



Project KAZ/5/004: "Developing Drought Tolerant and Disease Resistant Wheat Varieties with Enhanced Nutritional Content Using Mutation Breeding" 1 April-31 July 2019

PhD, Assoc. Professor Turasheva Svetlana

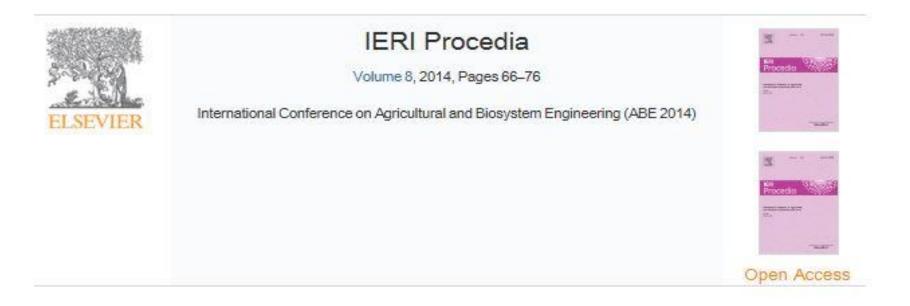
- Regional TC project KAZ/5/004: "Developing Drought Tolerant and Disease Resistant Wheat Varieties with Enhanced Nutritional Content Using Mutation Breeding", 1 April-31 July 2019
- Host institute: The John Innes Centre, Department of Metabolic Biology, Norwich, UK (Supervisor: Professor, Dr. Tony Miller)
- Fellowship Code No: FS-KAZ5004-1806382
- National coordinator: Professor, Dr. Kenzhebaeva Saule
- Financial support: International Atomic Energy Agency (Austria)

The goals of research were:

- 1) to find DNA polymorphism of mutant lines using SSR markers
- 3) to screen mutant wheat lines for resistance to drought
- 4) to determine drought resistance gene expression of mutant lines of soft spring wheat

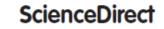
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Screening of Mutant Wheat Lines to Resistance for Fusarium Head Blight and Using SSR Markers for Detecting DNA Polymorphism

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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

Mutagenesis

Exploring genetic diversity of crops

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КазУУ хабаризасы. Эконогия серин

Конжобаева С.С., Турашева С.К. Оценка мутантных липой липкой пшеницы на устойчивость к фузариозу

Фузарноз пшенищы, возбудителем которого является фи тогенный гроб Fusarium graminearum Schwabe, вызвется одния опасных заболеваний пшевыща. Селещия сортов на устойние болезним в значительной степени связана с интрогрессиен « тенон устойчености от диких форм или адаптированных п INVESTIGATE DESCRIPTION AND APPENDENCE OF INTERNED OF ALL исследования заключалась и пшеное на устойчивость к фер трех родительских форм врсной миткой пшеницы казахстанской солекции «Женик», «Эритроглермум-35», «Ал 138 мутантных ляния вровой преницы (МЗ), полученных на г их генофонда в результате обработки различењими дозами раз (100- и 200 у муно). Значитичные размения в резистоятност затит head blight (FHB) были обнаружены среди исходных с постных и мутачтных довей. Сранитизаций акадиз показа мутантные минии (M3) Nº 6-(15), Nº 6(16), Nº 22(1), получи результате облучения семин сорта «Жемис», обладали наярезистентностью к фитопатолему уже на 14-день после инос Мутантная линия №89(4), полученная на основе сорта «Ан была определены как ННВ-толерантная. Три мутантные лини полученные путем иррадиации генофонда сорта «Эритроспери № 110(1), № 129(3) и № 150(5), имели более высокий и устоячиности к Гизапит graminearum, чем родительский сорг

Ключевые слова: мутантные линии мяткой пшеницы, и чентность, фузариса, fusarium graminearum Schwabe.

Kenzhebaeva S.S., Turasheva S.K. Evaluation of mutant wheat lines resistant to Fusarium head blight disease Towariam head hight, caused mainly be Fusianum gamineene of the most damaging diseases of wheat. Intereding duration mestance cultivars rely tangly on cantinually introgension new regenes, especially the genes with different defores mechanisms, insued varieties. The main objective of this research was to evolution spring wheat cultivars obtained by katabih breeders and 138 mulor spring wheat (MS generationed developed on their genetic base by irradiation treatment (100 and 200 - rays) for their resistance to um head highly disease and to use PCR-based DNA markers, such markers to investigate genetic diversity in wheat genetics. So indifferences in toefacture phenotype to Tousrinum bead blight weramong wheat cultivars and mutant lines. Comparing parent evdM structure (106 and 102 culti) and the Supher-Fusation resistance at 14-day after the introduction. M3 mutant lines 1944 developed on base of ex.

