Investigation of genes encoding iron (Fe) transporters in new mutant lines of spring wheat (M5)

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Iron deficiency is the most common eating disorder in the world. The imbalance of trace-element composition of food around the world, including in Kazakhstan, making iron by far the most commonly deficient nutrient in the world

Most preschool children and pregnant women in developing countries, and at least 30-40% in industrialized countries suffer from iron deficiency (WHO/ UNICEF/ UNU), 2001).

Medical iron supplements can rapidly replenish iron deficiency anemia in patients, but they are expensive and typically has unpleasant side effects

An alternative is to increase the iron content by conventional plant breeding or by genetic engineering methods

In plants, there are two strategies of iron absorption.

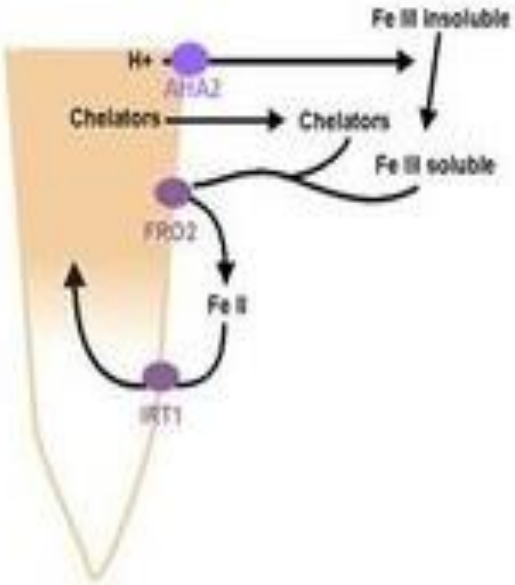
Strategy I – all plants except grasses employed the reduction-based strategy.

Insoluble soil ferric ions [Fe(III)] reduce into more-soluble ferrous ions [Fe(II)] by Ferric Reduction Oxidase 2 (FRO2), using a reducing agent NADPH.

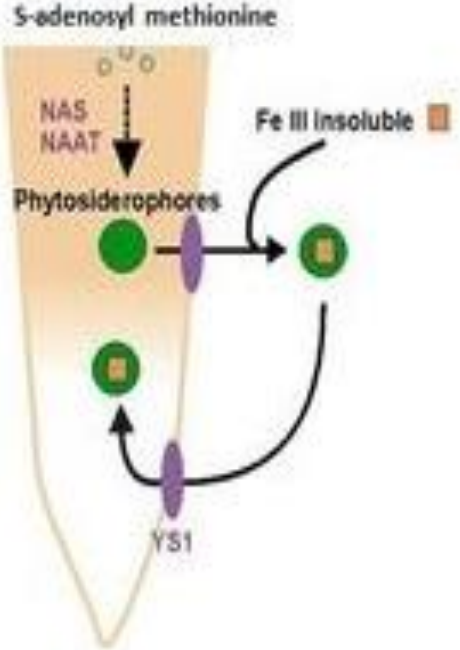
Strategy II -Graminaceous plants solubilize soil Fe by secreting mugineic acid family phytosiderophores (MAs), from their roots.

The resulting Fe(III)-MA complexes are then reabsorbed into the roots through a specific transporter.

- Strategy I (Fe reduction-based)
  - membrane-bound Fe chelate reductase
  - membrane-bound Fe II transporter
  - in angiosperms except grasses



- Strategy II (Fe chelation-based)
  - enzymes for phytosiderophore production
  - membrane-bound exporters for phytosiderophores
  - membrane-bound importers for Fe III-phytosiderophore complexes
  - in grasses



- **Major elements of the training programme:**

- **1. Growing the selected mutant lines of spring wheat (M5) that differ for their grain Fe content with their control varieties under greenhouse conditions in the hydroponic systems under normal and Fe-deficient conditions**

- 2. **Total RNA extraction and cDNA synthesis;**
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- **3. Data analysis and interpretation**

- **Task of the training:**
- **To investigate genes encoding iron (Fe) transporters (including YSL family, nicotianamine synthase (NAS) genes)**
- **in 3 new mutant lines of spring wheat (M5) that differ for their grain Fe content were chosen as experimental material;**

- **The selected mutant lines with their control varieties were grown under greenhouse conditions in the hydroponic systems and were subjected to variable iron supply (iron sufficient and iron deficient conditions).**

List of wheat genotypes

N	Wheat varieties/mutants	
1	Almaken St	Background
2	Almaken 95(7)	Mutant 1
3	Almaken 95(8)	Mutant 2
4	Eritrospermum St	Background
5	Eritrospermum 144(1)	Mutant 1
6	Eritrospermum 153(5)	Mutant 2
7	Zhenis St	Background
8	Zhenis 49(2)	Mutant 1
9	Zhenis 51 (8)	Mutant 2

