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Screening of Mutant Wheat Lines to Resistance for Fusarium Head Blight and Using SSR Markers for Detecting DNA Polymorphism

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Abstract

Fusarium head blight, caused mainly by *Fusarium graminearum* is one of the most damaging diseases of wheat. Breeding durable disease resistance cultivars rely largely on continually introgression 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 cultivars 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 Fusarium head blight disease and to use PCR-based DNA markers, such as SSRs markers to investigate genetic diversity in wheat germplasm. Significant differences in tolerance phenotype to Fusarium head blight were found among wheat cultivars and mutant lines. Comparing parent cv “Zhenis” M_3 mutant lines, № 6(15), № 6(16) and 22(1) had the highest means of Fusarium resistance at 15-day after the inoculation. M_3 mutant lines, №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” №110(1), №129(3) and №150(5) had higher level of Fusarium tolerance then parent cultivar. A total of 21 SSRs loci were used to determine genetic diversity among M_3 spring wheat lines and non-mutagenized plants.

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Keywords: mutant wheat lines; resistance to Fuzarium head blight disease; SSR markers

1. Introduction

Wheat is the second most important crop globally with a total estimated world production of ~663 million tonnes during 2011–2012 (http://faostat.fao.org/; http://www.igc.int/). Currently, about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum 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 total area of 13 million hectare. 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 nongenetic effects. To be useful, resistance advances must be achievable without negative effects on other important traits such as yield. Hence breeding wheat genotypes with 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 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 [1, 2, 3].

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 [3]. Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms [4, 5]. 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 [5]. 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 2500 varieties derived from mutagenesis programs have been released, as listed in the IAEA/FAO mutant variety database, including 534 rice lines, 205 wheat lines, and 71 maize lines (http://www-infocris.iaea.org/MVD/). 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 [6, 7].

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 England in the year 1884 where because of the chalky, lifeless appearance of the infected kernels, it was called wheat scab, and later

tombstone disease [8]. 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 [9]. The control of FHB with the use of management strategies like, crop rotation, tillage and the application of fungicide produces only limited results [10]. The most effective strategy for controlling FHB in wheat is through the development of resistant cultivars. Resistance to FHB exhibits quantitative variation and its inheritance involves several loci on different chromosomes [11]. Genotype x environment interaction complicates the phenotypic evaluation of FHB resistance and makes screening of FHB resistance laborious, time consuming and costly [12].

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

Molecular markers based on polymerase chain reaction (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 cultivars because they are plentiful, independent of plant tissue or environmental effects, and allow cultivar identification very early in plant development [13, 3]. Molecular characterization of cultivars 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 polymerase chain reaction (PCR) is much easier to perform than RFLP 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 map [14, 15], to identify alien chromatin [16] and to map agronomical important genes [17, 18, 19].

The aim of this work was to verify the susceptibility/tolerance phenotype to Fusarium head blight (FHB) of three spring wheat cultivars cv “Zhenis”, cv “Almaken” and cv “Erithrospermum-35” grown in Kazakhstan and advanced mutant lines which were developed on their genetic background by irradiation treatment (100 γ and 200 γ 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 to tolerance phenotype to FHB and applying of 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 to tolerance phenotype to FHB between these M_3 mutant lines and spring wheat cultivars, and (3) to evaluate the genetic relationships between M_3 spring wheat lines and non-mutagenized cultivars.

2. Material and methods

2.1 The plant material

The field experiment was carried out in the year 2011. The plant material used in the study consisted of 138 M_3 mutant lines of spring wheat which were developed using by irradiation treatment (100 and 200 Gy) on genetic base of three cultivars, cv “Zhenis”, cv “Almaken” and cv “Erithrospermum-35” and non-mutagenized plants. For screening resistance to FHB gemplasm were planted in pots in a in the greenhouse.

Based on radiation sensitivity studies, 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.

