Screening new mutant lines of spring wheat to resistance for Fusarium Head Blight

Associate Professor, PhD
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- Regional TC project KAZ/5002: «Improving Wheat and Maize Using Nuclear and Molecular Techniques”, April 2012-2018 (National coordinator: Professor, Dr. Kenzhebaeva S.S.)
- Theme of research: Screening new mutant lines of spring wheat to resistance for Fusarium Head Blight (FHB)
- Financial support: International Atomic Energy Agency (Austria)

The goals of research were:

1) to screen mutant wheat lines for resistance to Fusarium Head Blight

- 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
- to verify advanced mutant lines (M3 generations) that were got on their genetic background by irradiation treatment (100 - and 200-gamma rays).

2) to find DNA polymorphism of mutant lines using SSR markers

- The **main objective** of this research was to evaluate 3 spring wheat cultivars grown in Kazakhstan and the mutant lines (M3 generations) developed on their genetic background by irradiation treatment (100 and 200 gamma rays) for their resistance to FHB disease and to use PCR-based DNA markers, such as SSRs markers to investigate genetic diversity in wheat germplasm.

Mutagenesis

Spring wheat occupies 95% of the total wheat area in Kazakhstan. Breeding improvement of many agronomical traits requires genetic variation and these components of variation must be separable from non-genetic effects.

Genetic variability is very important for the improvement of many crop species, including wheat.

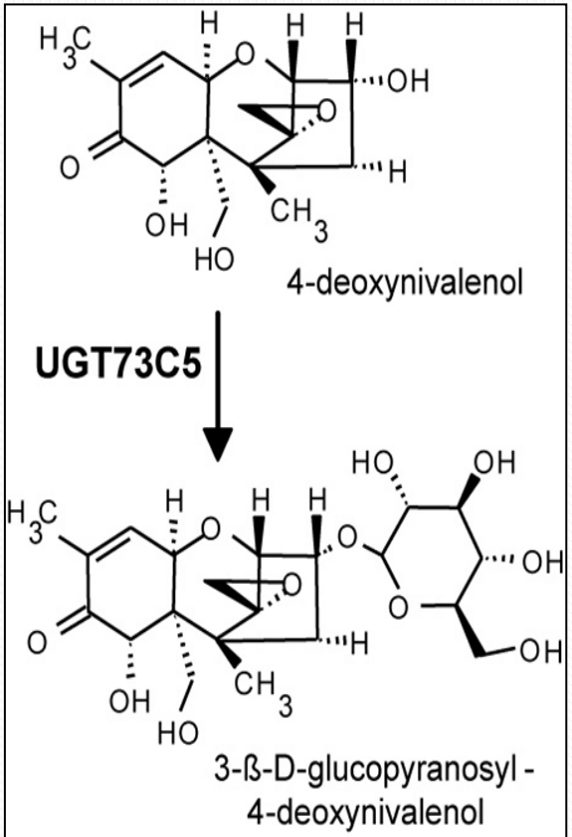
Mutagenesis is an important tool in crop improvement.

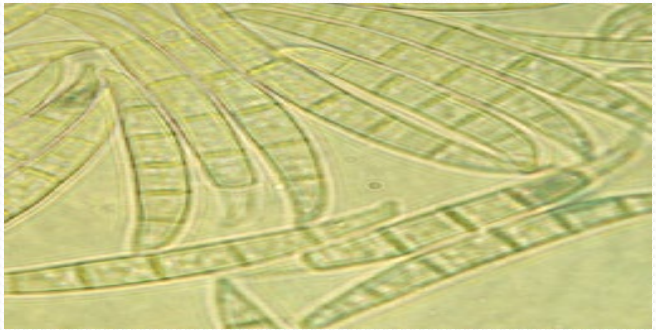
The induced mutation help to develop many agronomical important traits such as

- shorter growing period, suitable for rotation,
- increased tolerance or resistance to abiotic and biotic stresses (drought, salinity, diseases etc.)

Fusarium Head Blight (FHB)

- FHB, caused by Fusarium graminearum is one of the most damaging diseases of wheat.
- *F.graminearum* is a pathogenic fungus that commonly infects wheat and barley
- Infection with *Fusarium* results in severe reduction of crop yield and quality
- The trichothecene mycotoxin deoxinivalenol (DON) is produced by *F.graminearum*
- The mechanism causing high toxin resistance was shown to be the higher ability to convert DON into DON-3-O-glucoside





- The control of FHB using of management strategies like, crop rotation, tillage and the application of fungicide produce only limited results .
- The most effective strategy for controlling FHB in wheat is known as the development of resistant cultivars.
- Resistance to FHB exhibits quantitative variation and its inheritance involves several loci on different chromosomes

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

- Molecular markers based on PCR methods, such as **s**imple **s**equence **r**epeats **SSRs** or microsatellites, have provided a powerful approach to analyze genetic relationships among accessions in many crop species.
- 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.
- In most cases, microsatellites are inherited in a codominant manner and are chromosome-specific.
- Microsatellites have been successfully used to construct genetic map, to identify alien chromatin and to map agronomical important genes .

MATERIAL AND METHODS

- The plant material used in the study consisted of 138 M3 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. Initially, irradiation of 1000 seeds was performed in an ionizing device at the Kazakh Nuclear Center.
- Selection of individual plants was done every generation from M3 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.

