Chapter 12 – Screening of mutant wheat lines to resistance for *Fusarium* head blight and using SSR markers for detecting DNA polymorphisms

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Abstract

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum,* is one of the most damaging diseases of wheat. Breeding durable disease resistance varieties relies largely on continually introgressing new resistance genes, especially the genes with different defense mechanisms, into adapted varieties. The main objective of this research was to evaluate three spring wheat varieties grown in Kazakhstan and the mutant lines of spring wheat (M_3 generations) developed on their genetic background by irradiation treatment (100 and 200 γ rays) for their resistance to FHB and to use PCR-based DNA markers, such as simple sequence repeats (SSRs) markers to investigate genetic diversity in wheat germplasm. Significant differences in tolerance phenotype to FHB were found among wheat varieties and mutant lines. Comparing parent cv. Zhenis M_3 mutant lines, No. 6(15), No. 6(16) and No. 22(1) had the highest means of *Fusarium* resistance after 15 days of inoculation. M_3 mutant lines, No. 89(4) developed on base of cv. Almaken can be identified as FHB tolerant. Three M_3 mutant lines obtained on genetic background of cv. Erithrospermum-35, namely No. 110(1), No. 129(3) and No. 150(5) had higher level of *Fusarium* tolerance than the parent variety. A total of 21 SSRs loci were used to determine genetic diversity among M_3 spring wheat lines and non-mutagenized plants. Changes in the number of alleles on chromosomes 3A, 3DL, 3BS, 7A applying SSR markers *Barc12*, *Barc42, Gwm533* and *Gwm681* were determined for the mutant lines Zhenis No. 5(1), No. 25(9), No. 51(8), No. 5(4), No. 25(12), No. 43(4), No. 16(9), and No. 16(1).

Keywords: mutant, wheat, resistance, *Fusarium* head blight, SSR markers

12.1 Introduction

Wheat is the second most important crop globally with a total estimated world production of ~663 million tonnes during 2011-2012 (FAO, 2013). Currently, about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum

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wheat. The region of Central Asia is one of the most important wheat growing areas in the world. Kazakhstan is one of the largest wheat producers in Central Asia. In Kazakhstan wheat is the first food crop. The bread wheat (*Triticum aestivum* L.) accounts 91% and durum wheat is only 9% of the total area of 13 million ha. Spring wheat occupies 95% of the total wheat area in Kazakhstan.

To meet global food demand by 2020, wheat production should be increased by about 40%. Since its origin around 8,000 BC, hexaploid bread wheat (*Triticum aestivum* L.) has been subject to intense selection aimed at developing improved, high-yielding varieties that are adapted to diverse environmental conditions and agricultural practices.

Breeding improvement of many agronomical traits requires genetic variation and these components of variation must be separable from non-genetic effects. To be useful, resistance advances must be achievable without negative effects on other important traits such as yield. Hence breeding wheat genotypes with a diverse genetic base is a factor to achieve a level of self-sufficiency and sustainability. It is widely believed that the genetic diversity of major crops, including bread wheat, has suffered an overall reduction with time, primarily as a consequence of the domestication processes and, more recently, as a result of the recurrent use of adapted germplasm and the adoption of breeding schemes not favoring wide genetic recombination (Akfirat and Uncuoglu, 2013; Donini *et al.*, 2000; Reif *et al.*, 2005).

12.1.1 The use of mutagens for inducing genetic variation

Genetic variability is of prime importance for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm (Akfirat and Uncuoglu, 2013). Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms (Maluszynski and Szarejko, 2003; Parry *et al.*, 2009). Exploiting natural or induced genetic diversity is a proven strategy in the improvement of all major food crops, and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Parry *et al.*, 2009).

The use of physical mutagens, like X-rays, gamma and chemical mutagens for inducing variation, is well established. Over the past 70 years, more than 2,500 varieties derived from mutagenesis programmes have been released, as listed in the IAEA/FAO mutant variety database, including 534 rice lines, 205 wheat lines, and 71 maize lines (MVD, 2013) These induced mutation help to develop many agronomical important traits, such as shorter growing period, suitable for rotation, increased tolerance or resistance to abiotic and biotic stresses use in major crops such as wheat, rice, barley, cotton, peanuts and beans (Kenzhebayeva *et al.*, 2013; Maluszynski and Kasha, 2002; Singh and Balyan, 2009).