Investigated genes:

DMAS;
bHLH;
NAS1;
SAMS;
TOM;
POT;
Fer1A;
YSL;
NRAMP
NAA;

- **Total RNAs was extracted from the roots and leaves of 6 week seedlings of wheat background varieties and their mutant lines.**
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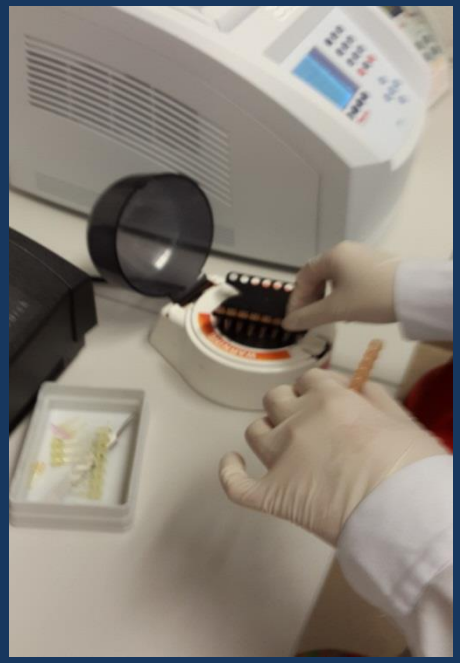
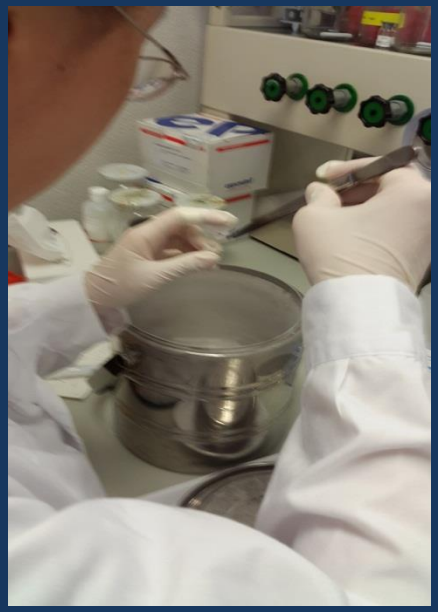
- **Total RNA was isolated from the shoots and roots. The RNA was denatured and electrophoresed on 1.5 % (v/v) agarose gels.**

- **Real-time quantitative PCRs (qRT-PCR) were performed**






Kumar

- **TOM gene**

- **TOM is an efflux transporter of dioxymugineic acids (DMA)**
- **In normal conditions in roots of mutants Zhenis 49(2) TOM gene was not expressed; in mutants Zhenis 49(2) and in roots of mutant Zhenis 51 (8) ($p < 0.01$) under Fe-deficient conditions the level of expression of TOM was higher in 3,53 times as compared to normal, but in background the differences were not significant .**

- **In normal conditions the differences in expression level in mutant Zhenis 49(2) was lower as compared to background; in Fe-deficient conditions the level of expression of TOM gene was higher ($p < 0.05$) as compared to background; in mutant Zhenis 51 (8) the expression level of TOM gene did not differ significantly.**

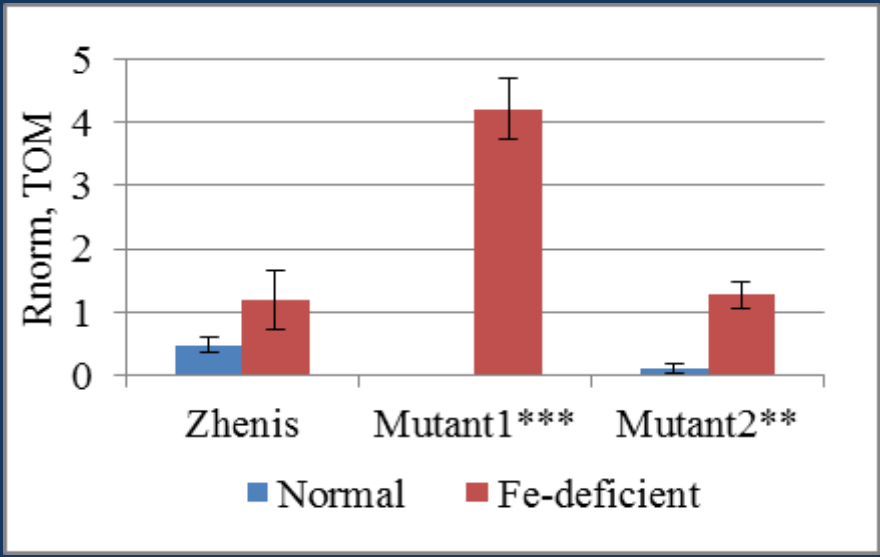


Fig.28A. The expression level of TOM gene in roots of Zhenis cv, mutant Zhenis 49(2) (mutant 1), Zhenis 51 (8) (mutant 2).

