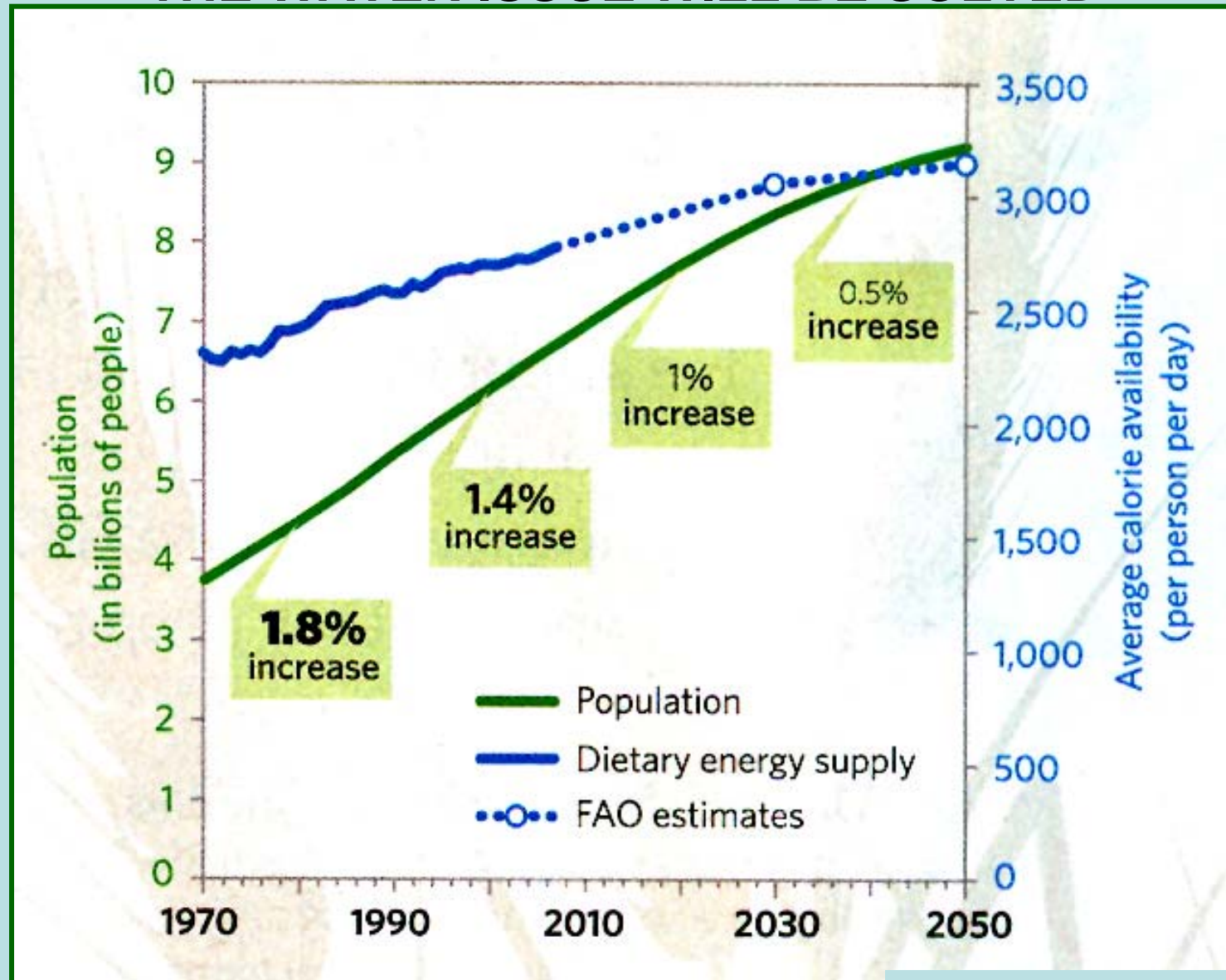




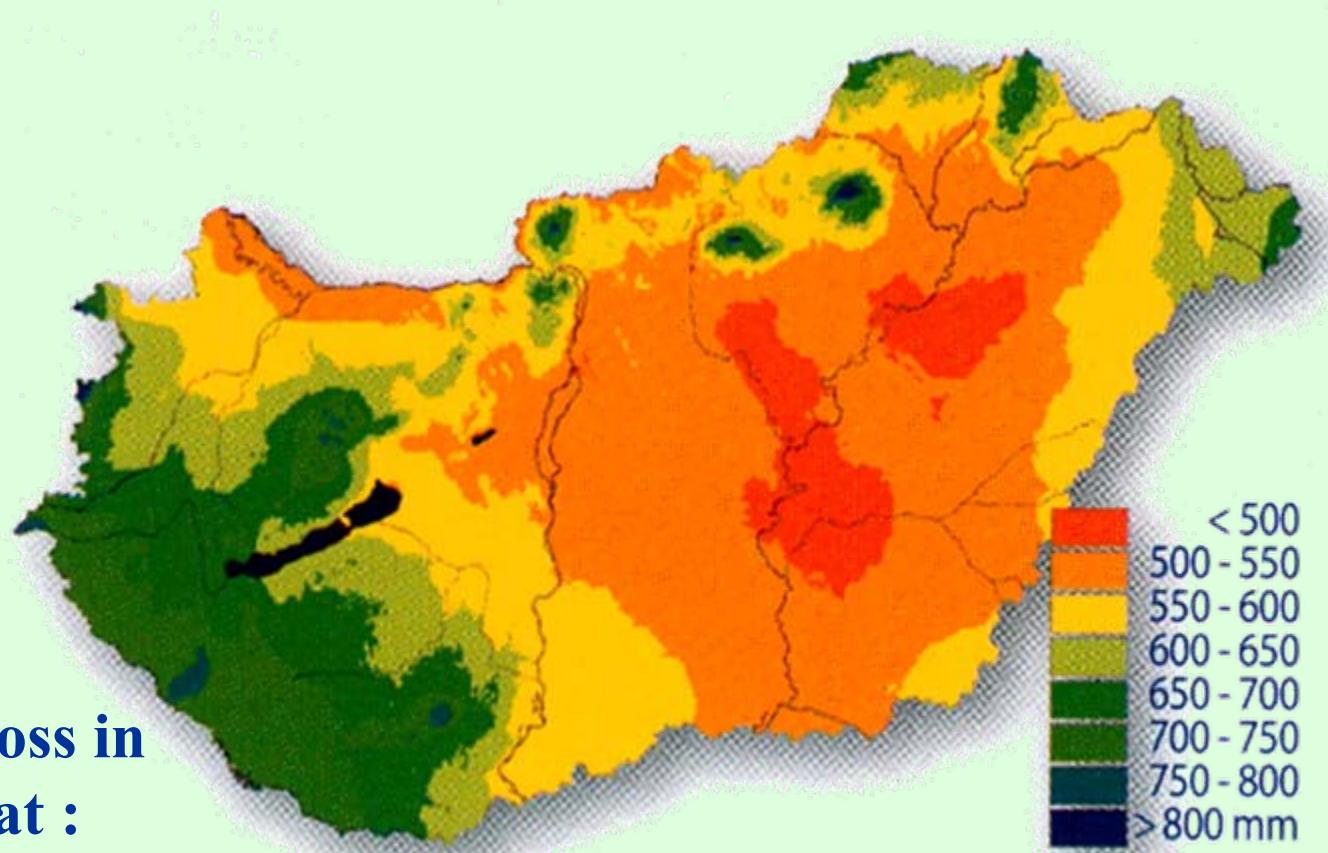
**COMBINED APPLICATION OF PHENOMICS AND GENOMIC TOOLS
IN THE ANALYSIS OF DROUGHT**