2.2 Fusarium resistance testing

Spores isolated from *Fusarium graminearum* were used for inoculations. Macroconidia of the *F. graminearum* were prepared as described by Snijders and Van Eeuwijk (1991) and Buerstmayr et al. (2000, 2002) [20, 21, 22]. 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 cambers under the microscope. The concentration of the conidia was 50.000 conidia/ml was produced and stored at - 80°C for the inoculation procedure [22].

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

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.

2.3 DNA extraction and SSR 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.

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 Units/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

(10µM, 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 X PCR buffer including 15mM MgCl2, 1 µl dNTP-mix (2mM each dNTP), 0.1 µl Taq polymerase (5 units/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 minutes and then 30 cycles of 94°C for 1 minute, 0.5°C s-1 to 51°C, 51°C for 30s, 0.5°C s-1 to72°C and 72°C for 1 minute, followed by 72°C for 5 minutes. 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 was performed using flouresecnt detection on Typhoon (GE Healthcare) fluorescences scanner.

3 Results and discussion

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. Tables 1-3 show the results of % of infected spikelets per spike of M_3 lines and non-mutagenized plants of different wheat cultivars. The M_3 mutant lines obtained on genetic background of cv "Zhenis" have different specter of variability of resistance to *Fusarium graminearum* (Table 1).

Table 1. Mean values of the visual scoring for FHB resistance of cv "Zhenis" and advanced M_3 mutant lines obtained on its genetic background by irradiation treatment of 100 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
№5(10)	8,65%	1.53	45	4.79
№6(15)	6,96%	1.31	34	3.72
№6(16)	6,98%	1.53	39	2.06
№ 6(12)	12,82%	1.63	49	1.81
№22(1)	5,15%	1.29	52	1.26
№22(2)	9,48%	1.18	43	3.21
№21(12)	11,60%	1.53	41	2.76

Screening of M_3 mutant lines developed on genetic base of cv "Zhenis" shows that treatment by 100 γ rays has a both positive and negative mutagenic effects on resistance to *Fusarium graminearum.* Visual scoring for resistance to *Fusarium graminearum* shows that the cv "Zhenis" has resistance level to disease with mean of

9,27% infected spikelet's. The infection rate of three M_3 mutant lines, № 6(15), № 6(16) and 22(1) was clearly below that it of cv "Zhenis" at 15-day after the inoculation. Comparing cv "Zhenis" these M_3 lines had the highest means of resistance to *Fusarium graminearum* and they are stable to the pathogenic fungus metabolites. The M_3 lines №6(12) and №22(12) had a high percentage of susceptibility to FHB (Table 1) then parent cv "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 showed in table 14.2 Like cv "Zhenis", cv "Almaken" has resistance level to disease with mean of 9,27% infected spikelet's. The infection rate of M_3 line, № 89(4) was clearly below that it of parent cv "Almaken" at 15-day after the inoculation. Screening results obtained indicated that the M_3 lines, №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 of these elements then non-mutagenized cv "Almaken" (Table 2).

Table 2. Mean values of the visual scoring for FHB resistance of wheat cv "Almaken" and advanced M_3 mutant lines obtained on its genetic background by irradiation treatment of 100 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 "Almaken"	9,27%	0.95±0.35	27±9.50	1.69±0.17
79(3)	16,6 %	1.02	37	2.0
№82(2)	11,1%	1.61	47	1.02
№81(2)	18,95%	1.03	39	0.72
№ 89(4)	6,96%	1.62	36	2.03
№84(6)	8,95%	1.41	38	2.41

As indicated screening results for FHB resistance of cv "Erithrospermum-35"comparing to other cultivars tested, cv "Zhenis" and cv "Almaken", a higher percent of infected spikelets per spike or low level of disease resistance with mean of 32,32%, was observed (Table 1-3). Three M_3 mutant lines obtained on genetic background of cv "Erithrospermum-35", namely №110(1), №129(3) and №150(5) had highest level of disease resistance. Other M_3 lines, expect the line №138(1) are characterized by lower means of % of infected spikelets per spike, or higher level of disease resistance then cv "Erithrospermum-35"(Table 3).