Fusarium resistance testing

- For screening resistance to FHB plants germplasm were planted in pots in the greenhouse.
- Spores isolated from Fusarium graminearum were used for inoculations. Macroconidia of the F. graminearum were prepared as described by Buerstmayr et al. (2002) .
- The concentration of the conidia was 50.000 conidia/ml was produced and stored at - 80°C for the inoculation procedure.
- 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, 12 th June 2012.
- After inoculation heads were covered by a plastic bags for 24 h in order to ensure high humidity. 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.

DNA extraction and SSR primer sources

The molecular characterization and genetic diversity of spring wheat genotypes was investigated using 21 SSRs primers.

We got screening results of mutant M3 spring wheat lines compared with non-mutagenized plants for tolerance to FHB and applying microsatellite markers for molecular genotyping of wheat and analyzed it.

- 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.

- For PCR amplification of M13-tailed microsatellites were used. For this method a forward primers with an M-13 tailed fluorescent primer was added to the PCR reaction.
- Microsatellite was performed using fluorescent detection on Typhoon (GE Healthcare) fluorescence scanner.

RESEARCH RESULTS

- **Fusarium resistance testing**

Each of the grown lines were artificially inoculated with Fusarium graminearum 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 M3 mutant lines and non-mutagenized plants of different wheat cultivars.

Table 1. Mean values of the visual scoring for FHB resistance of cv "Zhenis" and advanced M3 mutant lines obtained on its genetic background by irradiation treatment of 100 gamma rays at 15-day after 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

Table 2. Mean values of the visual scoring for FHB resistance of cv "Almaken" and advanced M3 mutant lines obtained on its genetic background by irradiation treatment of 100 gamma rays at 15-day after 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

Table 3. Mean values of the visual scoring for FHB resistance of cv "Erithrospermum-35" and advanced M3 mutant lines obtained on its genetic background by irradiation treatment of 100 gamma rays at 15-day after 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

Fig.1 SSR products amplified by Gwm359 (a), Gwm533(c), Barc56 (d) in cv.”Zhenis” (non-mutagenized plant) and advanced M3 mutant lines obtained on its genetic background by irradiation treatment of 200-gamma rays
1-cv “Zhenis”, 2-M3 line Zh48(3), 3-M3 line Zh49(6)

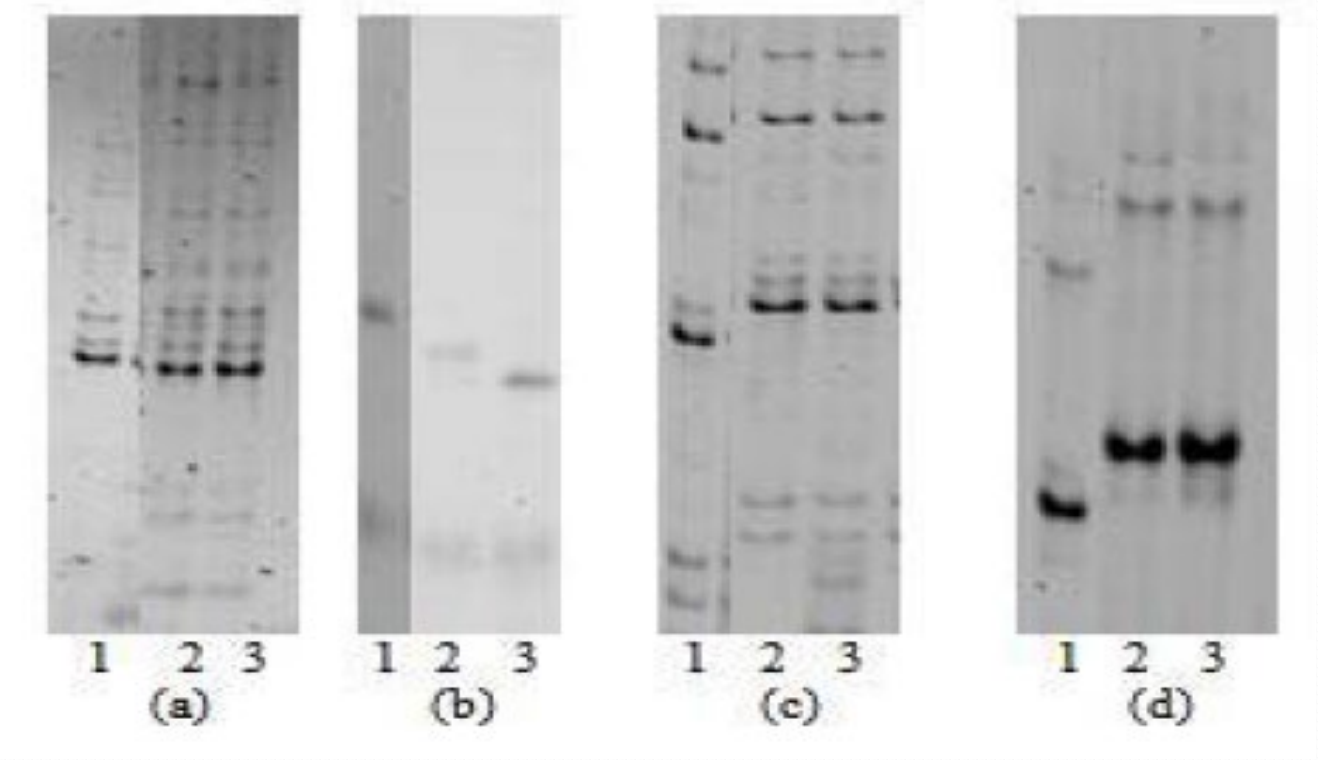


Fig.2 SSR products amplified by Barc12 (a), Barc42 (b), Gwm533 (c), Gwm681(d) cv.”Zhenis” and advanced M3 mutant lines obtained on its genetic background by irradiation treatment of 100- and 200gamma rays