Dénes Dudits Institute of Plant Biology, Biological Research Center, H.A.S. Szeged

FUTURE FOOD PRODUCTION CAN MEET THE NEEDS IF THE WATER ISSUE WILL BE SOLVED



SEMI- DESERT CONDITIONS IN HUNGARY



**Yield loss in
wheat :**

2003: 25%

2007: 12%

2012: 35%

Average precipitation/ year : 1971-2000

GENES AND ENVIRONMENT DETERMINE YIELD

PHENOTYPES

Yield - product

F1

F2

F3

F4

F5

Temperature

Precipitation

Sunlight

ENVIRONMENTAL FACTORS

Agrotechnologies

Use of fertilizers

Weed control

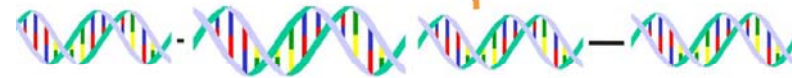
Plant protection

Irrigation



GENOME = GENES

Target for plant breeding



Methods:

Crossing (heterosis) → selection

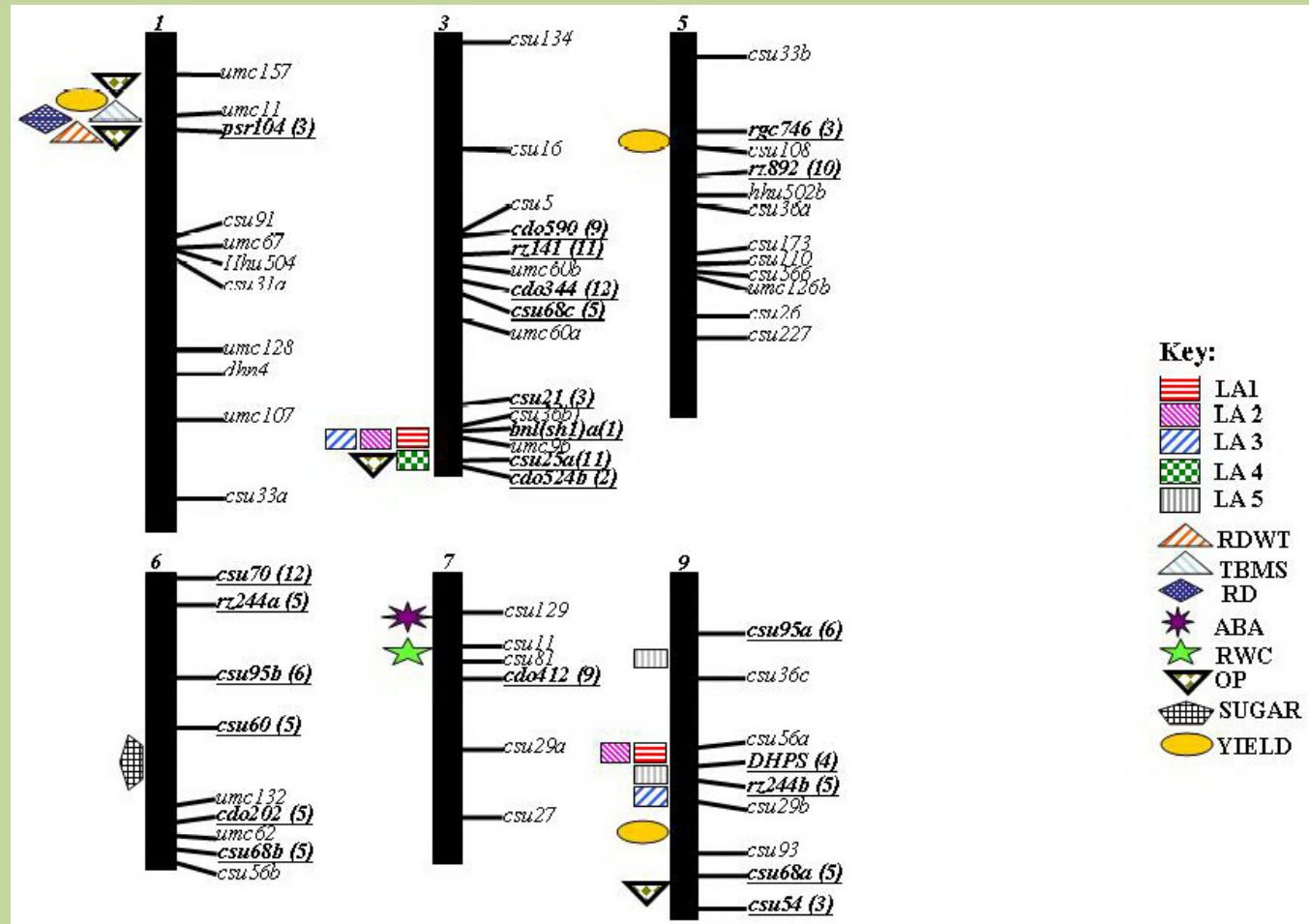
Gene technology (GM maize)

