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

Conference

International Conference on Agricultural and Biosystem Engineering (China, ABE 2014)

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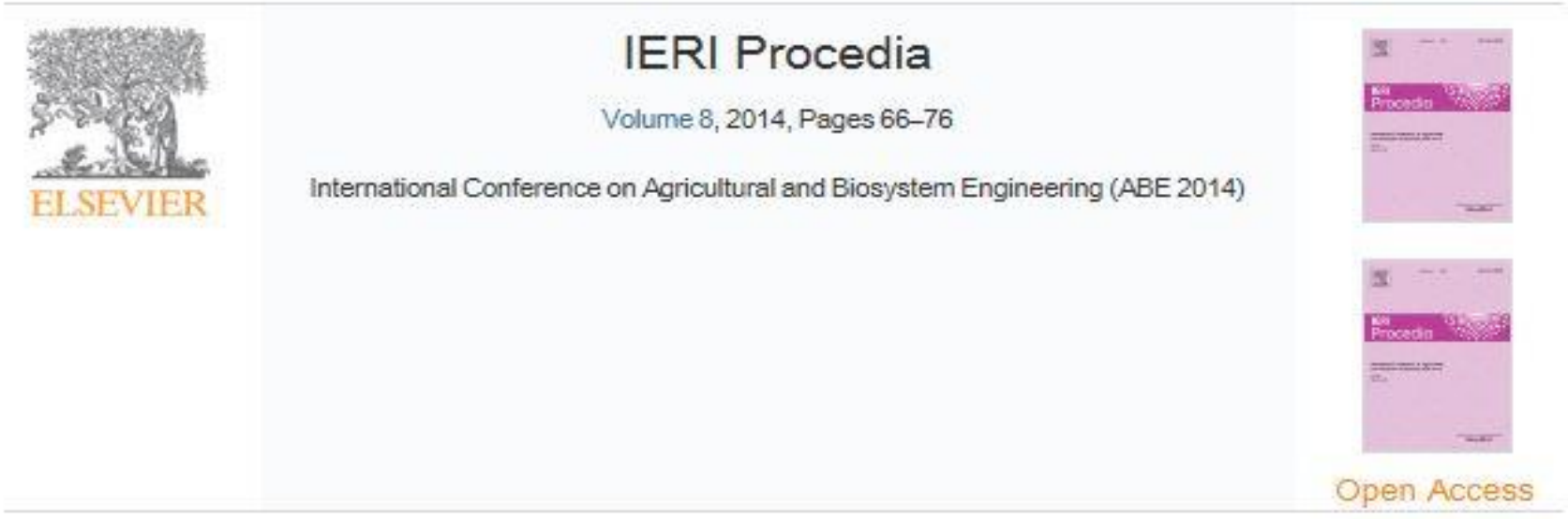

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Screening of Mutant Wheat Lines to Resistance for Fusarium Head Blight and Using SSR Markers for Detecting DNA Polymorphism

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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.

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- 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