1-cv “Zhenis”, 2-M3 line Zh5(1), 3-M3 line Zh25(9), 4-Zh51(8), 5-Zh5(4), 6-Zh25(12), 7-Zh43(4), 8-Zh16(9), 9-Zh16(1)

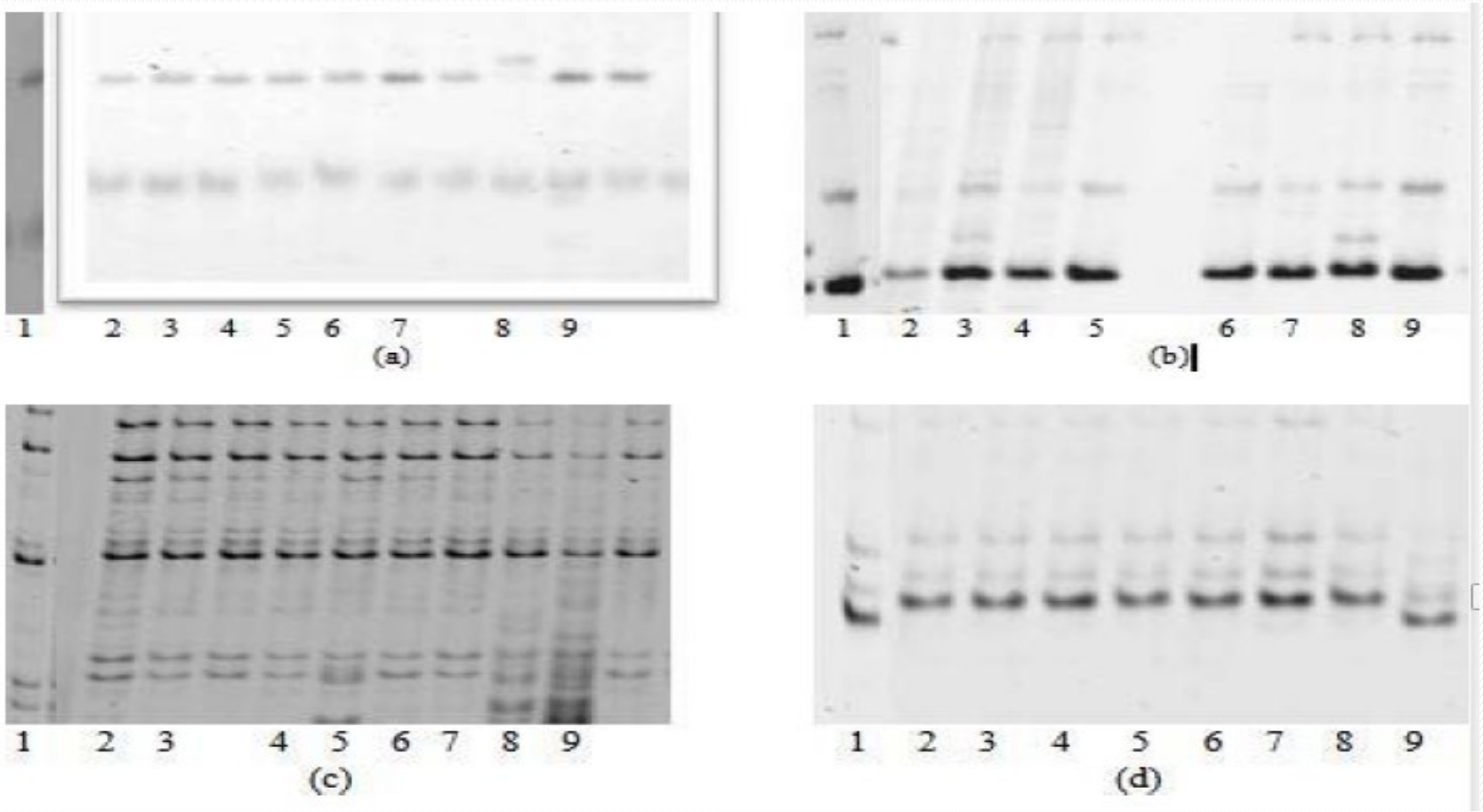
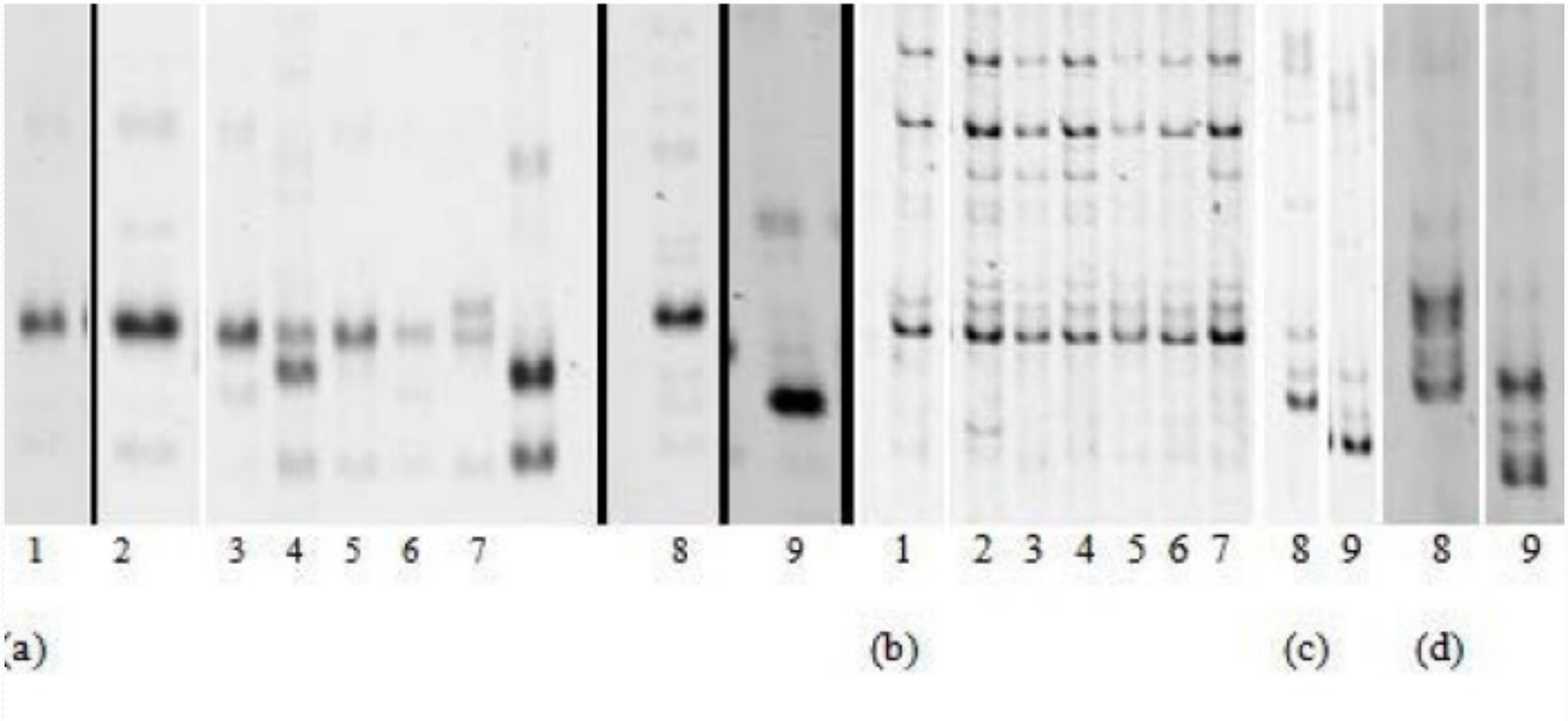


