 1993) (https://bds-bg.org/bg/project/show/bds:proj:17686).

Dry Gluten (DG) was done according to the ISO 21415-3:2006, BSS EN ISO 21415-4:2007 standards, to further understand the protein quality of wheat samples (https://www.iso.org/standard/35864.html).

Determination of nitrogen content and calculation of crude protein content (CP) – Part 1: Kjeldahl method (ISO 5983-1:2005) (https://www.iso.org/standard/39145.html).

**Legend for traits name:* VG – Grain vitreousness; SdV – Sedimentation value; FN-Fermentation number; CP – Crude protein; Wet gluten content – WGC; Gluten release – RG; Dry Gluten – DG; bread making strong index – BMSI.

Phytopathological evaluation of mutant lines under field conditions

Phytopathological evaluation was carried out in the breeding field, and in the infectious section of the IPGR „K. Malkov. Disease assessment was done under natural field conditions without application of artificial infestation. The reporting of the reactions to the causative agents of brown rust, yellow rust and powdery mildew was carried out by determining the type of infection and degree of attack, using the following methodology for the individual diseases:

– brown rust – according to Dimov (1988);
– yellow rust – according to Hayit et al. (2021);
– powdery mildew – according to Krivchenko (1980).

For the easier comparability of the results, an average coefficient of infection is calculated, or called adjusted relative attack rate P_0. Depending on the obtained P_0 values, the tested genotypes are grouped into 5 categories (Mihova et al., 1990):

I group – Highly resistant (HR), P_0 = 0–0-5.99%;
II group – Resistant (R), P_0 = 6.00–25.99%;
III group – Moderately resistant (MR) (R), P_0 = 26.00–45.99%;
IV group – Moderately susceptible (MS) (R), P_0 = 46.00–65.99%;
V group – Susceptible (S) (R), P_0 = 66.00–100%.

The evaluation of the studied breeding lines towards the causative agent of septoriosis is according to the method of Sanin et al. (2015), and the resistance of the samples was based on the degree of attack expressed as a percentage, corresponding to the leaf area occupied by the pathogen. In order to unify the obtained results, the data on immune responses to economically important diseases are presented as a score.

Molecular analyses

Genomic DNA was extracted from silicagel dried leaf tissue collected form 10 plant/plot in the field, by using Exgene™ Plant SV mini kit (Gene All, Korea) according to the manufacturer's instructions. The quantity and quality of the extracted gDNA was determined by Nanodrop™ 1000 and TAE agarose electrophoresis.

RGA-profiling

A novel NBS-TRAP method was developed, optimized and employed for amplification of PCR fragments, potentially linked to RGA genes. In this method, the PCR reaction mix contained one degenerative primer targeted to NBS domains (Table 1) and one Start Codon Targeted (SCoT) primer (Collard and Mackill, 2009). The PCR amplifications were conducted by two-step PCR. In the first step, to enrich the PCR products with RGA genes, each 5 µL PCR mixture consisted of 2.5 µL MyTaq HS 2× PCR mix (Bioline, London, UK), 30 ng of gDNA and 0.03 µL (10 µmol/L) of only the NGS primer. The cycling conditions included: initial denaturation for 3 min at 95°C; 35 cycles of 15 s at 95°C, 40 s at 50°C and 2 min at 72°C; and final extension for 10 min at 72°C. Upon completion of the first PCR step, 5 µL of PCR mix consisting of the same component as in the first PCR reaction, but supplemented with 0.4 µL (10 µmol/L) each of both NGS and SCoT primers was added, and the PCR was run for additional 45 cycles with annealing temperature elevated to 55°C. The rest of the PCR cycling conditions were identical to those in the first PCR step. All PCR amplifications were performed on a Veriti 96 well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA). The amplification products were separated on 2% TAE agarose electrophoresis, stained with GelRed™ and visualized on a UV transiluminator.

Microsatellite (SSR) analysis

Four Xgwm (Röder et al., 1998) and two Xwmc (Gupta et al., 2002) microsatellite loci, selected for their genome location close to fungal disease resistance genes, were amplified in 6 µL PCR reactions containing 30 ng g DNA, 1x My Taq HS PCR master mix, 5 pM of each forward (F) and reverse (R) primer. The F primer in each PCR was labelled with FAM or ATTO565. The PCR amplifications were performed on a Veriti 96 well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA), under the following cycling conditions: initial denaturation for 3 min at 95°C; 35 cycles of 15 s at 95°C, 60 s at a locus specific temperature °C and 2 min at 72 °C; and final extension for 10 min at 72 °C. The PCR fragments were separated on a 3130 Genetic Analyzer (Applied Biosystems) and the SSR allele sizes were recorded using Genemapper v4.0 software (Applied Biosystems).

Statistical section

Statistical processing of the results was done using the program SPSS 19. Multivariate analysis of variance (ANOVA) was used to determine the sources of variation (Lidansky, 1988). Duncan's test was used to detect significant differences between the variations at a significance level of $p<0.05$ (Duncan, 1955).

Results and Discussion

Wheat mutant lines (M and M5 generations), obtained by radiation induced mutagenesis of Bulgarian 6x wheat *cv. Fermer* were screened in 2 subsequent years for improved agronomic traits focusing on high yield, higher tolerance to biotic and abiotic stress, and quality traits for better bread production. In addition, molecular approaches were used to determine the mutagenesis induced genetic diversity in the wild type (*cv. Fermer*), and to select the mutant phenotype BC of highly susceptible to *P. graminis* mutant plants (M2 generation) and *cv Fermer.*

The selected mutant lines (M5 generation) showed valuable phenotype variation including low plant height, resistance to some economically important diseases, improved yield and bread making quality traits.

Chlorophyll content index

Higher value of CCI was measured in 80% of selected mutant lines in comparison to the standard *cv. Sadovo 1* and the parental genotype – *cv. Fermer*, in both low stem and resistant to diseases groups (Table 2 and Table 3). For the both crop seasons, the highest CCI index was determined in five mutant lines (№ 4, 5, 7, 9) and 10 belonging to low stem group and in five lines (№ 3, 4, 5, 8) and 10 originated from the group of resistant to diseases mutant lines. The observed relatively low CCI values were a result from the lack of drought stress before and during measurements. From these results it can be concluded that the low stemmed mutant lines № 4, 5, 9 and 10 (Table 2) and the diseases resistant lines № 3 and 8 (Table 3) possess high net photosynthetic rate and high chlorophyll content index. Le Roux Marlon-Schylor et al. (2020) reported that in mutant plants increased total chlorophyll and a higher photosynthesis rate compared to wild-type plants. Simova-Stoilova et al. (2020) established better reaction of seedlings originating from winter wheat mutant line compared to the initial sensitive variety to drought stress.