edited by:
N.B. Tomlekova
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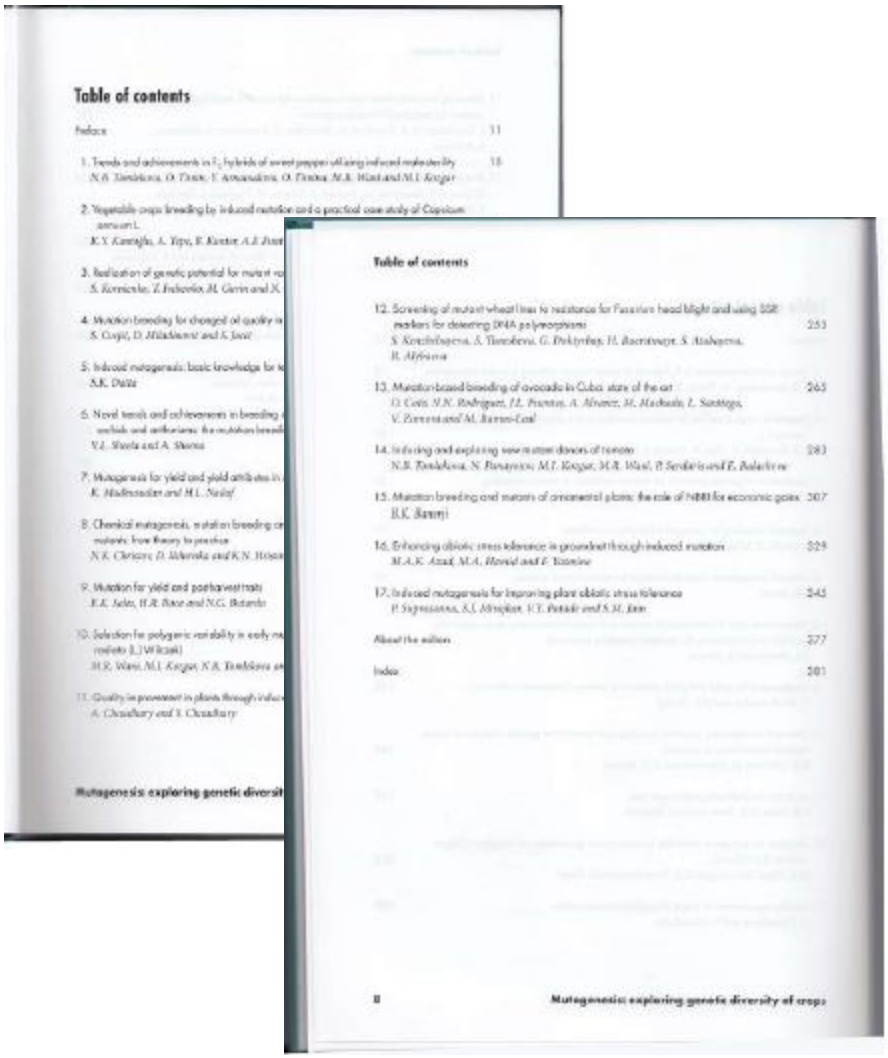

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КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ УНИВЕРСИТЕТ имени АЛЬ-ФАРАБИ
AL-FARABI KAZAKH NATIONAL UNIVERSITY

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

Ключевые слова: мутантные линии мягкой пшеницы, резистентность, 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, 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 obtained by Kazakh breeders and 138 mutant lines of spring wheat (M3 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 SSR 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», 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 disease, 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 бидайдың ең қауіпті ауруы болып саналады. Ауруларға төзімді сұрыптарының селекциясы әртүрлі қорғаныс механизмдері бар бейімделген дақыл сұрыптарының және жабайы түрлерінен алынған төзімді гендердің интрогрессиясымен байланысты. Бұл зерттеу жұмысының мақсаты – отандық селекциядағы «Женіс», «Эритроспермум-35», «Алмалы» жаздық жұмсақ бидай сұрыптарын және олардың генофонд негізінде сәулемен әртүрлі дозамен (100- және 200 γ сәулемен) өңдеу арқылы алынған 138 мутантты линияларын (М3) фузариозға тұрақтылығын анықтау.