3.2 PCR analysis of microsatellite (SSR) markers

SSR-markers were used for detecting DNA polymorphism in 3 cultivars of spring wheat, cv"Zhenis", cv "Almaken" and cv "Erithrospermum-35", used as genetic background for irradiation treatments of doses of 100 and γ and 138 M_3 mutant lines beveloped on their base. A total of 21 SSR loci were analyzed in all genotypes. For molecular analyses of wheat mutant lines have been used SSR primers *Barc 263*, *Gwm 11*, *Gwm 337*, *Barc 13*, *Barc 12*, *Barc 42*, *Gwm 359*, *Gwm 533*, *Barc 56, Gwm 681* for screening chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 5A, 6A, 6B, 6D, 7A, 7D of mutant lines genome.

Figure 1 shows the amplification pattern using SSR marker *Gwm359*, *Barc12*, *Gwm533*, *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 № 48(3) of cv "Zhenis" and № 49(6) of cv "Zhenis" had genetic differences compared to parental cultivar when tested with these microsatellite markers. These lines showed an additional allele at the SSR markers *Gwm359*, *Barc12*, *Gwm533*, *Barc56* (Fig. 1).

Table 3. Mean values of the visual scoring for fusarium resistance of cv "Erithrospermum-35" and advanced M_3 mutant lines obtained on its genetic background by treatment of 100 and 200 γ rays at 15-day after the inoculation, and their productivity components.

Wheat genotype and dose of irradiation , γ rays	% of infected spikelets per spike, %	Weight of grain per main spike, g	Number of grain per main spike	Grain yield per plant, g
cv "Erithrospermum-35"	32,32%	0.80±0.28	29.38±5.55	1.41±0.44
№109(1), 100	26,51%	1.59	46	2.54
№109(5), 100	21,57%	1.84	44	4.74
№110(1), 100	18,65%	1.7	41	1.6
№129(3), 100	14,27 %	0.87	39	1.86
№133(3), 100	24,12%	1.97	45	2.87
№ 35(3), 100	26,11%	2.06	53	1.75
№138(1), 100	32,32%	2.41	48	2.78
№150(5), 200	16,77%	1.01	39	0.92

It was reported that SSR marker GWM533 is closely linked to *Sr*2 gene [19]. Simple sequence repeat (SSR) based GWM533 and CAPS based CsSr2 markers reported for *Sr*2 gene were found promising for molecular confirmation of gene and have been used in stem rust breeding programmes in Australian, US and CIMMYT, Mexico [23, 24].

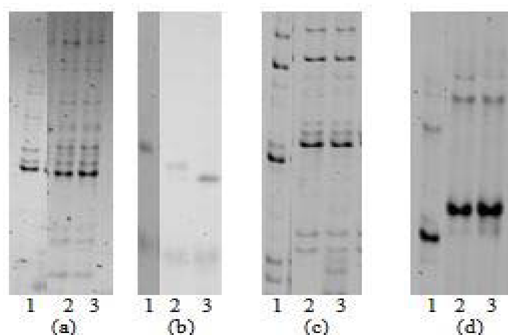

Fig. 1. SSR products amplified by *Gwm359* (a), *Barc12* (b), *Gwm533*(c), *Barc56* (d) in cv "Zhenis" (non-mutagenized plant) and advanced M_3 mutant lines obtained on its genetic background by irradiation treatment of 200 γ. 1 - cv "Zhenis" (non-mutagenized plant), 2 – M_3 line №48(3), 3 – M_3 line №49(6).

Molecular screening of cv “Zhenis” and its M_3 lines №5(1), 100 γ), Zhenis №25(9, 100 γ), Zhenis №51(8), 200 γ, Zhenis №5(4), 100 γ), Zhenis №25(12), 100 γ), Zhenis №43(4), 200 γ), Zhenis №16(9), 100 γ) Zhenis №16(1, 100 γ) by *Barc12(*3A), *Barc42 (*3DL), *Gwm533* (3BS) and *Gwm681* (7A) is shown on figure2. There were changes in the number of alleles on these chromosomes.