Table 1. NBS specific degenerate primer sequences

Primer	Sequence (5'-3')	Reference
NBS2	GTWGTYTTICCYRAICCISSCAT	(Calenge et al., 2005)
NBS3	GTWGTYTTICCYRAICCISSCATICC	(Calenge et al., 2005)
Nbs12-R	YTTSARSGCTAAAGGRAGRCC	(Verzaux et al., 2011)

Source: Authors' own elaboration

Table 2. Chlorophyll content index, leaf gas exchange and grain yield parameters of low stem mutant lines for the two-year period

Low stem mutant lines	CCI index	A µmol m^{-2} s^{-1}	E mmol m^{-2} s^{-1}	Ci vpm	Gs mmol m^{-2} s^{-1}	iWUE ratio	Yield, kg/da
St-Sadovo 1	29.3 ^{a}	8.10 ^{a}	1.47 ^{a}	270 ^{c}	0.13 ^{a}	5.51 ^{a}	380.0
Parental genotype cv. Fermer	33.6 ^{ab}	10.7 ^{ab}	1.61 ^{ab}	254 ^{ab}	0.15 ^{ab}	6.64 ^{a}	420.0
Mutant line 1	34.1 ^{ab}	11.5 ^{ab}	1.67 ^{ab}	252 ^{ab}	0.15 ^{ab}	6.89 ^{ab}	334.5
Mutant line 2	32.3 ^{ab}	11.0 ^{ab}	1.74 ^{b}	264 ^{ab}	0.16 ^{ab}	6.32 ^{a}	343.0
Mutant line3	32.9 ^{ab}	11.6 ^{ab}	1.76 ^{b}	269 ^{ab}	0.16 ^{ab}	6.59 ^{a}	425.5
Mutant line 4	39.4 ^{c}	12.4 ^{b}	1.81 ^{b}	248 ^{a}	0.16 ^{ab}	6.85 ^{ab}	404.5
Mutant line 5	37.6 ^{c}	13.0 ^{b}	1.68 ^{ab}	268 ^{ab}	0.17 ^{b}	7.74 ^{b}	347.0
Mutant line 6	33.4^{ab}	9.80 ^{a}	1.74 ^{b}	280 ^{c}	0.14 ^{a}	5.63 ^{a}	352.0
Mutant line 7	35.8^{bc}	11.2 ^{ab}	1.44 ^{a}	271 ^{ab}	0.16 ^{ab}	7.78 ^{b}	488.5
Mutant line 8	33.9^{ab}	10.6 ^{ab}	1.55 ^{ab}	278 ^{c}	0.16 ^{ab}	6.84 ^{a}	405.0
Mutant line 9	38.0^{c}	13.6 ^{b}	1.54 ^{ab}	242 ^{a}	0.16 ^{ab}	8.83 ^{b}	471.5
Mutant line 10	35.6^{bc}	12.8 ^{b}	1.45 ^{a}	235 ^{a}	0.18 ^{b}	8.83 ^{b}	272.5

Letters indicate differences significant at $P < 0.05$

A – CO_2 Photosynthetictic assimilation rate; E – H_2O Transpiration rate (E); intercellular (sub-stomatal) CO_2 concentration (Ci); stomatal conductance (Gs) and instantaneous water use efficiency (iWUE)

Source: Authors' own elaboration

Table 3. Chlorophyll content index, leaf gas exchange and grain yield parameters of tolerant to diseases mutant lines for the two- year period

Resistant to diseases mutant lines	CCI index	A µmol m^{-2} s^{-1}	E mmol m^{-2} s^{-1}	Ci	Gs	iWUE ratio	Yield, kg/da
St-Sadovo 1	29.3^{a}	8.10 ^{a}	1.47 ^{a}	270 ^{bc}	0.13 ^{a}	5.51 ^{a}	402.0
Parental genotype-cv. Fermer	33.6 ^{ab}	10.7 ^{ab}	1.61 ^{b}	254 ^{b}	0.15 ^{ab}	6.64 ^{ab}	440.0
Mutant line 1	30.1^{ab}	9.10 ^{a}	1.54 ^{ab}	266 ^{b}	0.14 ^{a}	5.91 ^{a}	391.5
Mutant line 2	35.2 ^{ab}	9.80 ^{ab}	1.61 ^{b}	254 ^{b}	0.14 ^{a}	6.09 ^{a}	489.5
Mutant line 3	39.0 ^{c}	12.1 ^{a}	1.45 ^{a}	234 ^{ab}	0.18 ^{b}	8.34 ^{b}	300.0
Mutant line 4	36.7 ^{bc}	10.9 ^{ab}	1.48 ^{a}	264 ^{b}	0.15 ^{ab}	7.36 ^{ab}	386.0
Mutant line 5	36.7 ^{bc}	10.1 ^{ab}	1.51 ^{ab}	274 ^{bc}	0.15 ^{ab}	6.69 ^{ab}	414.0
Mutant line 6	33.3 ^{ab}	10.7 ^{ab}	1.44 ^{a}	267 ^{b}	0.15 ^{ab}	7.43 ^{ab}	368.5
Mutant line 7	31.4^{ab}	8.50 ^{a}	1.51 ^{ab}	285 ^{c}	0.12 ^{a}	5.63 ^{a}	379.5
Mutant line 8	37.5 ^{c}	10.9 ^{ab}	1.59 ^{ab}	245 ^{ab}	0.16 ^{ab}	6.86 ^{ab}	529.0
Mutant line 9	36.2 ^{bc}	11.0 ^{ab}	1.43 ^{a}	183 ^{a}	0.16 ^{ab}	7.69 ^{b}	352.5
Mutant line10	36.7 ^{bc}	10.0 ^{ab}	1.33 ^{a}	258 ^{b}	0.17 ^{b}	7.52 ^{ab}	372.5

Letters indicate differences significant at $P < 0.05$

CO_2 Photosynthetictic assimilation rate; E – H_2O transpiration rate (E); intercellular (sub-stomatal) CO_2 concentration (Ci); stomatal conductance (Gs) and instantaneous water use efficiency (iWUE)

Source: Authors' own elaboration

Yield, as a major breeding trait, is extremely complex and controlled by multiple genes. It has a low broad sense heritability coefficient due to strong influence of the environmental conditions on its expression. The presence of an interaction makes selection difficult, as genotypes perform differently in different environments/years.

It is evident from Table 2 that five of the low stemmed mutants recorded for the 2 years period average grain yield lower than that of standard *cv. Sadovo 1* (№ 1, 2, 5, 6 and 10). The mutant lines 9 and 7 are characterized by yields above 4.7 t/ha), and they could be of interest for further breeding work. From the group of disease resistant mutants, lines № 2, 5 and 8 produced yields over 4.0 t/ha, that was higher than that of the parental *cv. Fermer* (Table 3). The trials on this trait continued for the M6 progenies of these lines under the conditions of competitive varietal testing.

The climatic conditions in the first year were not favourable for mass development of brown rust, yellow rust and powdery mildew. The lack of sufficient rainfall in the months of March, April, May and the high air temperatures in May did not allow the natural multiplication and spread of the mentioned phytopathogens.

For this reason, low morbidity among the studied mutant groups to the studied pathogens was reported for this year (Table 4). The only exception was observed when establishing the resistance of the studied breeding materials to Septoria, with 80% of the lines showing moderate sensitivity to the pathogen.

The evaluation of the resistance to economically important diseases in the second-year show that no disease-resistant mutant groups were reported. Regarding the leaf rust, the weakest attack by the pathogen was observed in the group of resistant mutant lines, where a sensitive immune reaction was recorded only in two lines (№ 4 and 6).

Yellow rust attack was most noticeable in the group of low stemmed lines compared to the resistant lines group. In these two groups, disease susceptibility was present in ten plots. Higher susceptibility to *Powdery mildew* and *Septoria* was reported in the group low stemmed mutants compared to mutant lines originating from tolerant plants.