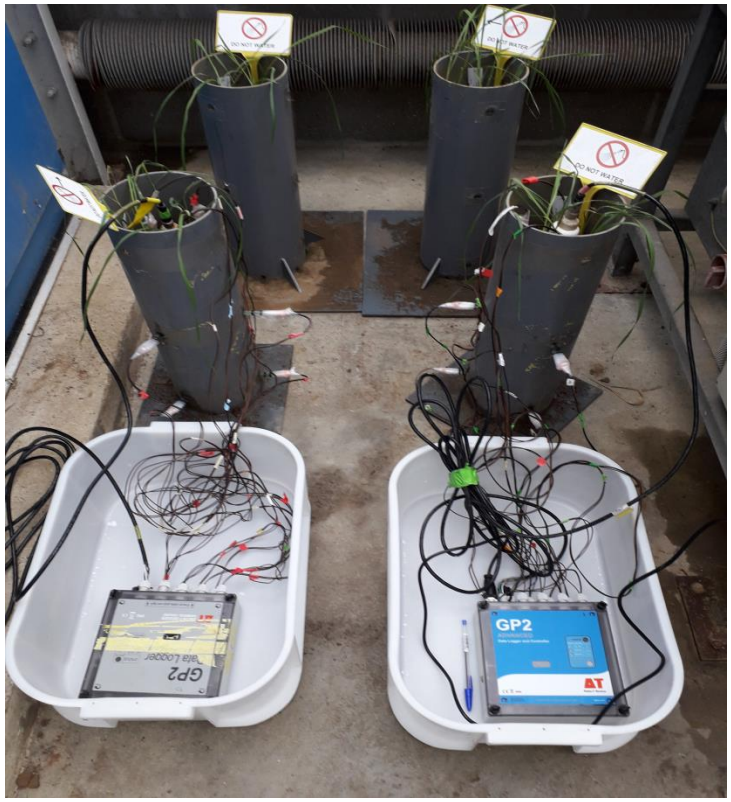
Project KAZ/5/004: “Developing Drought Tolerant and Disease Resistant Wheat Varieties with Enhanced Nutritional Content Using Mutation Breeding”

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.

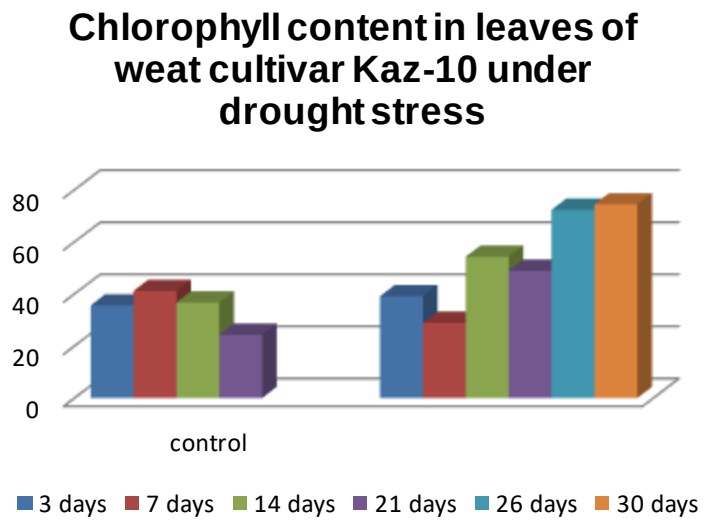
- There were built and created calibration curves for 36 nitrate selective soil sensors to compare and measure the nitrate concentrations in the soil water solution. The logger data will be compared with calibration to indicate the nitrate concentration in the soil. This data allow me to determine the 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.

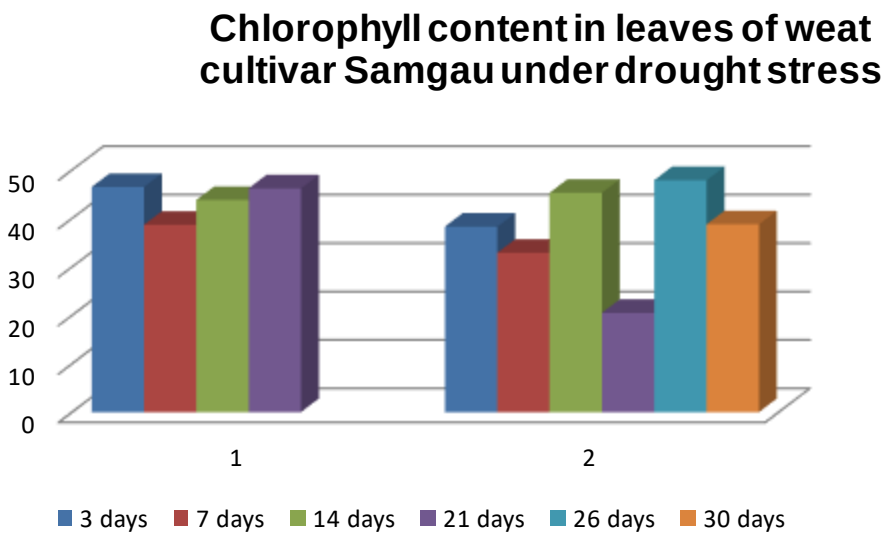
The techniques for measuring the water and nitrogen status of the wheat leaf