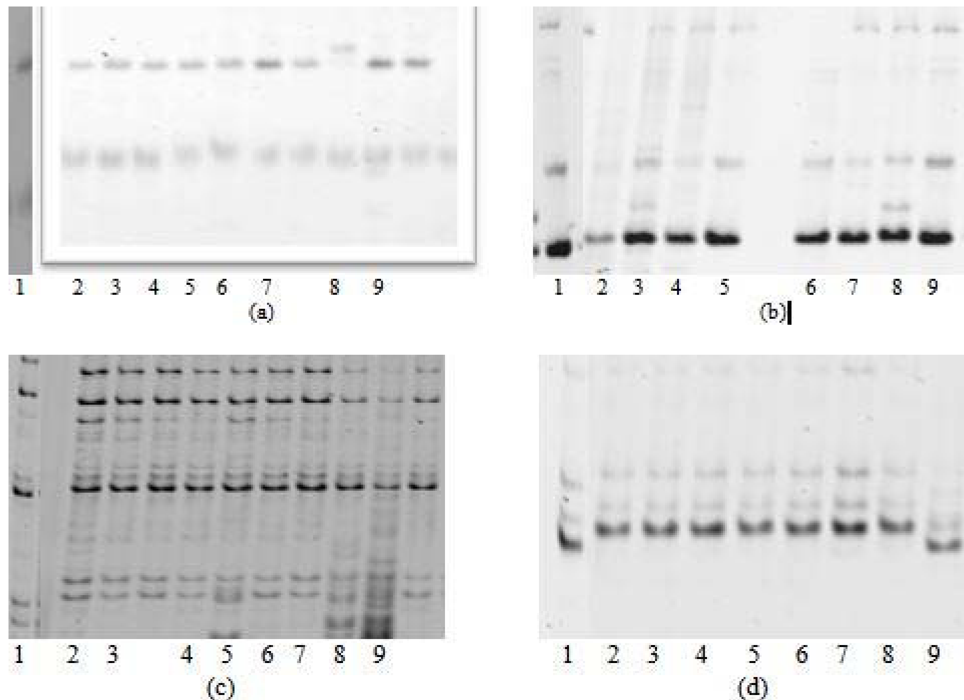

Fig.2. SSR products amplified for *Barc12* (a), *Barc42* (b), *Gwm533* (c), *Gwm681* (d) cv “Zhenis” and M_3 mutant lines obtained on its genetic background and by irradiation treatment of 100 and 200 γ. 1 - cv “Zhenis” (non-mutagenized plant), 2 - M_3 line №5(1), 3 - №25(9), 4 - № 51(8), 5 - №5(4), 6 - №25(12), 7 - №43(4), 8 - №16(9), 9 - №16(1).

Amplification profiles of cv “Almaken” and advanced M_3 line № developed on its genetic background for *Barc42* (3DL)*, Gwm533* (3BS), *Gwm681*(7A), *Barc273* DNA markers are shown on figure 3. Similar differences on chromosomes 3DL, 3BS have the lines №82(6), №101(8), №89(3), №101-3, №101(5, №94(2).

M_3 line №138(2) developed on cv “Erithrospermum-35” and by irradiation treatment of 100 γ revealed additional alleles for *Gwm681* and , *Barc 273* located on chromosomes 6DL, 7A) that (D) (Figure 3).

4. Conclusion and Future Perspective

Development and use of resistant wheat cultivars is the most practical and economic approach for control of FHB. 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 spring new M_3 mutant lines of spring wheat cultivar (138 lines) developed on genetic basis of three cultivars, cv "Zhenis", cv "Almaken" and cv "Erithrospermum-35" by irradiation treatment (100 and 200 γ rays) were used to evaluate the fusarium resistance. Genetic variation to resistance fusarium head blight disease among three studied cultivars of spring wheat grown in Kazakhstan was significant. Among Cv "Zhenis" has the greatest resistance to *Fusarium graminearum* with mean of 9,27% infected by *Fusarium* spikelet's per ear then other varieties, Almaken (20.53%) and Eritrospermum (38.81%, respectively). On genetic background of v. Zhenis three M_3 mutant lines, developed by irradiation treatment of 100 γ, № 6(15), № 6(16) and №22(1), were identified more resistant compared their non-mutagenized plants. The association between agronomic traits and resistance to FHB was also investigated in mutagenesis derived population. M_3 line, №89(4), which were developed on genetic background of v. Almaken and 100 γ rays irradiation treatment 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 of these elements then non-mutagenized variety.