The phytopathological assessment allowed selection of mutant lines resistant to some economically important for Bulgaria and the Balkan Peninsula region diseases, as the lowest degree of attack and relatively good resistance to economically important diseases were the following lines: mutant lines № 2, 8 and 9 from the resistant group and line No 5 of the low stemmed group. The resistance of mutant wheat lines to fungal pathogens has also been established by Boyd et al. (2006). For example, lines I3-48, I3-49 and I3-54 show resistance to yellow rust, and mutants I3-27, I3-30, I3-48 and I3-49 show resistance to leaf rust.

The results of the three-factorial ANOVA (Tables 5 and 6) showed that the values of the factor „*genotype*“ ranged from 9.88% for BMSI (Table 6) to 35.6% for the DG (Table 6). The factor „*year*“ (environmental conditions) shows the strongest effect for the trait FN (41.74%), followed by VG (40.51%), RG (38.7%) and BMSI (35.5%), but is non-significant for the traits SdV (4.34%) and WGC (1.80%). The group (Resistant or Low stemmed) to which they belong, did not significantly influence the values, and its effect ranged from 0.0% for FN to 15.26% for WGC. The interaction of „*genotype x group*“ is strong for all studied parameters, being decisive for SdV (49.55%), DG (36.79%) and CP (22.83%).

Table 4. Phytopathological evaluation of mutant groups, for the two- year period

№	Group	Phytopathogen							
		Puccinia recondite f.sp. *tritici*		*Puccinia striiformis* f.sp. *tritici*		*Blumeria graminis* f. sp. *tritici*		*Septoria tritici*	
		I year	II year	I year	II year	I year	II year	I year	II year
1	Resistant	7	3	9	3	9	3	3	3
2		9	3	9	3	9	3	3	3
3		9	3	9	1	7	3	3	3
4		7	1	9	3	7	3	1	1
5		9	3	9	3	7	3	3	1
6		7	1	9	3	7	1	3	3
7		9	3	7	1	7	1	3	3
8		9	3	9	3	9	3	3	3
9		9	3	9	3	9	3	3	3
10		9	3	7	3	9	3	3	3
1	Low stemed	9	3	7	1	5	3	3	3
2		9	3	7	1	7	3	3	3
3		7	1	7	1	5	1	3	3
4		7	1	9	1	7	3	1	3
5		7	3	9	3	7	3	3	1
6		9	3	9	1	7	1	3	3
7		7	1	9	1	9	3	3	3
8		7	3	9	1	9	3	3	1
9		7	1	9	1	9	3	3	3
10		9	3	9	3	9	1	1	3
Parental genotype-cv. Fermer		7	3	7	3	7	3	3	3

Legend: 9 (HR) – highly resistant; 7 (R) – resistant; 5 (MR) – moderately resistant; 3 (MS) – moderately susceptible; 1 (S) – highly susceptible
Source: Authors' own elaboration

The rest of the influence factors „*genotype x year*"(varies from 3.62% for the trait Sed. value to 13.84% for – BMSI). For „*group x year* is low from 0.30% for FN to 11.73% for CP, and „*genotype x group x year* (lowest at DG-1.5%, and highest at BMSI -16.19%). The influence of the three studied factors genotype, group, „*year"* have minimal influence on the studied traits (Tables 5 and 6).

The induced mutations in wheat were also widespread employed to modify some protein subunits determining the grain quality (Kiribuchi-Otobe et al., 1998; Yasui et al., 1998; Maluszynski et al., 2001). In the hybrid-mutant lines obtained with the help of chemically mutagenic sodium azide in a concentration of 1 mM on F2 seeds (Mangova and Rachovska 2004) in IPGR, Sadovo found differences in several technological characteristics. The values are positive and statistically significant.

Virtuousness (%) is an important quality trait. It is related to the density of the grain and the way of structuring the starch-protein aggregates. The differences between floury and vitreous grains are significant. This trait is very variable and is directly dependent on the conditions of cultivation and, in particular, the diverse impact of the environment during pouring and ripening of the grain, the nitrogen content in the soil, soil and air moisture (Filipov, 2004). In our study, it was found that in the first year of the experiment, higher values of this trait were recorded in the tolerant to diseases group of

Table 5. Three-factor ANOVA of the traits for the two- year period

Source	Grain vitreousness			Sedimentation value			Fermentation number			Crude protein		
	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.
Genotype	5745.1	0.000	12.3	14950.5	0.000	38.9	82982.7	0.000	19.5	47.4	0.000	18.7
Group	5031.1	0.000	10.8	114.0	0.000	0.3	1.2	0.283	0.0	16.4	0.000	6.4
Year	18825.1	0.000	40.5	1665.0	0.000	4.3	176947.2	0.000	41.7	31.2	0.000	12.3
„Genotype x Group"	9882.7	0.000	21.2	19028.1	0.000	49.5	98203.8	0.000	23.1	57.8	0.000	22.8
„Genotype x Year"	1962.7	0.000	4.2	1391.1	0.000	3.6	31282.8	0.000	7.3	35.1	0.000	13.8
„Group x Year"	516.7	0.000	1.1	279.0	0.000	0.7	1267.5	0.000	0.3	29.7	0.000	11.7
„Genotype x Year x Group"	4437.1	0.000	9.5	905.1	0.000	2.3	33184.5	0.000	7.8	34.8	0.000	13.7
Error	68.0		0.1	72.0		0.1	82.0		0.02	0.7		0.2
Corrected Total	46468.3		100	38405.3		100	423951.7		100	253.3		100

Source: Authors' own elaboration

Table 6. Three-factor ANOVA of the traits for the two- year period

Source	Wet gluten yield			Gluten release			Bread making strong index			Dry gluten		
	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.	Type III Sum of Squares	Sig.	Infl.
Genotype	1093.4	0.000	34.5	423.7	0.000	13.4	1212.0	0.000	9.8	111.8	0.000	35.6
Group	482.8	0.000	15.2	362.2	0.000	11.5	226.8	0.000	1.8	33.9	0.000	10.8
Year	57.1	0.000	1.8	1219.2	0.000	38.7	4356.0	0.000	35.5	27.4	0.000	8.7
„Genotype x Group"	1013.1	0.000	32.0	356.4	0.000	11.3	2539.8	0.000	20.7	115.5	0.000	36.7
„Genotype x Year"	310.3	0.000	9.8	411.4	0.000	13.0	1698.6	0.000	13.8	16.3	0.000	5.2
„Group x Year"	117.6	0.000	3.7	79.2	0.000	2.5	180.0	0.000	1.4	3.4	0.000	1.1
„Genotype x Year x Group"	90.0	0.000	2.8	245.7	0.000	7.8	1986.6	0.000	16.1	4.7	0.000	1.5
Error	0.6		0.02	52.2		1.6	72.0		0.5	0.703		0.2
Corrected Total	3165.2		100	3150.3		100	12272.3		100	314.0		100

Source: Authors' own elaboration

mutant lines, with an average of 58.2%. In the group of Low stemmed mutant lines, low values were observed in lines № 3, 5, 7 and 8. In the second year, the VG was lower in both experimental treatments. The average % for the Resistant to diseases group of mutant lines was reported 47.7%, and for the Low stem mutant lines group 30.6%. The coefficient of variation in the Resistant to diseases group of mutant lines was 16.1% (medium), while for the Low stemmed group of mutant lines was significantly high 31.4% (Table 7).