Phenotypic

Molecular marker assisted

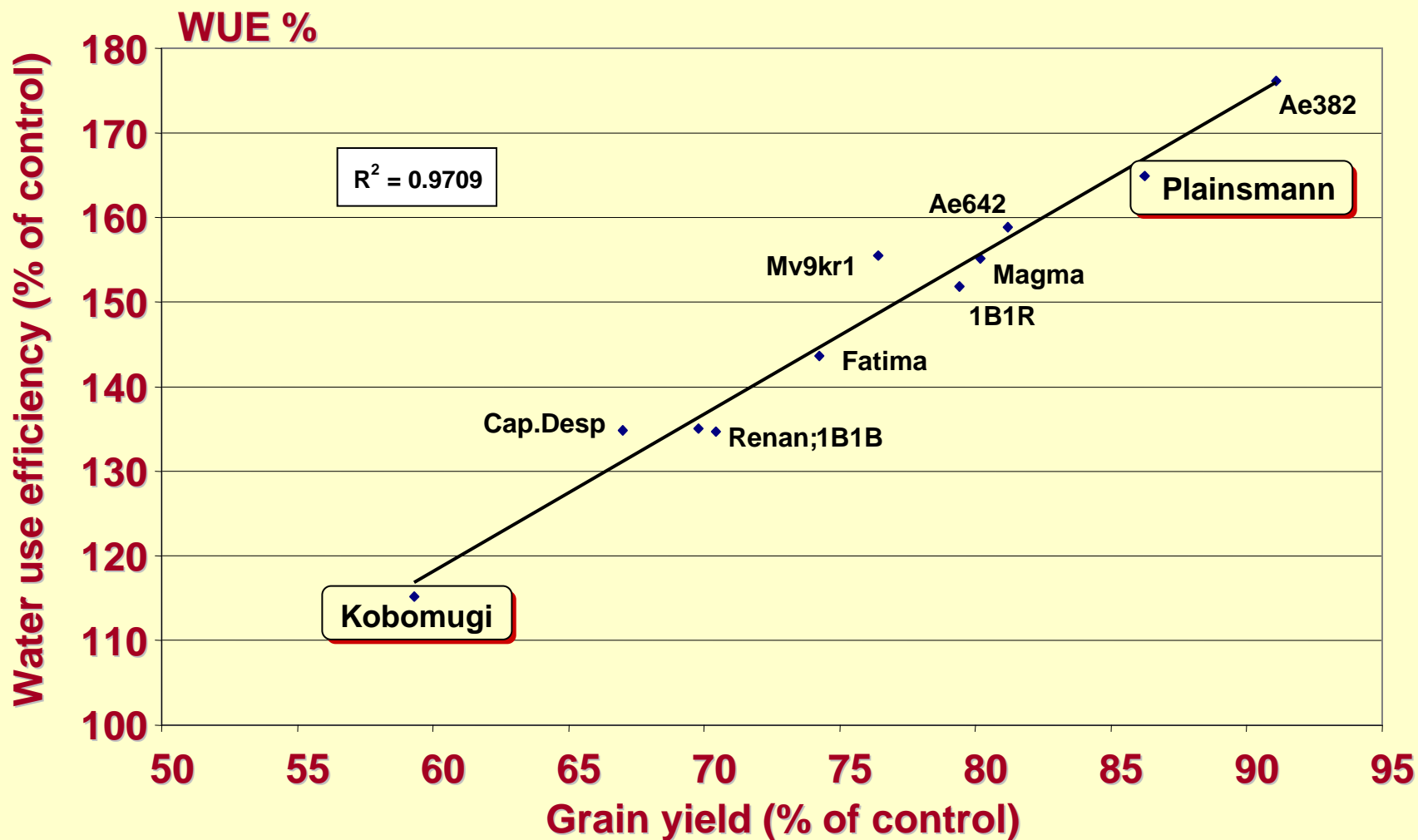
MAIZE CHROMOSOMAL REGIONS CONTROLLING DROUGHT STRESS RELATED TRAITS :