Fig.3 SSR products amplified by Barc42 (a), Gwm533 (b), Gwm681(c) Barc273 (d) in cv.”Almaken” and M3 mutant lines obtained on its genetic background by irradiation treatment of 100gamma rays 1-cv “Almaken”, 2-M3 line A82(6), 3-M3 line A101(8), 4-A89(3), 5-A101(3), 6-A101(5), 7-A94(2), 8-cv “Erithrospermum-35”, 9-A138(2) developed on genetic background of cv “Erithrospermum-35” by irradiation treatment of 100gamma rays



Conclusion and Future Perspective

- In this study new M3mutant 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 gamma rays) were used to evaluate the Fusarium resistance.
- Genetic variation for resistance to 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 cv. Zhenis three M3mutant lines, developed by irradiation treatment of 100 gamma rays, Zh6(15), Zh6(16) and Zh22(1), were identified more resistant compared their non-mutagenized plants.

Conference

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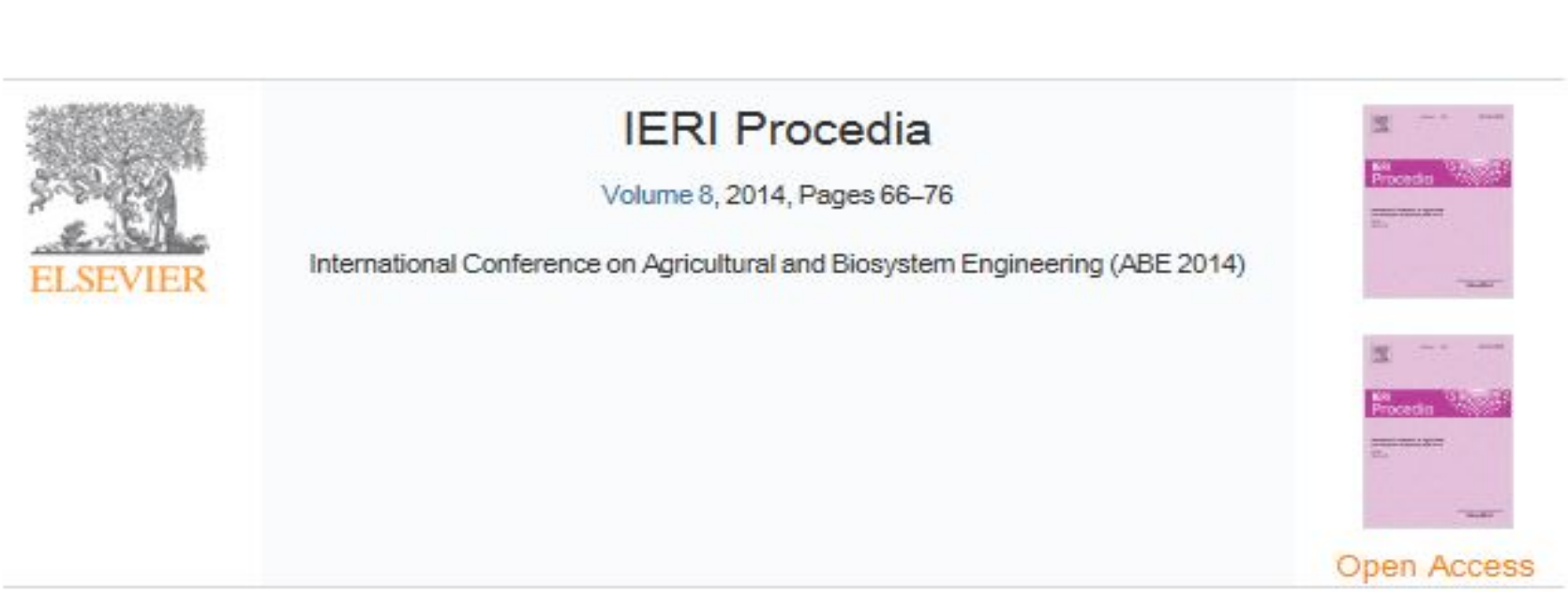

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Screening of Mutant Wheat Lines to Resistance for Fusarium Head Blight and Using SSR Markers for Detecting DNA Polymorphism

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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