The sedimentation value (cm^3) is a method for studying the swelling of ground wheat grain products in water and weak acid solutions, which accurately indicates the quality of gluten and dough. It was found that the sedimentation value correlated with the gluten content, gluten quality and

Table.7 Variance analysis of observed traits

Resistant group								
Line number	1. VG	2. SdV	3. FN	4. CP	5.WGC	6. RG	7. BMSI	8. DG
Farmer	50 b	59.5 d	84.5 cd	14.31 d	35.1 bc	13 b	51 gf	11.54 bcd
1	51.5 c	74.5 g	138.5 j	9.78 a	39.4 h	10.3 a	59 i	12.76 f
2	38.0 a	35 a	35 a	10.57 b	31.5 a	19.0 e	42 a	9.52 a
3	61.0 e	55 b	98 g	11.77 cde	37.6 f	16.5 d	48.5 e	11.99 cde
4	56.0 d	58 c	100.5 h	11.17 bc	35.5 c	17.3 d	46.5 d	11.41 bc
5	61.0 e	67.5 e	93 e	12.46 ef	36.9 de	16.3 d	49 ef	11.01 b
6	61.5 e	76 h	96.5 f	12.77 f	38.4 g	14.5 c	51.5 h	12.19 e
7	51.0 c	56 b	50.5 b	11.97 de	36.5 d	14.5 c	50 fg	11.98 cde
8	64.5 f	69 f	124.5 i	10.58 b	34.7 b	12.5 b	52 h	11.17 b
9	67.0 g	78 i	85.5 d	11.37 cd	37.6 ef	18.8 e	43.5 b	12.14 de
10	70.0 h	79 i	83.5 c	11.97 de	37.6 ef	20.5 f	45 c	11.59 bcde
Mean	58.2	64.8	90.6	11.44	36.6	16.0	48.7	11.58
Minimum	38	35	35	9.78	31.5	10.3	42	9.52
Maximum	70	79	138.5	12.77	39.4	20.5	59	12.76
St. dev.	3.0	13.9	30.6	0.93	2.2	3.1	4.9	0.89
St. error	9.4	4.4	9.7	0.29	0.7	1.0	1.6	0.28
Coeff.var,%	16.1	21.4	33.8	8.1	6.1	19.6	10.1	7.7
Low stemmed group								
Line number	1. VG	2. SdV	3. FN	4. CP	5.WGC	6. RG	7. BMSI	8. DG
Farmer	50 e	59.5 f	84.5 e	14.31 f	35.1 f	13 cde	51 d	11.54 c
1	65 h	82 i	147 h	12.97 de	37.9 gh	12.8 cd	52 de	12.85 e
2	60 g	89 k	152 i	13.47 e	37.5 g	12.0 c	53 e	12.13 d
3	30.5 b	48.5 d	45.5 c	11.27 ab	30.4 d	14.0 ef	49 c	9.61 b
4	50 f	84.5 j	99.5 f	12.97 de	38.6 hi	14.5 f	47.5 b	12.20 d
5	38.5 c	43 c	40 b	11.77 bc	28.4 c	16.0 g	45.5 a	9.02 b
6	48.5 e	75.5 g	72 d	13.57 e	34.6 f	13.0 cde	49.5 c	11.24 c
7	30.5 b	39.5 b	35.5 a	10.87 a	24.6 b	9.8 b	52.5 e	8.27 a
8	23.5 a	36 a	39.5 b	10.77 a	23.4 a	10.3 b	45.5 a	7.90 a
9	45.5 d	51.5 e	136.5 g	11.77 bc	31.2 e	8.5 a	69 f	9.54 b
10	60 g	79 h	136 g	12.37 cd	38.9 i	13.5 def	51 d	12.36 de
Mean	45.2	62.85	90.4	12.18	32.6	12.4	51.45	10.51
Minimum	23.5	36	35.5	10.77	23.4	8.5	45.5	7.90
Maximum	65	89	152	13.57	38.9	16	69	12.85
St. dev.	4.5	20.9	49.2	1.04	5.8	2.3	6.7	1.85
St. error	14.2	6.6	15.6	0.33	1.8	0.7	2.1	0.58
Coeff.var,%	31.4	33.3	54.5	8.5	17.9	18.6	13.1	17.6

*Mean values (in each column), followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiplerange test

Legend for traits names: VG – Grain vitreousness; SdV – Sedimentation value; FN-Fermentation number; CP – Crude protein; Wet gluten content – WGC; Gluten release – RG; Dry Gluten – DG; bread making strong index – BMSI

Source: Authors' own elaboration

bread volume depended on the protein content (Boyadjieva and Mangova, 2007; Pshenichnaya and Dorokhov 2017; Galushko, et. al., 2021).

Therefore, measuring this value is extremely useful for grain quality. It has also been established that sedimentation value is influenced by heredity, and is determined mostly by genotype, but environmental factors are also of significant importance (Lorenzo and Kronstad, 1987; Compbell et al., 1987; Grausgruber et al., 2000; Hruskova and Famera, 2003; Kibkalo, 2022). For the group of mutant lines Resistant to diseases, the mean values ranged from 35 cm^3 to 79 cm^3, with higher values in the first year of cultivation. For this period, six of the mutant lines exceeded the standard cultivar *Sadovo1*. For the low stemmed group of lines, five of the accessions had values above the standard for the study period, the mean values ranged significantly from 36 cm^3 to 89 cm^3, and remained high during the two-year period in mutant lines № 1, 2, 4, and 10. The coefficient of variation was high in both groups (21.4% in the resistant to diseases mutant lines and 33.3% in low stemmed mutants). The high values of sedimentation coefficient in the resistant to diseases mutant lines is a confirmation of the very high wet gluten content in this group (Table 7). Similar results were obtained in the studies by Rahemi et al. (2015) and Oğuz Bilgin et al. (2022)

The joint consideration of the trait sedimentation number together with the fermentation number of Pelshenke (min) (Pelshenke et al., 1953) of the magnitude of their values for the interdependence between the quality and quantity of gluten (Hermuth, 2019; Angelova et al., 2020; Galushko and Sokolenko, 2021). The results obtained in the present study show high values of this trait in the first crop year. In the group of resistant to diseases eight mutant lines had higher values than the parental variety. On average, the maximum value for the mutant resistant to diseases was 132.2 min in the first year of cultivation, and 125.5 min for the low stemmed lines. For the second year, the values of the trait were lower below 100 min for both groups. In the resistant to diseases mutant lines only one line (No 8) was above the parental *cv. Fermer*, and in the group of low stemmed lines four. The mean values for the study period were similar for both experimental treatments (90.6 min – in resistant to diseases mutant lines and 90.4 min in low stemmed mutant lines). The coefficient of variation for this trait was high for both groups (33.8 % – in the resistant to diseases mutant lines and 54.5% – in low stemmed mutant) (Table 7).

The content of crude protein in wheat is the most important quality criteria. It plays key roles in determining the quality of wheat grain, and has a strong influence on the end-use products obtained from it (Balyan et al., 2013). For crude protein, the results obtained did not exceed the parental *cv. Fermer* (14.31%). Mean values ranged from 9.78% to 12.77% for resistant to diseases mutant lines and from 10.77% to 13.57% for low stemmed group. The coefficient of variation was low in both groups of mutant lines (8.1% for the disease resistant group of mutant lines and 8.5% for low stemmed group) (Table 7). The positive relationship between crude protein and wet gluten yield is confirmed by the studies of several authors Balyan et al. (2013), Laidig et al. (2017).