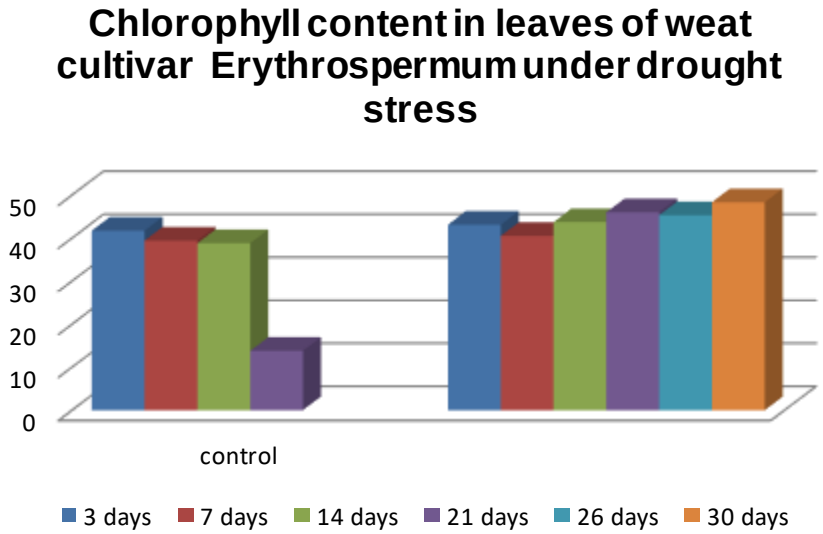
It was determined the relative water content (RWC) for five genotypes of spring wheat growing under drought.

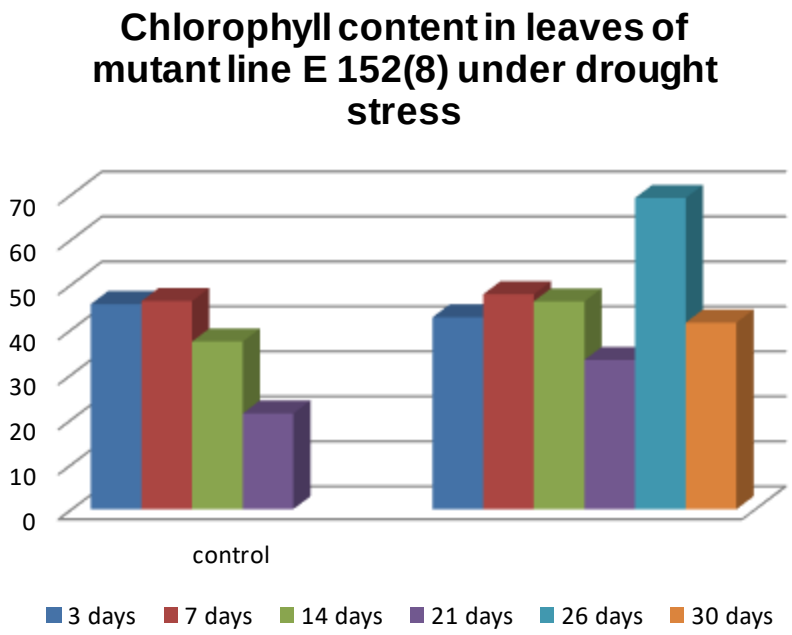
For this experiment three leaf samples were taken from each pot, sealed in plastic bags and taken to the laboratory. After fresh weight determination, the leaf parts were floated in distilled water for 3 h at room temperature with no illumination. Then leaf surfaces were dried with filter paper and the turgid weight was determined. To measure the dry weight the leaf parts were oven-dried at 85^0 C overnight and then reweighed. The water status was evaluated before drought (control) and during drought stress for 13 days.