12.1.2 *Fusarium* head blight

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a fungal disease that mostly affects wheat, corn and barley, including other crops. It was first discovered in the United Kingdom in the year 1884, where because of the chalky, lifeless appearance of the infected kernels, it was called wheat scab, and later tombstone disease (Clear and Patrick, 2010). FHB can cause significant yield losses due to floret sterility and reduced grain weight, as well as quality reductions due to the production of mycotoxins. The occurrence of such natural contaminants in wheat (and other cereals) is of great concern because their presence in feeds and foods is often associated with chronic or acute mycotoxicoses in livestock and could threaten human health (Visconti and Pascale, 2010). The control of FHB with the use of management strategies like, crop rotation, tillage and the application of fungicides produces only limited results (Paul *et al.*, 2005). The most effective strategy for controlling FHB in wheat is through the development of resistant varieties. Resistance to FHB exhibits quantitative variation and its inheritance involves several loci on different chromosomes (Kolb *et al.*, 2001). Genotype × environment interaction complicates the phenotypic evaluation of FHB resistance and makes screening of FHB resistance laborious, time consuming and costly (Rudd *et al.*, 2001).

12.1.3 Molecular markers for detecting DNA polymorphism, genotype identification and genetic diversity

Molecular markers based on PCR methods, such as simple sequence repeats (SSRs) or microsatellites, have provided a powerful approach to analyze genetic relationships among accessions in many crop species. Molecular markers are a useful complement to morphological and physiological characterization of varieties because they are plentiful, independent of plant tissue or environmental effects, and allow variety identification very early in plant development (Akfirat and Uncuoglu, 2013; Manifesto *et al.*, 2001; Salem *et al.*, 2008). Molecular characterization of varieties is also useful to evaluate potential genetic erosion, i.e. a reduction of genetic diversity along the breeding process. DNA-based markers are particularly useful in wheat and other crops with an apparent narrow genetic background.

Microsatellites are tandem repeats of short DNA sequences (2-6 bp), which are highly polymorphic in various animal and plant species. The analysis of microsatellites based on PCR is much easier to perform than restriction fragment length polymorphism analysis and is highly amenable to automation. In most cases, microsatellites are inherited in a codominant manner and are chromosome-specific. Microsatellites have been successfully used to construct genetic maps (Huang *et al.*, 2002; Roder *et al.*, 1998), to identify alien chromatin (Francis *et al.*, 1995) and to map agronomically important genes (Barakat *et al.*, 2012; Korzun *et al.*, 1998; Malik *et al.*, 2013).

12.1.4 Study aims

The aim of this work was to verify the susceptibility/tolerance phenotype to FHB of three spring wheat varieties 'Zhenis', 'Almaken' and 'Erithrospermum-35' grown in Kazakhstan and advanced

mutant lines which were developed on their genetic background by irradiation treatment (100 and 200 Gy gamma rays). The molecular characterization and genetic diversity of spring wheat genotypes was investigated using 21 SSRs primers. We described screening results on the developed more productive mutant M_3 spring wheat lines compared to non-mutagenized plants for tolerance to FHB using wheat microsatellite markers for molecular genotyping. The objectives of this work were: (1) to develop M_3 mutant lines of spring wheat for components of productivity, (2) to evaluate the genetic variation of tolerance to FHB between these M_3 mutant lines and spring wheat varieties, and (3) to evaluate the genetic relationships between M_3 spring wheat lines and non-mutagenized varieties.

12.2 Material and methods

12.2.1 The plant material

The field experiment was carried out in 2011. The plant material used in the study consisted of 138 M_3 mutant lines of spring wheat based on the cultivars Zhenis, Almaken and Erithrospermum-35 and non-mutagenized plants. For screening resistance to FHB germplasm was planted in pots in the greenhouse.

Based on radiation sensitivity studies, a dose of 100 and 200 Gy was chosen to irradiate of the Zhenis variety of spring wheat (*Triticum aestivum* L.) to obtain M_3 mutant lines. Initially, irradiation of 1000 seeds was performed in an ionizing device (PXM-γ 20, ^{60}Co gamma rays) at the Kazakh Nuclear Center. The plants were grown in field experimental plots. The selection of high-yielding potential mutant lines was made under field conditions. Selection of individual plants was done every generation from M_3 taking into account the following yield parameters: grain yield per plant, greater number of grain per main spike, greater weight of grains per main spike compared to the parental variety. The best genotypes were chosen according to their elements of yield.