The quantity and quality of gluten are considered the most important quality parameters of wheat flour (grain). The gluten-forming proteins play an important role in the baking quality of wheat. They improve the extensibility and elasticity of the dough, as well as the ability to absorb water (Wieser, 2007). The content of gluten is directly related to the content of protein in the grain, which is strongly influenced by the growing conditions, and especially, by the climatic conditions. However, wheat genotype appears to be the most important factor affecting gluten quality characteristics (Simic et al., 2006). In our study, the mean wet gluten content-WGC values of eight of the group of Resistant mutant lines exceeded those of the parental *cv. Fermer* (35.1%) (Table 7). The coefficient of variation for the group was low – 6.1%. For the Low stemmed group, six of the lines had period means below the parental genotype. In this group, the variance of the trait is average (17.9%). The gluten release (Table 7) in both studied groups had a mean variation (19.6% resistant to diseases and 18.6% for low stemmed). The bread making strength index (Table 7) also had a mean variation (10.1% for tolerant to diseases and 13.1% for low stemmed). For dry gluten, a low variation of values (7.7%) was observed in the resistant to diseases mutant lines, but it was significant (average) – 17.6% in the group of low stemmed mutants. The gluten content (Table 7) is directly related to the protein content of the grain, which is strongly influenced by wheat genotype and growing conditions (Hajas et al., 2018; Kenzhebayeva et al., 2018; Öztürk et al., 2020).

Molecular analyses

1. RGA-profiling of M5 mutant lines

In total 94 combinations of 3 degenerate NBS primers (NBS2, NBS3 and NBS12) with 36 SCoT primers were tested for amplification of polymorphic fragments in bulk samples of ten M5 lines (10 Low stemmed, 10 Resistant and 10 Susceptible each) (Fig. 1). Only five of the tested primer combinations produced polymorphic fragments in some of the bulk samples. The polymorphic fragments were confirmed by PCR amplification in individual M5 lines (Fig. 2). The AFLP based NBS-profiling method has been efficient for targeting RGA genes in potato (Calenge et al., 2005; Verzaux et al., 2011). Moreover, it has been demonstrated that NBS-profiling with

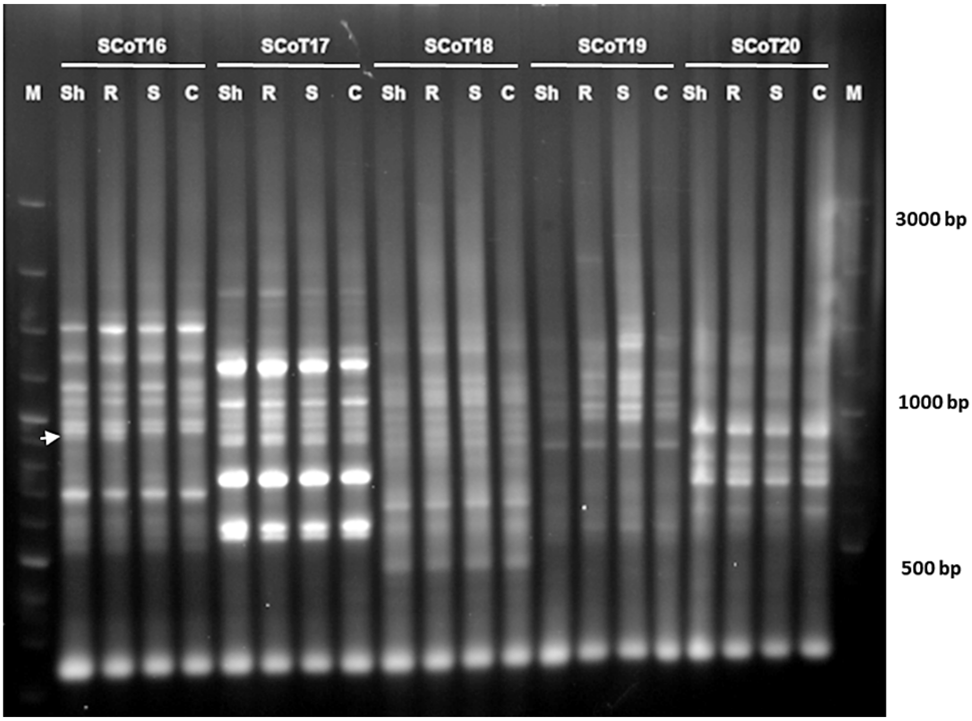

Fig. 1. Example of PCR screening using combinations of different SCoT primers with the degenerative primer NBS12. Sh – bulk sample of 10 low stemmed M_5 lines, R – bulk sample of 10 Resistant M_5 lines, S – bulk sample of 10 Susceptible M_5 lines, C – control *cv. Fermer* **M – "100 bp +" DNA ladder. White arrow on the left shows polymorphic fragment amplified in the „R – bulk sample with primers NBS12/SCoT16**
Source: Authors' own elaboration

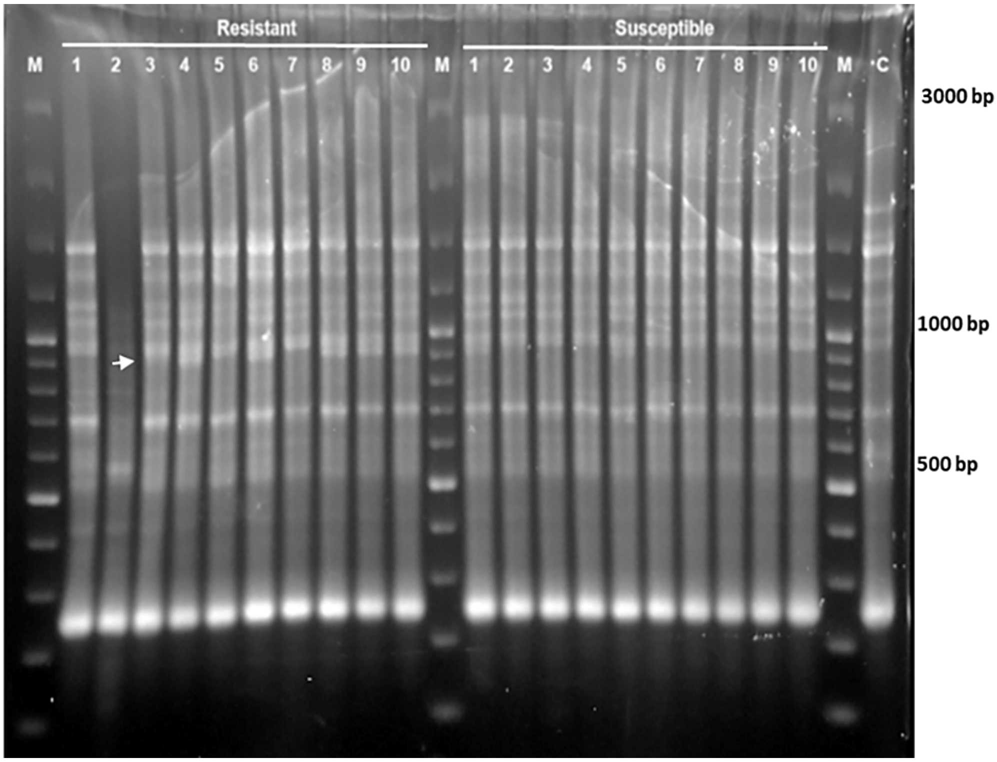

Fig. 2. Electrophoresis separation of PCR products amplified in 10 Resistant and 10 Susceptible M_5 lines with the primer combination NBS12/SCoT16 C – control *cv. Fermer* **M – "100 bp +" DNA ladder. White arrow on the left shows polymorphic fragment amplified in some of the resistant (tolerant) M_5 lines**
Source: Authors' own elaboration

the same NBS primers, designed for potato, allowed identification of polymorphic amplified fragments of RGA genes in wheat (Tufan et al., 2019). Therefore, the newly developed RGA-profiling method, presented here is a promising approach to detect mutations in disease resistance genes.