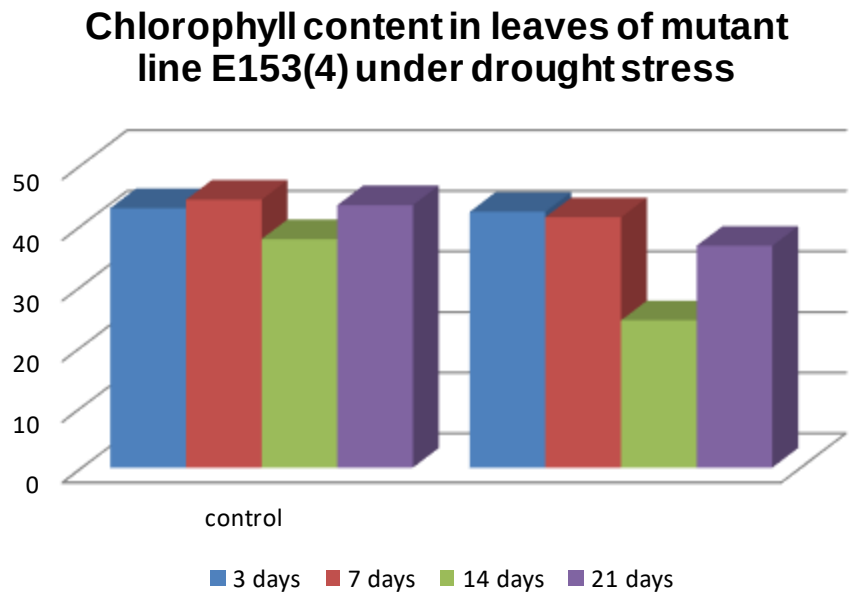
- One of the physiological parameter related to resistance to abiotic stress factors is level of photosynthesis and also chlorophyll content. Chlorophyll content is one indicator of plant health and can be used to optimize the timing and quantity added fertilizer to provide the largest crop yields. For 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
80
60
40
20
0
control
3 days
7 days
14 days
21 days
26 days
30 days


Chlorophyll content in leaves of weat cultivar Samgau under drought stress
50
40
30
20
10
0
1
2
3 days
7 days
14 days
21 days
26 days
30 days


Chlorophyll content in leaves of weat cultivar Erythrospermum under drought stress
50
40
30
20
10
0
control
3 days
7 days
14 days
21 days
26 days
30 days