QUANTITATIVE TRAIT LOCI: QTLs



SUGAR = sugar concentration; GY = grain yield under drought; L-ABA = leaf abscisic acid content; OP = osmotic potential; RWC = relative water content; RD = root density; RDWT = root dry weight; TBMS = total biomass; LA = leaf surface area.

CORRELATION BETWEEN AGRONOMIC WATER USE EFFICIENCY (WUE) AND YIELD AFTER WATER STRESS (50% WATER SUPPLY) FROM FLOWERING

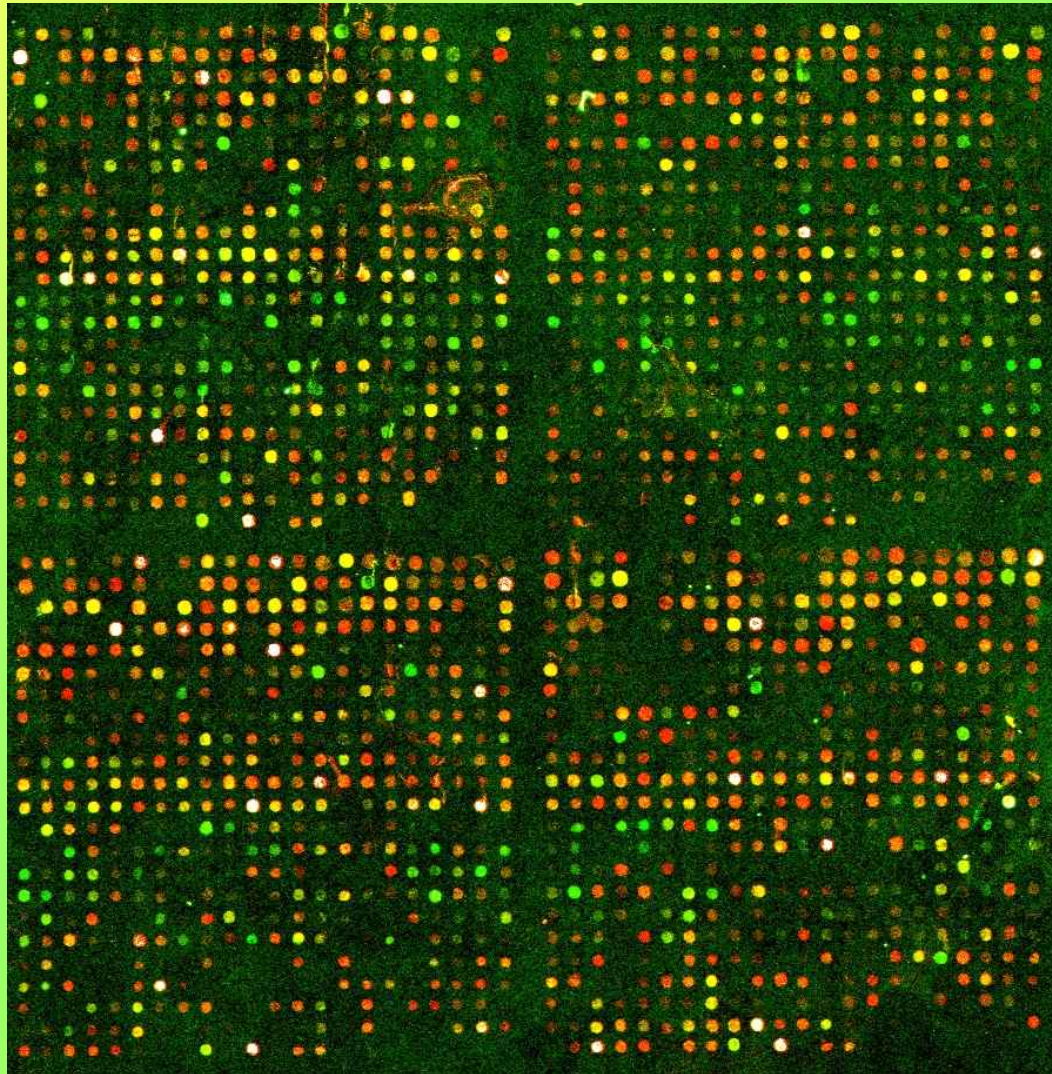


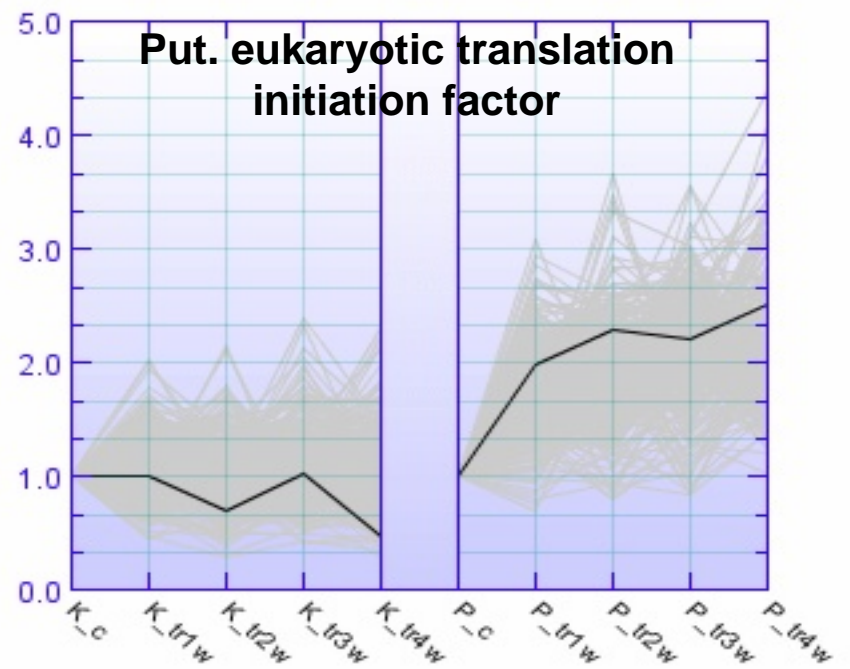
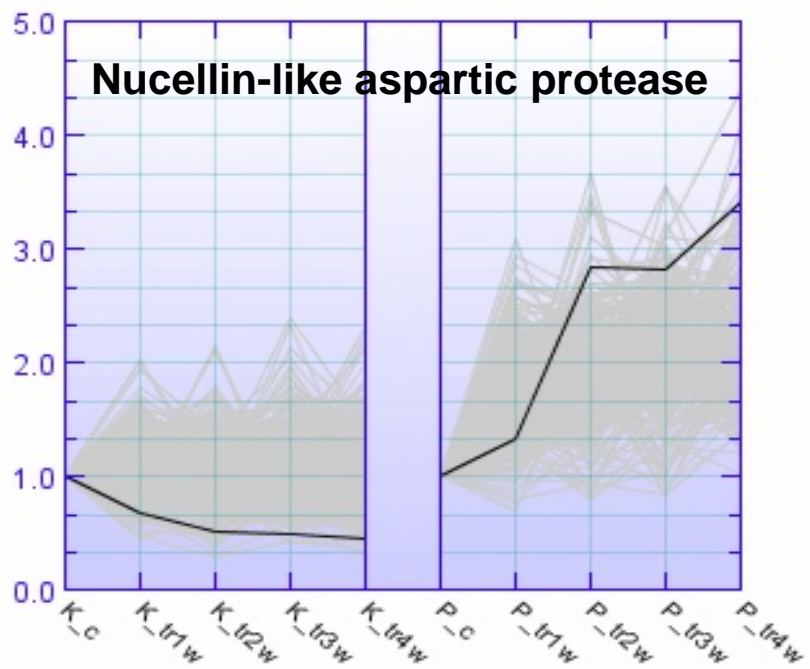
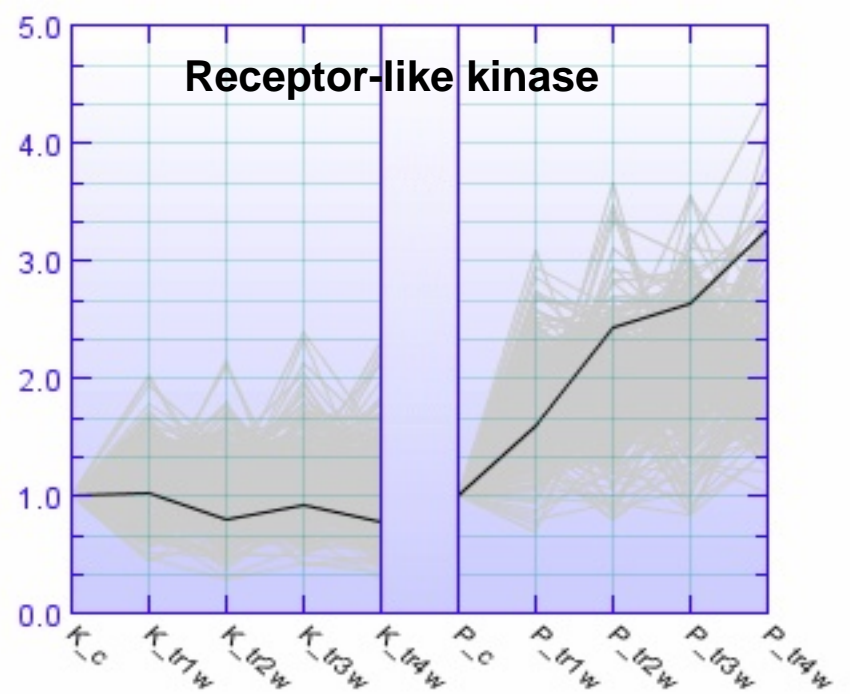
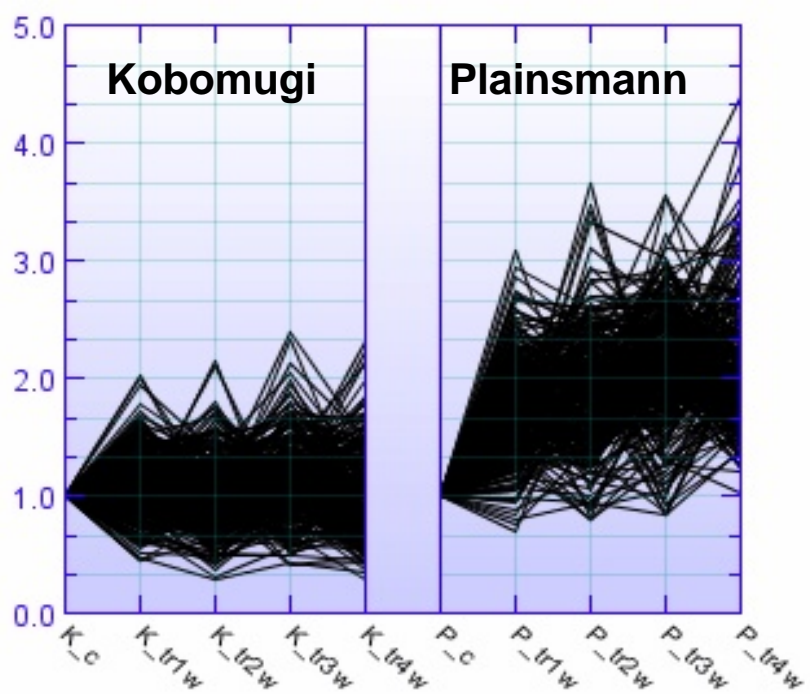