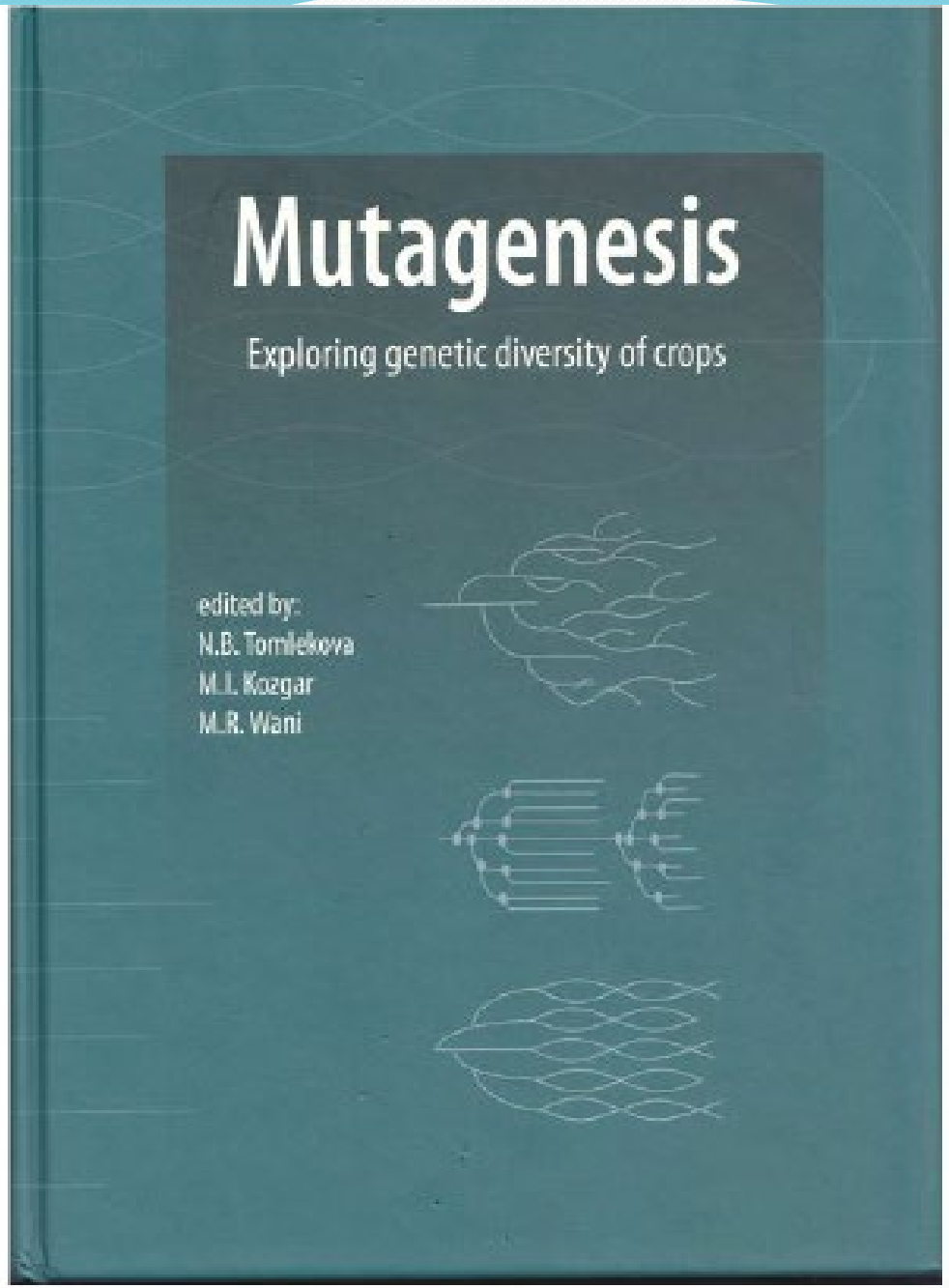
Publications

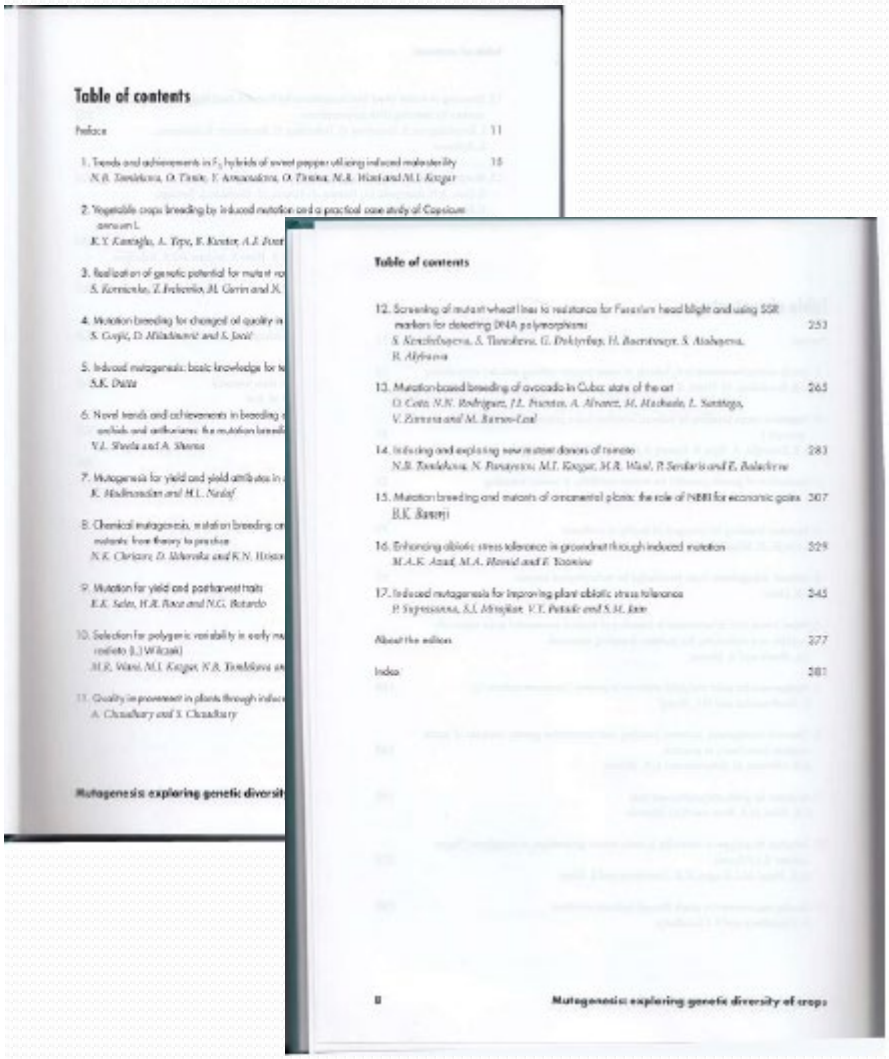
- 1. Saule Kenzhebayeva, Svetlana Turasheva, Gulina Doktyrbay, Hermann Buerstmayr, Saule Atabayeva, Ravilya Alybaeva. **Screening of mutant wheat lines to resistance for Fusarium Head Blight and using SSR markers for detecting DNA polymorphism** //IERI Procedia . Vol.8. P.66-76. 2014

- 2. **Mutagenesis: Exploring genetic diversity of crops**. Chapter 12. P.253-265. 2014.

The book edited by: N.B.Tomlekova, M.I.Kozgar, M.R.Wani. Wageningen Academic Publishers, The Netherlands. 2014. ISBN: 978-90-8686-244-3

- 3. Saule Kenzhebayeva, Svetlana Turasheva. **Evaluation of mutant wheat lines resistant to Fusarium Head Blight disease** // Bulletin Al-Farabi KazNU. Ecology Series. Vol. 2(45). P. 146-156. 2015