Key words: mutant wheat lices, resistance, Fuzarium head block arease, Fuzarium gramineanam Schwabe.

Kenskeflaena C.C., Typasiena C.K.

Жұмсақ бидай мутантты лишияларының фузариоз ауруына төзімділігін бағалау Евизнала длатіянскала Schwale финопалогесны Саннун с сертенен пайда бозалын фусаркога аруу бизайлан (н. к. у бозап санахады. Аррхадига тозімаї сурыппармяны сеалин забанка трурсерінен замаган тозамаї стемараді неграмен Баданська, бур заелге у жумыстных максала – оталаль заміт жабанка, это разерттеу жумыстных максала – оталаль бездаї сурыттарин жене одаралет геовораді него бадах за бездаї сурыттарин жене одаралет геоворад него бадах за бездаї сурыттарин жене одаралет геоворад него бадах с бездаї сурыттарин жене одаралет геоворад него бадах с бездаї сурыттарин жене одаралет геоворад него бадах с 118 магантъ миникарны (943) фударновог турытальната Project KAZ/5/004: "Developing Drought Tolerant and Disease Resistant Wheat Varieties with Enhanced Nutritional Content Using Mutation Breeding" August 2019

Drought resistance

Methods used for determine resistance to drought of mutant wheat lines:

- Soil sensors method;
- RWC method (relative water content);
- Nitrogen status in leaf (SPAD measurement);
- qPCR analysis







Note: A-seedlings after 3 days of germination; B-seedlings of wheat in the pots (after 18 days of watering); C,D-seedlings of wheat in the soil column (after 18 days of watering) Figure. The experiments with soil columns and pots in the glass-house

- Nitrate is the main form of nitrogen available for wheat crop and it usually limits growth and yield. Soil sensor gives a real-time measurement of nitrate availability in the soil. Other methods involve extraction of soil and chemical measurements of nitrate in soil
- The purpose of training is to manufacture a cost-effective ion selective sensor to measure the nitrate concentration of the soil water solution. We are going to apply this method for screening mutant wheat germplasm collections from Kazakhstan for nitrate uptake efficiency under differing levels of water supply.

The seeds of local cultivars tolerant (Kaz-10) and sensitive to drought (Samgau), also mutant line (E-152, M7) and its parent cultivar (Erythrospermum standart) planted in the soil columns after pre-germination in the petri dishes. The experiment was carried out in controlled conditions in the glasshouse during one month.



- There were built and created calibration curves for 36 nitrate selective soil sensors to compare and measure the nitrate concentrations in the soil water solution. The logger data will be compared with calibration to indicate the nitrate concentration in the soil. This data allow me to determine the real time nitrate concentration in the soil at different stages of plant development during the onset of drought conditions.
- The mutant lines (M7, M8) are derived from the cultivar Almaken, Zhenis, Erythrospermum (soft spring wheat varieties of Kazakhstan breeding) will be screened for growth and nitrate uptake efficiency under changing levels of water supply in the Almaty region of Kazakhstan.

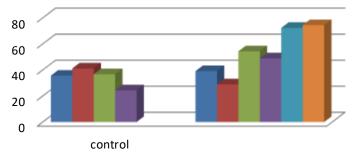
The techniques for measuring the water and nitrogen status of the wheat leaf

It was determined the relative water content (RWC) for five genotypes of spring wheat growing under drought. For this experiment three leaf samples were taken from each pot, sealed in plastic bags and taken to the laboratory. After fresh weight determination, the leaf parts were floated in distilled water for 3 h at room temperature with no illumination. Then leaf surfaces were dried with filter paper and the turgid weight was determined. To measure the dry weight the leaf parts were oven-dried at 85° C overnight and then reweighed. The water status was evaluated before drought (control) and during drought stress for 13 days.

 One of the physiological parameter related to resistance to abiotic stress factors is level of photosynthesis and also chlorophyll content. Chlorophyll content is one indicator of plant health and can be used to optimize the timing and quantity added fertilizer to provide the largest crop yields. For this aim was used easy measurements of the chlorophyll content of plant leaves by the SPAD-502Plus (Konica Minolta, Osaka, Japan).