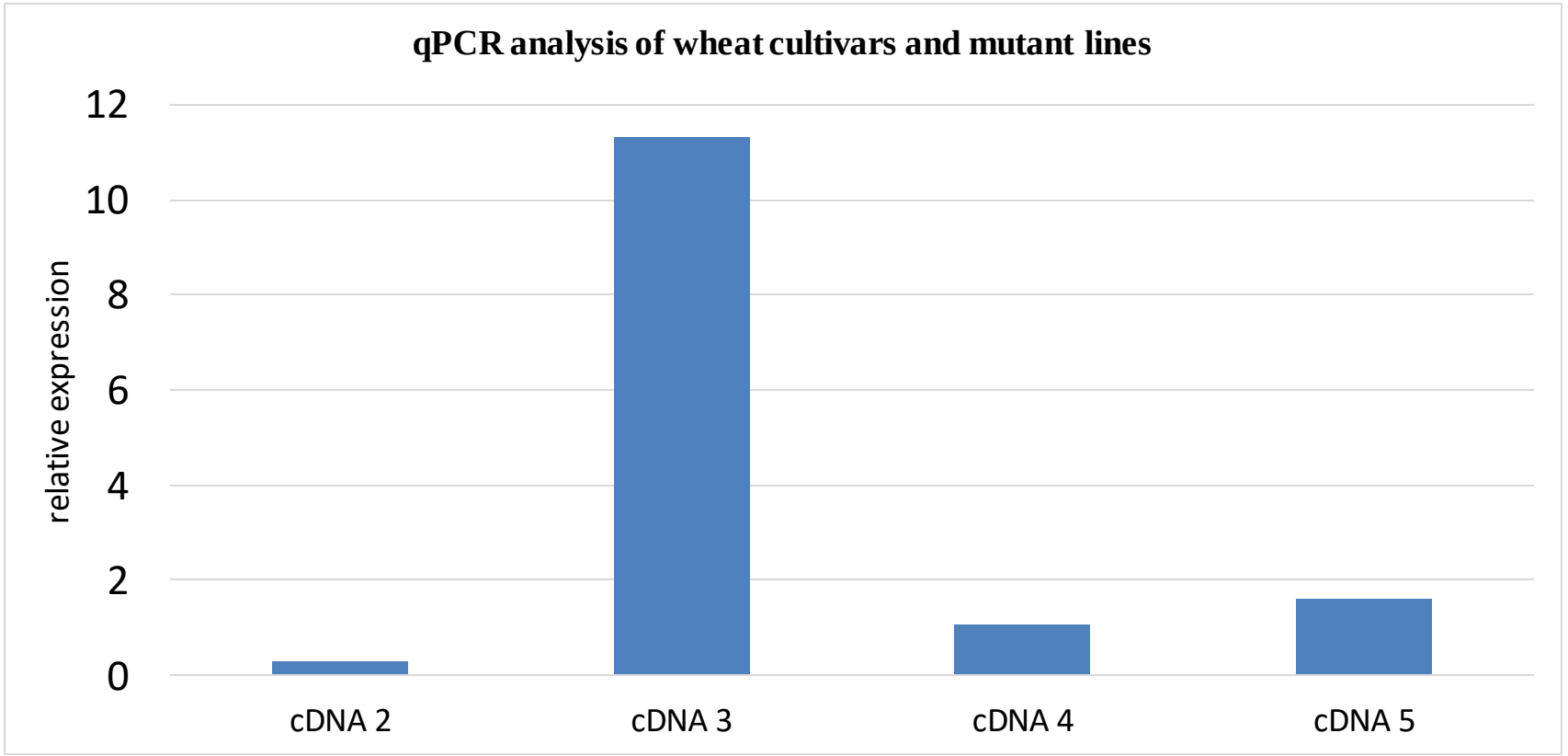

Chlorophyll content in leaves of mutant line E 152(8) under drought stress
70
60
50
40
30
20
10
0
control
3 days
7 days
14 days
21 days
26 days
30 days


Chlorophyll content in leaves of mutant line E153(4) under drought stress
50
40
30
20
10
0
control
3 days
7 days
14 days
21 days

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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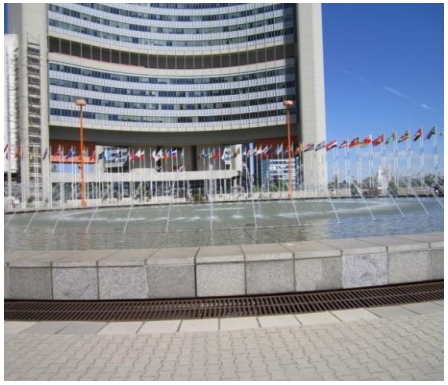
- The John Innes Centre, Department of Metabolic Biology
- National coordinator, Professor Saule Kenzhebayeva



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