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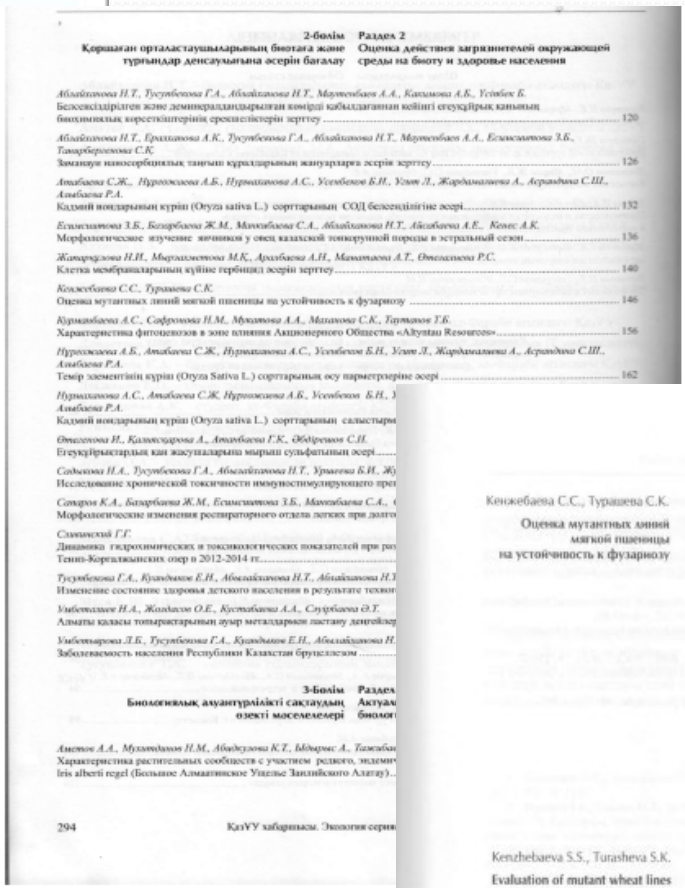

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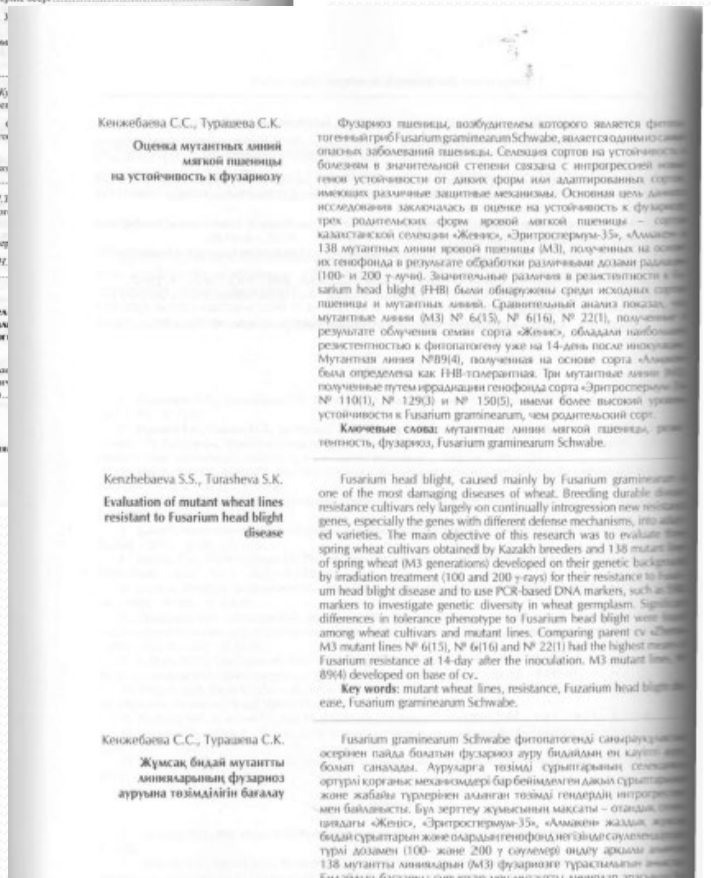


Кенжебаева С.С., Турашева С.К.

Оценка мутантных линий мягкой пшеницы на устойчивость к фузариозу

Фузариоз пшеницы, возбудителем которого является фито
тогенный гриб Fusarium graminearum Schwabe, является одним из самы
опасных заболеваний пшеницы. Селекция сортов на устойчивость
болезням в значительной степени связана с интрогрессией новы
генов устойчивости от диких форм или адаптированных сорто
имеющих различные защитные механизмы. Основная цель данно
исследования заключалась в оценке на устойчивость к фузариоз
трех родительских форм яровой мягкой пшеницы – сорто
казахстанской селекции «Женис», «Эритростермум-35», «Алмакен
138 мутантных линии яровой пшеницы (М3), полученных на основе
их генофонда в результате обработки различными дозами радиации
(100- и 200 γ-лучей). Значительные различия в резистентности к ф
sarium head blight (FHB) были обнаружены среди исходных сорто
пшеницы и мутантных линий. Сравнительный анализ показал,
мутантные линии (М3) № 6(15), № 6(16), № 22(1), полученные
результате облучения семян сорта «Женис», обладали наиболь
резистентностью к фитопатогену уже на 14-день после инокуляции
Мутантная линия №89(4), полученная на основе сорта «Алмакен
была определена как FHB-толерантная. Три мутантные линии (М3
полученные путем ирадиации генофонда сорта «Эритростермум
№ 110(1), № 129(3) и № 150(5), имели более высокий уровень
устойчивости к Fusarium graminearum, чем родительский сорт.

Ключевые слова: мутантные линии мягкой пшеницы, рези
тентность, фузариоз, Fusarium graminearum Schwabe.

Kenzhebaeva S.S., Turasheva S.K.