2. Microsatellite (SSR) analysis

Thirty mutant M5 lines (10 Low stemmed, 10 Resistant and 10 Susceptible to fungal pathogens) were analysed at 6 SSR loci (Xgwm389, Xgwm46, Xgwm513, Xgwm539, Xwmc44 и Xwmc607), that were previously reported to be linked to fungal pathogens resistance genes. On the locus Xgwm513 one sample ("Low stemmed №2") showed null allele ("0") and the other samples showed segregation of the two parental alleles. In all other analysed loci, mutant alleles were observed in some of the M5 lines (Table 8), suggesting the presence of induced mutations in the vicinity or inside the resistance genes. The SSR locus Xgwm389 has been mapped on chromosome 3BS at genetic distance of 2 cM from the yellow rust resistance gene Yr57 (Randhawa et al., 2015). Notably, in the locus Xgwm389 lack of amplification product is observed in 60% of Susceptible and 50% of Low stemmed lines while only one of the tolerant lines (10%) shows null allele at this locus (Table 8). This result suggests

Table 8. Microsatellite analysis of M5 mutant wheat lines.

Sample/ SSR alleles (Resistance genes)	Xgwm389 (Yr57, FHB, Septoria)	Xgwm46 (FHB)	Xgwm513 (Yr 62)	Xgwm539 (FHB)	Xwmc44 (Lr19)	Xwmc607 (FHB)
Low stemmed 1	0	177	142	137	262/264	0
Low stemmed 2	0	0	142	137	262/264	0
Low stemmed 3	142	177	144	137	206/208	0
Low stemmed 4	0	177	142	137	262/264	0
Low stemmed 5	142	177	144	137	206/208	0
Low stemmed 6	276	177	0	137	262/264	0
Low stemmed 7	142	177	144	137	206	0
Low stemmed 8	142	177	144	137	206	0
Low stemmed 9	0	177	142	137	0	0
Low stemmed 10	0	177	142	137	208/262/264	0
Resistant 1	120	177	142	137	262/264	0
Resistant 2	142	173	144	137/150/152	206/208	148
Resistant 3	121	173/175	142	137	206/208	146
Resistant 4	121	174/175	142	137	206/208	0
Resistant 5	121	177	144	137	208/256/258	144/146
Resistant 6	121	177	142	137	206/208/262/264	146
Resistant 7	0	177	142	137	206/208	0
Resistant 8	121	177	142	137	206/208	146
Resistant 9	121	177	142	0	206/208	146
Resistant 10	121	176/177	142	137	206	144
Susceptible 1	0	177	144	137	206	175
Susceptible 2	0	173	142	137	206	146
Susceptible 3	0	176/177	142	137	206	146
Susceptible 4	120	177	142	137	262/264	0
Susceptible 5	0	177	142	0	262	0
Susceptible 6	121	177	142	137	262/264	0
Susceptible 7	121	177/179	142/144	137	206/262	144
Susceptible 8	121	177	142	137	206/262	0
Susceptible 9	0	177	142	137	206	0
Susceptible 10	0	177	142	137/139	206	0
St Fermer 2	121	176/177	142/144	137	206/262/264	137/139/153/155
St Fermer 1-5 (bulk)	121	176/177	142	137	206/262/264	127/137/139/153/155

The length of mutant SSR alleles in bp is indicated in red. Lack of amplified SSR fragment is shown as „0".

Source: Authors' own elaboration

a possible deletion of the chromosome region containing this SSR locus, as well as genes conferring multiple fungal pathogen resistances in the lines showing null alleles. However, further research is needed to confirm this hypothesis.

Conclusions

The study of the mutant lines shows:

– mutant lines with low stem № 4, 5, 9 and 10 and № 3 and 8 (Resistant to disease) possess high net photosynthetic rate and high chlorophyll content index.

Higher yielding lines were obtained compared to the parent variety Fermer, with the highest yield for the period at № 3, 7 and 9 (low-stemmed group) and at № 2, 5 and 8 (disease-resistant group).

Influence of genotype was the strongest for the trait DG (35.6%). For the factor year (environmental conditions), the influence was strongest for the trait FN (41.74%), followed by VG (40.51%), RG (38.7%) and BMSI (35.5%).

The factors genus*year, group*year, genus*group*year had minimal influence on the studied traits.

Presented results demonstrate that the applied doses of Gamma ray induced useful variation in wheat *cv. Fermer* at both phenotype and DNA level.

The molecular characterization of M5 lines using SSR and other DNA markers identified mutant alleles in some of the selected lines thus confirming that the observed phenotype differences reflect the changes at DNA level.

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References

Adamu A. & Aliyu, H. (2007). Morphogical effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). *Sci. World J., 2*(4). http://doi/10.4314/swj.v2i4.51755 .

Angelova, T., Dimitrov, E. & Uhr, Z. (2020). Evaluation of selection materials of ordinary winter wheat by express methods. *Proceedings of the National Scientific and Technical Conference with International Participation Ecology and Health*, 25 – 26 June 2020 Plovdiv, 6 – 12.

Arumuganathan, K. & Earle, E. (1991) Nuclear DNA content of some important plant species. *Plant. Mol. Biol Rep*., 9, 208 – 218.

Balyan, H. S., Gupta, P. K., Kumar, S., Dhariwal, R., Jaiswal, V., Tyagi, S. & Kumari, S. (2013). Genetic improvement of grain protein content and other health-related constituents of wheat grain. *Plant Breeding*, *132*(5), 446 – 457. http://doi.org/10.1111/pbr.12047.

Borzouei, A., Kafi, M., Khazaei, H., Naseriyan, B. & Majdabadi, A. (2010). Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pak. J. Bot*., *42*(4), 2281 – 2290.

Boyadjieva, D. & Mangova, M. (2007). Study on wheat germplasm (*T. aestivum*L.) for breeding of grain quality. *Bulg. J. Agric. Sci, 13*(3), 273 – 280.

Boyd, L. A., Smith, P. H. & Hart, N. (2006). Mutants in wheat showing multipathogen resistance to biotrophic fungal pathogens. *Plant Pathology, 55*(4), 475 – 484. https://doi.org/10.1111/j.1365-3059.2006.01402.x.

Calenge, F., Van der Linden, C. G., Van de Weg, E., Schouten, H. J., Van Arkel, G., Denancé, C. & Durel, C. E. (2005). Resistance gene analogues identified through the NBS-profiling method map close to major genes and QTL for disease resistance in apple. *Theoretical and Applied Genetics*, *110*, 660 – 668. https://doi.org/10.1007/s00122-004-1891-6.

Collard, B. C. Y. & Mackill, D. J. (2009). Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. *Plant Molecular Biology Reporter, 27*, 86 – 93. https://doi.org/10.1007/s11105-008-0060-5.