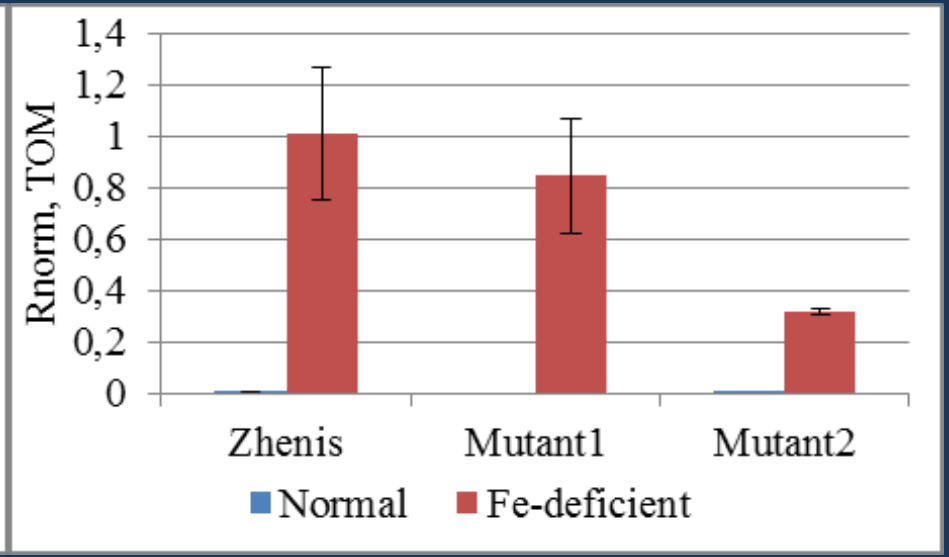


Fig. 28B. The expression level of TOM gene in shoots of Zhenis cv, mutant Zhenis 49(2) (mutant 1), Zhenis 51 (8) (mutant 2).

In Fe-deficient conditions the expression level of TOM gene did not differ significantly (Fig.28B) as compared to normal conditions.

In mutants the expression level of TOM gene in normal and Fe-deficient conditions as compared to background did not differ significantly ($p > 0.05$).

Summary of expression differencies obtained between background Zhenis cv and mutants (Zhenis 49(2), Zhenis 51(8) in normal and Fe-deficiency conditions

Zhenis cv, mutant Zhenis 49(2), Zhenis 51(8)										
Genes	Zhenis cv Background		Zhenis 49(2)		Background/ Zhenis 49(2),		Zhenis 51(8)		Background/ Zhenis 51(8)	
	Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
SAMS	-	-	16.27↑↑	-	- / -	-/ -	8.8↑	-	-/ -	- / -
NAAT	-	-	-	-	- / -	- /-	7.12↑	-	2.6 ↓/ -	- /-
NAS1	-	-	0/(66.8)↑	-	(16.56) 0↓↓/-	- / -	13.36↑	-	3.63↓/-	- /-
DMAS	-	-	6.59↑	-	2.53↑/ -	- / -	12.13↑↑-	-	3.26 ↓↓/-	- /-
Fer1A	2.7↓	-	-	10.13↓	- / -	- / -	6.27↓	17.89↓	-/-	- /-
POT	-	5.04↑↑↑	3.84↑	-	- / -	- / -	3.06↑↑	4.63↑↑↑	- / -	- /1.2↓
bHLH	-	(0)/(0.92)↑ ↑	7.21↑	-	- / -	-/ -	(0)/(0.05)↑	(0)/(0.29)↑	-/-	-/3.16↓↓
TOM	-	0/-	(0)/(4.2)↑	0/-	(0.48) (0) ↓/3.53↑	- / -	-/10.96↑	(0)/-	- / -	- / -
YSL		4.24↑-	5.06↑	-	- / -	- / -	5.94↑↑-	-	2.49↓ / -	1.45↓↓/ -
NRAMP	-	1.95↑↑	2.33↑	-	- / -	- / -	2.12↑	-	- / -	- / -