12.2.2 *Fusarium* resistance testing

Spores isolated from *F. graminearum* were used for inoculation. Macroconidia of *F. graminearum* were prepared as described by Snijders and Van Eeuwijk (1991) and Buerstmayr *et al.* (2000, 2002). A mixture of wheat and oat kernels (3:1) was soaked overnight in water and then autoclaved and inoculated. The mixture was then incubated for 2 weeks at 25 °C followed by 3 weeks at 5 °C in the dark, leading to production of macroconidia. Macroconidia were washed off the colonized grains with deionised water. The concentration of the conidia was calculated with a Bürker-Türk counting chamber under the microscope. A concentration of 50,000 conidia/ml was used and stored at -80 °C for the inoculation procedure (Buerstmayr *at al.,* 2002).

The plants were artificially inoculated by fungus suspension. The first inoculation of the ears of mutant lines and non-mutagenized plants was made at the flowering stage in a controlled

greenhouse, at 20 °C on 12 June 2012. They were inoculated at a time when about 50% of the ears were flowering. This was seen when the anthers appeared from the middle of the ears. Using a motor driven back-pack sprayer, 5 ml of inoculum was sprayed on the heads. After inoculation heads were covered by plastic bags for 24 h in order to ensure high humidity. Inoculations were carried out in the evening. After three days the same procedure was repeated twice.

12.2.3 Scoring of *Fusarium* Head Blight severity

For scoring we assumed an average head-size of 24-28 spikelets per spike as the basis for estimating FHB severity, e.g. an average of one infected spikelet per spike was rated as 5% FHB severity. Disease symptoms were recorded on the 10, 14, 17, 21 and 24 days after inoculation. In each plot the percentage of visually infected spikelets was estimated according to a linear scale 0 to 100% infected spikelets on a whole plot basis. The *Fusarium* severity level was calculated as the average percent of *Fusarium* damaged spikelets per ear.

12.2.4 DNA extraction and single sequence repeat primer sources

Genomic DNA was isolated from young leaves using the CTAB extraction method described by Saghai-Maroof *et al.* (1984). DNA concentration was determined by the use of BioSpecNanoDNA spectrophotometer. A total of 21 pairs of microsatellite primers were used for detecting of DNA polymorphism in wheat mutant lines and non-mutagenized plants. The primers *Barc 263*, *Gwm 11*, *Gwm 337*, *Barc 13*, *Barc 12*, *Barc 42*, *Gwm 359*, *Gwm 533*, *Barc 56, Gwm 681* were used for estimating variation on DNA level of the chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 5A, 6A, 6B, 6D, 7A, 7D.

12.2.5 PCR analysis of microsatellite markers

For PCR amplification of M13-tailed microsatellites were used. One microsatellite primer is extended by a M13 sequence at the 5' end. 40-150 ng genomic were digested with R-, F-Primers, 0.2 mM dNTP mix (MBI Fermentas), 0.05 U/µl Taq-polymerase and its corresponding reaction buffer. The F-Primer has a M13-30 sequence at the 5'end. The M13-30 sequence is: 5'-CCCAGTCACGACGTTG-3'. It is labeled with a fluorescent dye at the 5'-end. For this method a forward primers with an M-13 tailed fluorescent primer was added to the PCR reaction. The reaction mix for the M13-tailed SSRs contained 0.02 µl forward primer (10 µM, with M13-tail at the 5'-end: CCCAGTCACGACGTTG), 0.18 µl M13-primer (with a fluorochrome, IRD700 or IRD800, at the 5'-end) 0.2 µl reverse primer (10 µM), 1 µl 10× PCR buffer including 15 mM $MgCl_2$, 1 µl dNTP-mix (2 mM each dNTP), 0.1 µl Taq polymerase (5 U/µl) and 2 µl of template DNA for a 10 µl reaction.