Compbell, W. P., Wrigley, C. W., Cressy, P. J. & Slack, C. R. (1987). Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. *Cereal Chemistry, 64*, 293 – 299.

Daryanto, S., Wang, L. & Jacinthe, P. A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. Agric. *Water Management 179*, 18 – 33. https://doi.org/10.1016/j.agwat.2016.04.022.

Dimov, A. (1988). Wheat resistance to brown rust, stem rust, powdery mildew and its use in breeding. *Doctoral Dissertation, IPGR, Sadovo* (Bg).

Duncan, D. (1955). Multiple Range and Multiple F-tests. *Biometrics, 11*(1), 1 – 42.

Figueroa, M., Hammond-Kosack, K. & Solomon, P. (2018). A review of wheat diseases – a field perspective. *Molecular plant pathology, 19*(6), 1523 – 1536. https://doi.org/10.1111/mpp.12618.

Filipov, H. (2004). Evaluation of wheat quality by grain appearance. *Academic publishing house of Agrarian University*, Plovdiv.

Galushko, N. A. & Sokolenko, N. I. (2021). The most important selection criteria for grain quality in winter wheat breeding of the Federal State Budgetary Scientific Institution „North Caucasian Federal Scientific Agrarian Center“. *Tauride Bulletin of Agrarian Science, 28*(4), 50 – 57 (Ru). DOI: 10.33952/2542-0720-2021-4-28-50-57.

Galushko, N., Taradina, D. & I. Donets, I. (2021). Grain quality of new genotypes of winter wheat. *Agricultural journal, 14*(3), 18 – 24 (Ru). https://doi.org/10.25930/2687-1254/003.3.14.2021.

Grausgruber, H., Oberfoster, M., Werteker, M., Ruckenbauer, P. & Vollman, J. (2000). Stability off quality traits in Austrian–grown winter wheats. *Field Crops Research, 66*, 257 – 267. https://doi.org/10.1016/S0378-4290(00)00079-4.

Gupta, P., Balyan, H. S., Edwards, K. J., Isaac, P., Korzun, V., Röder, M. S. & Leroy, P. (2002). Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and applied genetics*, *105*, 413 – 422. https://doi:10.1007/s00122-002-0865-9.

Hajas, L., Scherf, K. A., Török, K., Bugyi, Z., Schall, E., Poms, R. E. & Tömösközi, S. (2018). Variation in protein composition among wheat (*Triticum aestivum L.*) cultivars to identify cultivars suitable as reference material for wheat gluten analysis. *Food chemistry, 267*, 387 – 394. https://doi.org/10.1016/j.foodchem.2017.05.005.

Hayit, T., Erbay, H., Varĉın, F., Hayit, F. & Akci, N. (2021). Determination of the severity level of yellow rust disease in wheat by using convolutional neural networks. *J. Plant Pathol., 103*(3), 923 – 934. https://doi.org/10.1007/s42161-021-00886-2.

Hermuth, J., Leišova-Svobodova, L., Bradova, J., Kosová, K., Dvořáček, V., Prášil, I. & Dotlačil, L. (2019) Genetic characterization and evaluation of twenty Chinese winter wheat cultivars as potential sources of new diversity for breeding. *Czech J. Genet. Plant Breed, 55*(1), 8 – 14. (Cz). https://doi.org/10.17221/192/2017-CJGPB**.**

Hruskova, M. & Famera, O. (2003). Prediction of wheat and flour Zeleny sedimentation value using NIR technique. *Czech Journal of Food Sciences, 21*, 91 – 96. https://DOI:10.17221/3482-CJFS.

Kenzhebayeva, S., Doktyrbay, G., Sarsu, F., Omirbekova, N., Abekova, A. & Tashenev, D. (2018). Mutant Resources of Spring Wheat to Improve Grain Quality and Morphology. In: *Fahad S., Basir A. and Adnan M. (eds) Global Wheat Production, IntechOpan*, London, UK, 165 – 181.

Kibkalo, I. (2022). Effectiveness of and perspectives for the sedimentation analysis method in grain quality evaluation in various cereal crops for breeding purposes. *Plants, 11*(13), 1640. https://doi.org/10.3390/plants11131640.

Kiribuchi-Otobe, C., Yanagisawa, T., Yamaguchi, I. & Yoshida, H. (1998). Wheat mutant with waxy starch showing stable hot paste viscosity. *Cereal Chemistry., 75,* 671 – 672. http://dx.doi.org/10.1094/CCHEM.1998.75.5.671.

Knott, D. R. (1991). What determines the success of mutation breeding? *IAEA, 5*(1), 111 – 118.

Kodym, A. & Afza, R. (2003). Plant Functional Genomics. *Springer, Berlin, Germany. Physical and chemical mutagenesis*, 189 – 203. DOI: 10.1385/1-59259-413-1:189.

Konzak, C. F. (1987). Mutations and Mutation Breeding. In: *E.G. Heyne (ed.), Wheat and Wheat Improvement, Second Edition*, 428 – 443.

Kozgar, M. I., Khan, S. & Wani, M. R. (2012). Variability and correlations studies for total iron and manganese contents of chickpea (*Cicer arietinum* L.) high yielding mutants. *Am. J. Food Technol., 7*, 437 – 444. https://doi.org/10.3923/ajft.2012.437.444**.**

Krivchenko, V. (1980). Study of resistance of cereal crops to powdery mildew. *In methodological guidelines*. Leningrad (Ru).

Laidig, F., Piepho, H. P., Rentel, D., Drobek, T., Meyer, U. & Huesken, A. (2017). Breeding progress, environmental variation and correlation of winter wheat yield and quality traits in German official variety trials and on-farm during 1983 – 2014. *Theoretical and Applied Genetics*, *130*, 223 – 245. https://doi.org/10.1007/s00122-016-2810-3**.**

Le Roux Marlon-Schylor, L., Burger, N., Francois, V., Vlok Maré, Kunert, K. J., Cullis Christopher, A. & Botha, A. (2020). Wheat Line "RYNO3936" Is Associated With Delayed Water Stress-Induced Leaf Senescence and Rapid Water-Deficit Stress Recovery. *Frontier in Plant Sciences, 11*, 1053. https://doi.org/10.3389/fpls.2020.01053.

Lidansky, T. (1988). Statistical methods in biology and agriculture. *Zemizdat*, 374 Sofia.

Lorenzo, A. & Kronstad, W. E. (1987). Reliability of two laboratory techniques to predict bread wheat protein quality in nontraditional growing areas. *Crop Sci., 27*, 247 – 252.

Maluszynski, M., Szareiko, I. & Maluszinska, J. (2001). Induced mutations in wheat. The world wheat book: A history of wheat breeding. *Lavoisier, Londres-ParisNew York*, 939 – 977.

Mangova, M. & Rachovska, G. (2004) Technological characteristics of newly developed mutant common winter wheat lines. *Plant soil environ.*, *50*(2), 84 – 87.

Marcussen, T., Sandve, S. R., Heier, L., Spannagl, M., Pfeifer, M., Jakobsen, K. S., Wulff, B. B., Steuernagel, B., Mayer, K. F. & Olsen, O. A. (2014). Ancient hybridizations among the ancestral genomes of bread wheat. *Science, 345*, 6194. DOI: 10.1126/science.1250092.