Summary of expression differencies obtained between parent Almaken cv and mutant Almaken 95/7, mutant Almaken 95/8 at normal and Fe-deficient conditions

cv. Almaken (Background), Almaken 95/7 , Almaken 95/8										
Genes	Almaken cv Background		Almaken 95/7		Background/ Almaken 95/7		Almaken 95/8		Background/ Almaken 95/8	
	Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
SAMS	-	-	6.27↑	-	3,19↓/ -	-/ -	-	-	-/ -	- / -
NAAT	-	-	4.02↑	-	-/ -	- /1.89↓	-	-	- / -	- / -
NAS1	-	-	4.51↑	-	-/ -	- / -	-	-	-/-	- / -
DMAS	-	-	4.62↑	-	3.35↓/ -	- / -	-	-	- /-	- / -
Fer1A	0.36↓	12.5↓	0.35↓	-	1.87↑/ -	- / -	-	5.99↓	-/-	- / -
POT	1.74↑	5.04↑	2.45↑	6.12↑	- / -	- / -	-	3.9↑	- / -	- / -
bHLH	-	-	6.94↑	241.19 ↑	- / -	12.09↓/ -	0/-	-	(0.02)(0) ↓/-	- / -
TOM	-	0/-	3.16↑	0/0	3.35↓/ -	- / -	-	0/-	- / -	- / -
YSL	-	-	2.64↑	1.3↑	- / -	1.22↑/ -	--	-	- / -	1.47↑ / -
NRAMP	1.56↑		-	1.52↑	- / -	- / -	-	9.14↑	- / -	- / -

Summary of expression differencies obtained between parent Eritrospermum cv and mutants (Eritrospermum 144/1, Eritrospermum153/5 in normal and Fe-deficient conditions

Eritrospermum (Background), Eritrospermum 144/1, Eritrospermum153/5										
Genes	Almaken cv Background		Eritrospermum 144/1		Background/ Eritrospermum 144/1		Eritrospermum 153/5		Background/ Eritrospermum153/5	
	Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient		Normal/ Fe-deficient	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
SAMS	-	-	-	-	-/ -	-/ -	-	-	-/ -	- / -
NAAT	-	3.04↑	-	-	-/ -	- /3.9↓	-	-	- / -	- /2.77↓
NAS1	-	19.77↑	2.71↑	-	-/ -	1.48↓- /4.45↓	-	-	-/-	1.31↓ /8.91↓
DMAS	-	3.14↑	-	-	-/ -	- /2.39↓	-	-	-/ -	-- /2.22↓-
Fer1A	4.44↓	6.85↓	-	15.76↓	-	3.46↑↑/ -	-	-	-/-	- / -
POT	2.73↑↑	5.98↑↑	2.73↑↑	5.59↑↑	- / -	3.46↑↑/ -	2.00↑↑/-	5.03↑↑	- / -	- / -
bHLH	-	0/-	-	0/-	- / -	-/ -	- / -	--/ -	- / -	- / -
TOM	-	0/-	-	0/-	- / -	- / -	- / -	0/-	- / -	- / -
YSL	-	2.84↑↑	-	-	- / -	-/3.01↓↓	1.84 ↑	-	2.72↑ / -	-/2.18↓↓
NRAMP	-	-	-	-	- / -	- / -	-	-	- / -	- / -

- **Thus, under Fe-deficient conditions all studied genes responsible for iron uptake and transport (except *Fer1A*) were up-regulated in roots of mutant Almaken 45/7.**
- **It means that under Fe-deficient conditions the high expression level of these genes could increase grain iron content.**

- **NAS1 and POT genes in roots of mutant Eritrspermum144/1 and POT and YSL and in roots of mutant Eritropspermum153/5 were up-regulated under Fe-deficient conditions. Fer1A and POT genes in shoots of mutant Eritrspermum144/1 were expressed in normal conditions at higher level as compared to parent Erotrospermum cv.**

- **All studied genes, except Fer1A (down-regulated) and NAAT genes, in roots of mutant Zhenis 49(2) were up-regulated under Fe-deficient conditions.**

- **In roots of mutant Zhenis 51(8) all studied genes, except Fer1A (down-regulated) and TOM gene, were up-regulated under Fe-deficient conditions. Only in mutant Zhenis 49(2) the expression level of TOM gene was higher (10.86 fold) as compared to parent Zhenis cv.**

- **In mutant Zhenis 49(2) the up-regulation of TOM gene and it's higher expression as compared to background means that this mutant have high ability to release phytosiderophores and iron uptake under Fe-deficient conditions.**
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- **Other genes didn't show the high expression as compared to control under Fe-deficient conditions. These data are of interest for further analysis and investigation.**