The PCR programme for the M13-tailed primer was 94 °C for 2 min and then 30 cycles of 94 °C for 1 min, 0.5 °C s-1 to 51 °C, 51 °C for 30s, 0.5 °C s-1 to72 °C and 72 °C for 1 min, followed by 72 °C for 5 min. PCR were performed on a 384 well Eppendorf Mastercycler. The cycle profile for amplification was as follows: after an initial denaturation step (95 °C/2 min) 6

cycles of 95 °C/50 s, 63 °C/1 min 30 s, and extension 72 °C/1 min 30 s 25 cycles. PCR product was diluted with ddH_2O(1:5 or 1:10), added 5 µl formamide loading buffer (95% formamid deionisiert, 0.5 mM EDTA, 0.1 mg/ml new fuchsin red), denatured 10 min at 95°C and loaded on gel. Microsatellite measurements were performed using fluorescent detection on Typhoon (GE Healthcare) fluorescence scanner.

12.3 Results and discussion

12.3.1 *Fusarium* resistance testing

Each of the grown lines were artificially inoculated with *Fusarium culmorum* during flowering. The lines all reacted to the inoculums and showed different symptoms. Table 12.1 shows the results of percentage of infected spikelets per spike of M_3 lines and non-mutagenized plants of different wheat varieties. The M_3 mutant lines obtained on genetic background of cv. Zhenis have a different spectre of variability of resistance to *F. graminearum*. Screening of M_3 mutant lines developed on the genetic base of cv. Zhenis show that treatment by 100 Gy γ rays had both positive and negative mutagenic effects on resistance to *F. graminearum.* Visual scoring for resistance to *F. graminearum* showed that cv. Zhenis has a resistance level to FHB with a mean of 9.27% infected spikelets. The infection rate of the three M_3 mutant lines, No. 6(15), No. 6(16) and 22(1) was clearly below that of Zhenis at 15-day after the inoculation. Compared to cv. Zhenis these M_3 lines had the highest means of resistance to *F. graminearum* and they are stable to the pathogenic fungus metabolites. The M_3 lines No. 6(12) and No. 22(12) had a higher percentage of susceptibility to FHB (Table 12.1) than parent Zhenis.

Screening of M_3 mutant lines obtained on genetic background of cv. Almaken by treatment of 100-γ rays irradiation for resistance to FHB are shown in Table 12.1. Like cv. Zhenis, Almaken has a resistance level to the disease with a mean of 9.27% infected spikelet's. The infection rate of M_3 line No. 89(4) was clearly below that it of parent Almaken at 15-day after the inoculation. Screening results obtained indicated that the M_3 line No. 89(4) can be identified as FHB-tolerant. The productivity components, weight of grain per main spike and grain yield per plant show that this M_3 line is characterized by higher means for these traits than the parent.

As indicated in Table 12.1, screening results for *Fusarium* resistance of cv. Erithrospermum-35 showed a higher percent of infected spikelets per spike or a lower level of disease resistance with a mean of 32.32%, compared to the other varieties Zhenis and Almaken. Three M_3 mutant lines obtained on the genetic background of Erithrospermum-35, No. 110(1), No. 129(3) and No. 150(5) had the highest level of disease resistance. Other M_3 lines, except line No. 138(1) are characterized by lower means of % of infected spikelets per spike than cv. Erithrospermum-35.

Table 12.1. Mean values of the visual scoring for *Fusarium* resistance of wheat cultivars Zhenis, Almaken and Erithrospermum-35, and advanced M_3 mutant lines obtained on its genetic background by irradiation treatment of 100 Gy γ rays at 15-day after the inoculation and their productivity components.