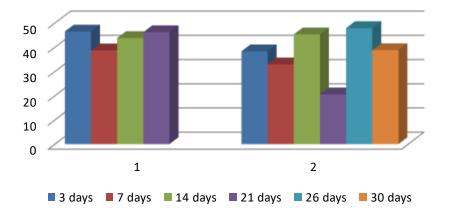


Chlorophyll content in leaves of weat cultivar Kaz-10 under drought stress

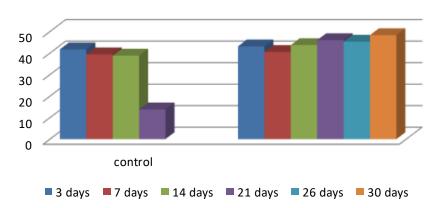


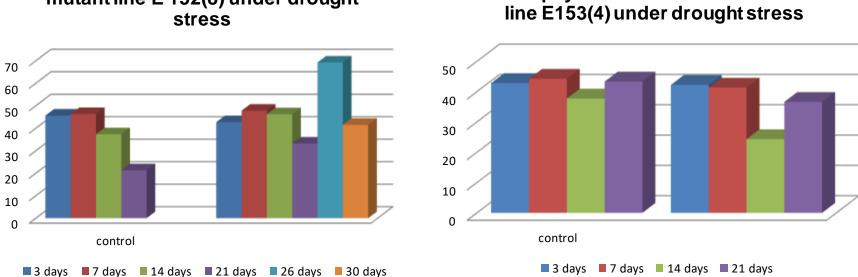
■ 3 days ■ 7 days ■ 14 days ■ 21 days ■ 26 days ■ 30 days

Chlorophyll content in leaves of weat cultivar Samgau under drought stress



Chlorophyll content in leaves of weat cultivar Erythrospermum under drought stress





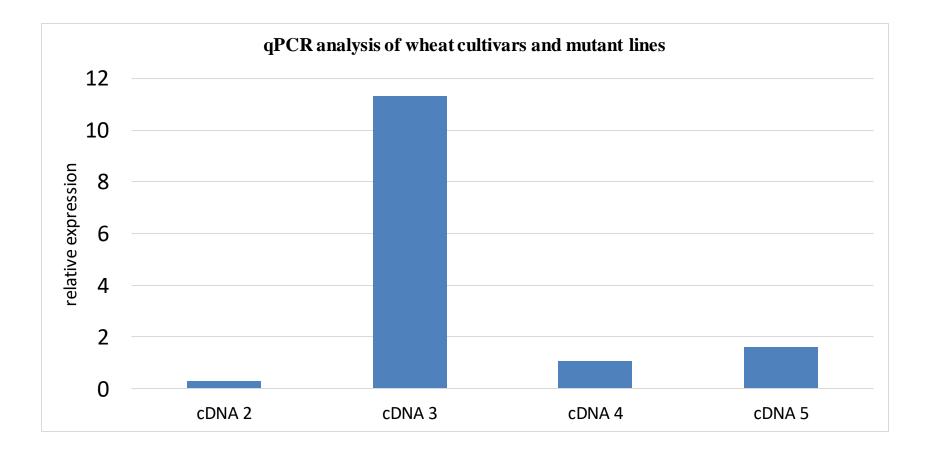
Chlorophyll content in leaves of mutant

Chlorophyll content in leaves of mutant line E 152(8) under drought stress

PCR screening of wheat mutant lines

- To indicate the drought status of the wheat plants at the molecular level samples were taken for RNA expression analysis and quantitative RT-PCR. For this aim the fresh plant samples were harvested before drought stress and then after 3-d, 7, 14 days without watering.
- This will include identifying and testing a suite of drought marker genes that can be used for PCR screening wheat growing under stress.

• The fresh plant samples harvested, quickly frozen in liquid nitrogen and then stored at -80°C before use. Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen, Melbourne, Victoria, Australia). Nucleic acid quantity was analysed with a NanoDropND-1000UV-VisSpectrophotometer (Nano Drop Technologies, Wilmington, DE). Quantiscript Reverse Transcriptase and RT primer mix were used for cDNA synthesis (Qiagen). As a reference gene were used Actin and Tubulin primers. Real-time quantitative RT-PCR(qRT-PCR) were performed using double-stranded DNA binding dye SYBR Green PCR master mix (Applied Biosystems, Scoresby, Victoria, Australia) in ViiA[™] 7 system (Applied Biosystems). Each reaction was run in triplicate. The first experiment data presented in Figure 3.



Note: cDNA 2- cultivar Samgau, cDNA 3 – cultivar Erythrospermum, cDNA 4 – mutant line E 152-8, cDNA 5 – mutant line E 153-4

Figure 3. Relative expression of marker gene DREB in leaf samples of wheat cultivars and mutant lines

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- University of Natural Resources and Life Sciences, (Vienna), Institute for Biotechnology in Plant Production, Department for Agrobiotechnology, IFA
- The John Innes Centre, Department of Metabolic Biology
- National coordinator, Professor Saule Kenzhebayeva













Thank you for your attention