Evaluation of mutant wheat lines resistant to Fusarium head blight disease

Fusarium head blight, caused mainly by Fusarium graminearum
one of the most damaging diseases of wheat. Breeding durable dise
resistance cultivars rely largely on continually introgression new resist
genes, especially the genes with different defense mechanisms, into ad
ed varieties. The main objective of this research was to evaluate the
spring wheat cultivars obtained by Kazakh breeders and 138 mutant lin
of spring wheat (M3 generations) developed on their genetic backgroun
by irradiation treatment (100 and 200 γ-rays) for their resistance to Fusa
um head blight disease and to use PCR-based DNA markers, such as
markers to investigate genetic diversity in wheat germplasm. Signific
differences in tolerance phenotype to Fusarium head blight were fou
among wheat cultivars and mutant lines. Comparing parent cv «Zhen
M3 mutant lines № 6(15), № 6(16) and № 22(1) had the highest mean
Fusarium resistance at 14-day after the inoculation. M3 mutant lines,
89(4) developed on base of cv.

Key words: mutant wheat lines, resistance, Fuzarium head blight dis
ease, Fusarium graminearum Schwabe.

Кенжебаева С.С., Турашева С.К.

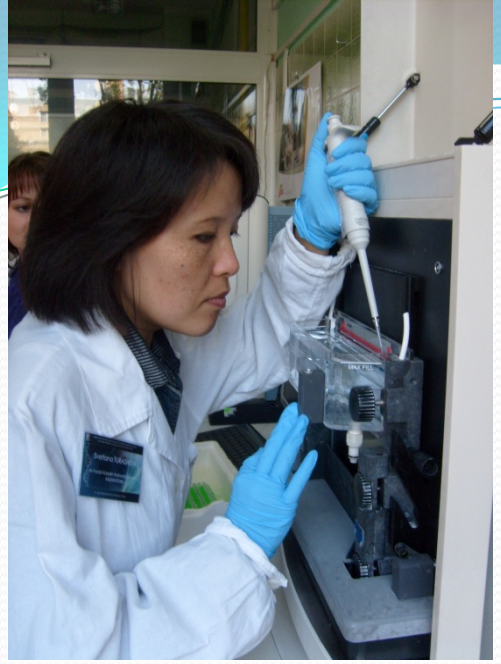
Жұмсақ бидай мутантты линияларының фузариоз ауруына төзімділігін бағалау

Fusarium graminearum Schwabe фитопатогенді саңырауқұлақ
әсерінен пайда болатын фузариоз ауру биліктің ең қауіпті ауру
болып саналады. Ауруларға төзімді сұрыптарының селекциясы
ортүрлі қорғаныс механизмдерін бар бейімделген дақыл сұрыпта
және жабайы түрлерінен алынған төзімді гендердің интрогрессия
мен байланысты. Бұл зерттеу жұмысының мақсаты – отандық
шаяқты «Женіс», «Эритростермум-35», «Алмакен» жазық жұмса
бидай сұрыптары және олардың генофонд негізінде сәулеленд
түрлі дозамен (100- және 200 γ сәулелері) индуу арқылы алын
138 мутантты линияларын (М3) фузариозге тұрақтылығын анық
Бастапқы бағалау нәтижелері сорт «Женіс» негізінде алынған
мутантты линиялар (М3) № 6(15), № 6(16), № 22(1) инокуляциядан
14 күн өткеннен кейін фузариозға ең жоғары деңгейде төзімділік
көрсетті. Сорт «Алмакен» негізінде алынған мутантты линия №89(4)
FHB-толерантты деп анықталды. Генофонд сорт «Эритростермум
негізінде алынған үш мутантты линия (М3) № 110(1), № 129(3) және
№ 150(5) Fusarium graminearum-ға ата-аналық сортқа қарағанда
жоғары деңгейде төзімділік көрсетті.

Түйін сөздер: мутантты линиялар, жұмсақ бидай, резистентті
фузариоз, Fusarium graminearum Schwabe.

Acknowledgement

- I would like to thanks International Atomic Energy Agency (Austria) for financial support of National TC project KAZ/5002 «Improving Wheat and Maize Using Nuclear and Molecular Techniques" and
- University of Natural Resources and Life Sciences, (Vienna), Institute for Biotechnology in Plant Production, Department for Agrobiotechnology, IFA –Tulln (Tulln, Austria) and Professor Hermann Buerstmayr

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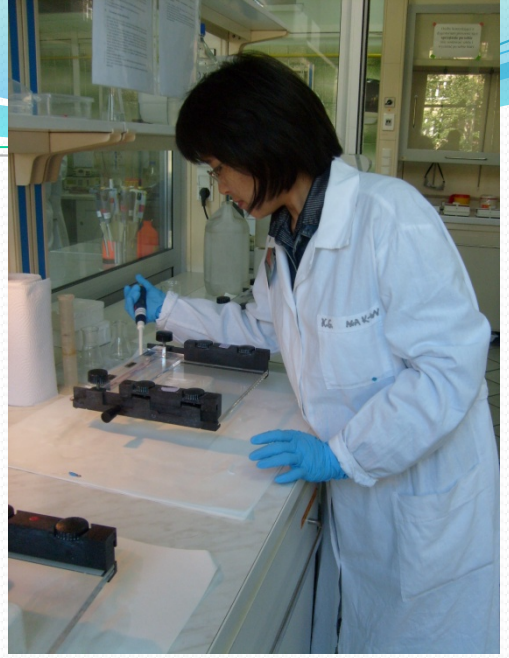


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