Wheat genotype	% of infected spikelets per spike	Weight of grain per main spike (g)	Number of grain per main spike	Grain yield per plant (g)
cv. Zhenis	9.27%	1.30±0.32	36.2±6.78	2.34±0.82
No. 5(10)	8.65%	1.53	45	4.79
No. 6(15)	6.96%	1.31	34	3.72
No. 6(16)	6.98%	1.53	39	2.06
No. 6(12)	12.82%	1.63	49	1.81
No. 22(1)	5.15%	1.29	52	1.26
No. 22(2)	9.48%	1.18	43	3.21
No. 21(12)	11.60%	1.53	41	2.76
cv. Almaken	9.27%	0.95±0.35	27±9.50	1.69±0.17
No. 79(3)	16.6%	1.02	37	2.00
No. 82(2)	11.1%	1.61	47	1.02
No. 81(2)	18.95%	1.03	39	0.72
No. 89(4)	6.96%	1.62	36	2.03
No. 84(6)	8.95%	1.41	38	2.41
cv. Erithrospermum-35	32.32%	0.80±0.28	29.38±5.55	1.41±0.44
No. 109(1)	26.51%	1.59	46	2.54
No. 109(5)	21.57%	1.84	44	4.74
No. 110(1)	18.65%	1.70	41	1.60
No. 129(3)	14.27%	0.87	39	1.86
No. 133(3)	24.12%	1.97	45	2.87
No. 35(3)	26.11%	2.06	53	1.75
No. 138(1)	32.32%	2.41	48	2.78
No. 150(5)[a]	16.77%	1.01	39	0.92

[a] Irradiation treatment for this mutant line was at 200 Gy.

12.3.2 PCR analysis of microsatellite markers

SSR-markers were used for detecting DNA polymorphisms in the 3 varieties of spring wheat, Zhenis, Almaken and Erithrospermum-35, that were used as parents for irradiation treatments and the 138 M_3 mutant lines developed on their base. A total of 21 SSR loci were analyzed in all genotypes. For molecular analyses of wheat mutant lines the SSR primers *Barc 263*, *Gwm 11*, *Gwm 337*, *Barc 13*, *Barc 12*, *Barc 42*, *Gwm 359*, *Gwm 533*, *Barc 56, Gwm 681* were used for screening chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 5A, 6A, 6B, 6D, 7A, 7D of the genomes of the mutant lines.

Figure 12.1 shows the amplification pattern using SSR marker *Gwm359*, *Barc12*, *Gwm533*, and *Barc56* (D) located on wheat chromosome 2AS, 3A, 3BS and 5A, respectively. An analysis of the data for molecular screening of selected lines revealed that M_3 lines No. 48(3) and No. 49(6) of cv Zhenis had genetic differences compared to the parental variety when tested with these SSR markers. These lines showed an additional allele for the SSR markers *Gwm359*, *Barc12*, *Gwm533*, *Barc56* (Figure 12.1). SSR marker GWM533 has been reported to be closely linked to *Sr*2 gene (Malik *et al.*, 2013). SSR-based GWM533 and CAPS-based CsSr2 markers reported for *Sr*2 gene were found promising for molecular confirmation of the gene and have been used in stem rust breeding programmes in Australia, the USA and CIMMYT (Mexico) (Mago *et al.*, 2011; Spielmeyer *et al.*, 2003).

Molecular screening of cv. Zhenis and its M_3 lines Zhenis No. 5(1), No. 5(4), No. 16(1), No. 16(9), No. 25(9) and No. 25(12) (100 Gy γ rays), and Zhenis No. 43(4) and No. 51(8) (200 Gy γ rays) by *Barc12* (3A), *Barc42* (3DL), *Gwm533* (3BS) and *Gwm681* (7A) is shown in Figure 12.2. There were changes in the number of alleles on these chromosomes.

Amplification profiles of cv. Almaken and advanced M_3 lines developed on its genetic background for *Barc42* (3DL), *Gwm533* (3BS), *Gwm681*(7A), *Barc273* DNA markers are shown in Figure 14.3. The lines No. 82(6), No. 101(8), No. 89(3), No. 101-3, No. 101(5) and No. 94(2) have similar differences on chromosomes 3DL and 3BS. M_3 line No. 138(2) developed on the genetic background of cv. Erithrospermum-35 revealed additional alleles for *Gwm681* and *Barc 273* located on chromosomes 6DL (Figure 12.3c,d).

Figure 12.1. Simple sequence repeat products amplified by (a) *Gwm359*, (b) *Barc12*, (c) *Gwm533*, and (d) *Barc56* in cultivar Zhenis (non-mutagenized plant) and advanced M_3 mutant lines obtained by irradiation treatment of 200 Gy γ rays. 1 = cv. Zhenis (non-mutagenized plant), 2 = M_3 line No. 48(3), 3 = M_3 line No. 49(6).