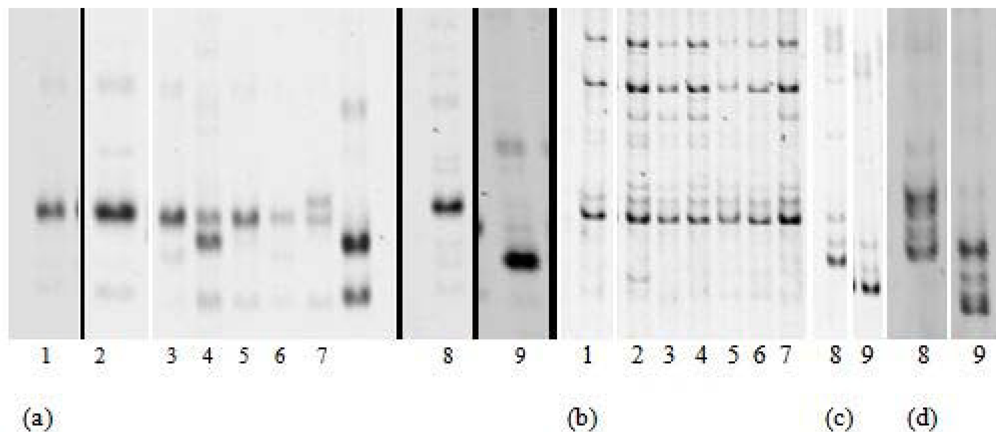

Fig. 3. SSR products amplified for *Barc42* (a), *Gwm533* (b), *Gwm681* (c), *Barc 273* (d) DNA markers in cv "Almaken" and M_3 mutant lines obtained on its variety genetic background by irradiation treatment of 100 γ. 1- cv "Almaken" (non-mutagenized plant), 2- №82(6), 3 - №101(8), 4 - №89(3), 5 - №101(3), 6 - №101(5), 7 - №94(2), 8 – cv "Erithrospermum-35" (non-mutagenized plant),); 9 - M_3 line №138(2) developed on genetic background of cv "Erithrospermum-35" by irradiation treatment of 100 γ.

Three M_3 mutant lines obtained on v. Erithrospermum-35 genetic background, №110(1), №129(3), and №150(5) are characterized by higher level of disease resistance comparing to non-mutagenized variety. New M_3 lines of spring wheat developed can be classified as new source of *Fusarium graminearum* resistance genes in future wheat varieties to accelerate Kazakhstan breeding program for r FHB resistance. ifferent genes from these sources might be pyramided into single lines, and then the lines could serve as parents in wheat improvement programs for Fusarium head blight resistance caused mainly by Fusarium graminearum. The final goal of a breeding program, however, should be to develop cultivars with the greatest degree of resistance possible.

SSR markers were used for study of genetic diversity in 138 wheat mutant lines developed on basic of 3 wheat varieties grown in Kazakhstan. A total of 21 SSR loci were analyzed in all genotypes. For the mutant lines Zhenis №5(1), Zhenis №25(9), Zhenis №51(8), Zhenis №5(4), Zhenis №25(12), Zhenis №43(4), Zhenis №16(9), Zhenis №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" are very variability than mutant lines derived from cv "Almaken" and Erithrospermum-35". This finding also indicates

potential of mutagenesis for crop improvement. The identification of fusarium resistant genes in mutany wheat germplasm will help in accelerating the breeding program in future, including pyramiding of different wheat resistant genes in wheat genotypes and varieties.

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