Mba, C. (2013) Induced mutations unleash the potentials ofplant genetic resources for food and agriculture. *Agronomy,3*(1), 200 – 231. doi.org/10.3390/agronomy3010200.

Mihova, S., Stoyanov, I. & Iliev, I. (1990). New lines of winter soft wheat with complexdisease resistance. *Plant Sciences, XXVII*(3), 39 – 44.

Morgun, V. V. & Logvinenko V. F. (1995). Mutational selection of wheat. Kyiv, *Naukova dumka,* 652. (Ru).

Mostafa, G. G. (2011) Effect of Sodium Azide on the Grovvth and Variability Induction in. *Int. J. Plant Breed. Genet.,5*, 76 – 85. http://DOI:10.3923/ijpbg.2011.76.85.

Oğuz Bilgin, Alpay Balkan, Damla Balaban Göçmen and Başer İs. (2022). Elicitation of high-quality and yield in bread wheat (triticum aestivum l.) mutant lines improved by gamma rays**.** *Int. J. Adv. Res.* 10(04), 723-732: ISSN: 2320-5407; http://dx.doi.org/10.21474/IJAR01/14607

Oladosu, Y., Mohd, Y. R., Abdullah, N., Hussin, Gh., Ramli, A., Rahim, H. A., Miah, G. & Magaji, U. (2016). Principle and application of plant mutagenesis in crop improvement: a review, *Biotechnology & Biotechnological Equipment*, *30*(1), 1 – 16. https://doi.org/10.1080/13102818.2015.1087333.

Öztürk, İ., Şen, A., Kılıç, T. H. & Şili, Ş. (2020). Selection of advanced mutant wheat (*Triticum aestivum* L.) lines based on yield and quality parameters. *Turkish Journal of Agricultural and Natural Sciences*, *7*, 87 – 95. https://doi.org/10.30910/turkjans.680019.

Pelschenke, P., Hampel, G., Schafer, W., Kleber, W., Ludecke, H. & Heuer, E. (1953). *Methodenbuch, Band XV*.

Pshenichnaya, I. A. & Dorokhov, B. A. (2017). The useof the sedimentation method in assessing the quality of grain lines of a breeding nursery. *Central Scientific Bulletin, 2*(22), 36 – 37.

Pumpyanskiy, A. Y. (1971). Tekhnologicheskie svoystva myagkikh pshenits. *L. Kolos, 320*.

Rachovska, G. (2010). Winter common wheat cultivar „Fermer". *Plant Science*, 47(4), 357–362.

Rahemi, M. R., Yamchi, A., Navabpour, S. & Soltanloo, H. (2015). The effect of Gamma ray on baking properties of the bread wheat Roshan. *In Biological Forum, 7*(1), 1616 – 1621. ISSN No. (Print): 0975-1130.

Randhawa, M. S., Bariana, H. S., Mago, R. & Bansal, U. K. (2015) Mapping of a new stripe rust resistance locus Yr57 on chromosome 3BS of wheat. *Molecular Breeding 35*, 65. https://doi.org/10.1007/s11032-015-0270-0.

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P. & Ganal, M. W. (1998). A microsatellite map of wheat. *Genetics*, *149*(4), 2007 – 2023. https://doi.org/10.1093/genetics/149.4.2007.

Sakin, M. A., Yıldırım, A. & Gökmen, S. (2004). The evaluation of agronomic traits of durum wheat (Triticum durum Desf.) mutants. *Pak. J. Biol. Sci.*, *7*(4), 571 – 576.

Sakin, M. A., Yıldırım, A. & Gökmen, S. (2005). Determining some yield and quality characteristics of mutants induced from a durum wheat (*Triticum durum* Desf.) cultivar. *Turk. J. Agric. For., 29,* 61 – 67.

Sanin, S., Korneva, L. & Polyakov, T. (2015). Forecasting the risk of epiphytotics developing of Septoria leaf and ear on wheat. *Plant Protection and Quarantine, Volume 3*, 33-36. (Ru)

Semenov, M. A. & Stratonovitch, P. (2013). Designing high-yielding wheat ideotypes for a changing climate. *Food Energy Secure, 3*, 185 – 360.

Shah, T. M., Atta, B. M., Mirza, J. I. & Haq, M. A. (2012). Radio-Sensitivity of various chickpea genotypes in M1 generation II-field studies. *Pak. J. Bot*., *44*(2), 631 – 634.

Sikora, P., Chawade, A., Larsson, M., Olsson, J. & Olsson, O. (2011). Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *Int. J. Plant Genom*, 314829. doi: 10.1155/2011/314829.

Simic, G., Horvat, D., Jurkovic, Z., Drezner, G., Novoselovic, D. & Dvojkovic, K. (2006). The genotype effect on the ratio of wet gluten content to total wheat grain protein. *Journal of Central European Agriculture* 7(1), 13 – 18.

Simova-Stoilova, L., Pecheva, D. & Kirova, E. (2020). Drought stress response in winter wheat varieties –changes in leaf proteins and proteolytic activities. *Acta Botanica Croatica, 79*(2), 121 – 130. https://doi.org/10.37427/botcro-2020-018.

Thapa, C. B. (2004). Effect of acute exposure of gamma rays on seed germination and seedling growth of Pinus kesiya Gord and P. wallichiana A. B. Jacks*. Our Nature,* 2, 13 – 17. https://doi.org/10.3126/on.v2i1.318.

Tomlekova, N. B. (2010). Induced Mutagenesis for Crop Improvement. *Pl. Mut. Rep, 2*, 4 – 27. INIS-XA--11I0997.

Tufan, H. A., Göçmen Taşkin, B., Maccormack, R., Boyd, L. A., Kaya, Z. & Türet, M. (2019). The utility of NBS-profiling for characterization of yellow rust resistance in an F 6 durum wheat population. *Journal of Genetics*, *98*, 1 – 12. https://doi.org/10.1007/s12041-019-1143-9**.**

Uhr, Zl., Angelova, T., Dimitrov, E. & Dragov, R. (2023). Technological evaluation and determination of the traits stability in breeding materials in the early generations. *Bulgarian Journal of Crop Science, 60*(5), 17 – 27. https://doi.org/10.61308/DBOD8638.

Verzaux, E., Budding, D., de Vetten, N., Niks, R. E., Vleeshouwers, V. G., van der Vossen, E. A. & Visser, R. G. (2011). High resolution mapping of a novel late blight resistance gene Rpi-avl1, from the wild Bolivian species Solanum avilesii. *American journal of potato research*, *88*, 511 – 519. https://doi.org/10.1007/s12230-011-9218-z.

Vitanova, M., & Rachovska, G. (2009). Resistance of mutant and hybrid-mutant germplasm to *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*. *Field Crops Studies*, V(2), 239–244

Wieser, H. (2007). Chemistry of gluten proteins*. Food Microbiol 24*, 115 – 119.

Yasui T., Sasaki J. & Matsuki, J. (1998). Waxy bread wheat mutants, K107W × 1 and K107W × 2, have a new null allele on Wx-D1 locus. *Japanese Journal of Breeding, 48*, 405 – 407. https://doi.org/10.1270/jsbbs1951.48.405.

https://apps.fas.usda.gov/psdonline/circulars/production.pdf

https://bds-bg.org/bg/project/show/bds:proj:17686

https://bds-bg.org/bg/project/show/bds:proj:17689

https://www.iso.org/standard/35864.html

https://www.iso.org/standard/39145.html

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