Figure 12.2. Simple sequence repeat products amplified for (a) *Barc12*, (b) *Barc42*, (c) *Gwm533*, and (d) *Gwm681* in cultivar Zhenis and M_3 mutant lines obtained on its genetic background and by irradiation treatment of 100 and 200 Gy γ rays. 1 = Zhenis (non-mutagenized plant), 2 = M_3 line No. 5(1), 3 = No. 25(9), 4 = No. 51(8), 5 = No. 5(4), 6 = No. 25(12), 7 = No. 43(4), 8 = No. 16(9), 9 = No. 16(1).

Figure 12.3. Simple sequence repeat products amplified for (a) *Barc42*, (b) *Gwm533*, (c) *Gwm681*, and (d) *Barc 273* DNA markers in cv. Almaken, cv. Erithrospermum-35 and M_3 mutant lines by irradiation treatment of 100 Gy γ rays. 1 = cv. Almaken (non-mutagenized plant), 2 = No. 82(6), 3 = No. 101(8), 4 = No. 89(3), 5 = No. 101(3), 6 = No. 101(5), 7 = No. 94(2), 8 = cv. Erithrospermum-35 (non-mutagenized plant), 9 = No. 138(2).

12.4 Conclusion and future perspectives

Development and use of resistant wheat varieties is the most practical and economic approach for control of FHB (Yang *et al.*, 2005b). Research on FHB resistance as well as breeding efforts have mainly focused on introgressing resistance from Chinese sources. The 3BS QTL from the resistant Chinese line 'Sumai 3' and its derivatives, which confers resistance to disease spread within the spike, is widely used in wheat breeding programs. To avoid complete dependence on limited sources of resistance, finding new and different sources of resistance is a critical goal.

In this study, new M_3 mutant lines of spring wheat variety (138 lines) developed on genetic basis of three varieties, cv. Zhenis, cv. Almaken and cv. Erithrospermum-35 by irradiation treatment (100 and 200 Gy γ rays) were used to evaluate the *Fusarium* resistance. Genetic variation to resistance FHB disease among the three studied varieties of spring wheat grown in Kazakhstan was significant. Among them, cv. Zhenis had the greatest resistance to *F. graminearum* with a mean of 9.27% infected spikelet's per ear, followed by cv. Almaken (20.53%) and Eritrospermum (38.81%).

On the genetic background of cv. Zhenis three M_3 mutant lines No. 6(15), No. 6(16) and No. 22(1), developed by irradiation treatment with 100 Gy γ rays, were identified as more resistant compared their non-mutagenized plants. The association between agronomic traits and resistance to FHB was also investigated in the mutagenesis derived population. M_3 line, No. 89(4), developed on genetic background of cv. Almaken (100 Gy γ rays) can be identified as tolerant to disease infection. The productivity components, weight of grain per main spike, g, and grain yield per plant show that this M_3 is characterized by higher means for these elements than the non-mutagenized variety. Three M_3 mutant lines, No. 110(1), No. 129(3) and No. 150(5), obtained on the cv. Erithrospermum-35 genetic background, are characterized by a higher level of disease resistance comparing to the non-mutagenized variety.

The developed M_3 lines of spring wheat can be classified as new source of *Fusarium graminearum* resistance genes for future wheat varieties to accelerate the Kazakhstan breeding program for FHB resistance. Different genes from these sources might be combined into single lines, and then the lines could serve as parents in wheat improvement programs for FHB resistance caused mainly by *Fusarium graminearum*. The final goal of a breeding program, however, should be to develop varieties with the greatest degree of resistance possible.

SSR markers were used to study the genetic diversity in the 138 mutant wheat lines. A total of 21 SSR loci were analyzed in all genotypes. For the mutant lines Zhenis No. 5(1), No. 25(9), No. 51(8), No. 5(4), No. 25(12), No. 43(4), No. 16(9) and No. 16(1) changes in the number of alleles on chromosomes 3A, 3DL, 3BS, 7A for SSR markers *Barc12*, *Barc42, Gwm533, Gwm681*) were determined. The mutant lines of cv. Zhenis show more variability than mutant lines derived from cv. Almaken and cv. Erithrospermum-35. This finding also indicates the potential of mutagenesis for crop improvement. The identification of *Fusarium* resistant genes in mutant wheat germplasm

will help in accelerating the breeding program in future, including combining of different wheat resistant genes in wheat genotypes and varieties.

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