Morphological comparison of the two wheat genotypes grown at low (40%) and high (80%) relative field capacity.

Picture taken 22 days after germination. Sečenji et al., 2011

HYBRIDIZATION OF cDNA CHIP WITH PROBES FROM ROOTS OF WHEAT SEEDLING AFTER OSMOTIC STRESS





Conceptional Basis of Phenomics as an Independent Discipline:

Interpretation of high-dimensional phenomic data from different levels of organization:
from morphology through physiological state to molecular scale

External Phenotype

morphology, growth, development

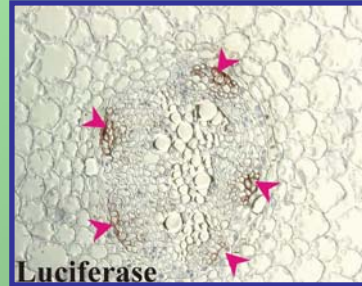


yield potential

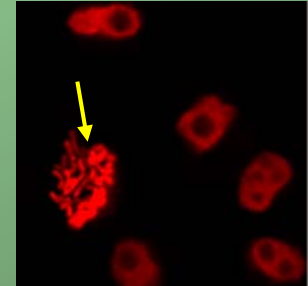


Internal Phenotype

tissue/organ
structure-anatomy

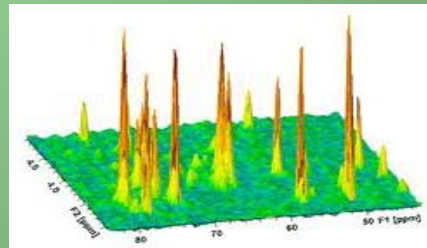


cellular structure-function

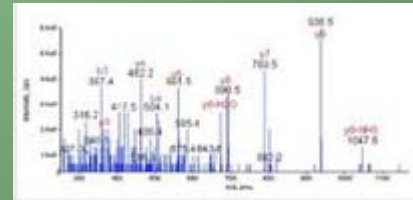


molecular phenotyping

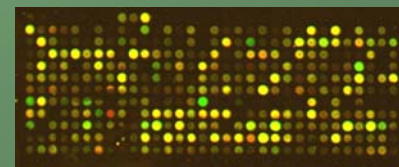
metabolomics



proteomics



transcriptomics



Genetic variants: **germplasm collections**; mutant libraries; mapping populations; **transgenic lines**

HIGH THROUGHPUT PHENOTYPING OF MAIZE PLANTS



Australian Plant Phenomics Facility



The Plant Accelerator®

HIGH-DIMENSIONAL PHENOTYPING OF MAIZE PLANTS

Australian Plant Phenomics Facility



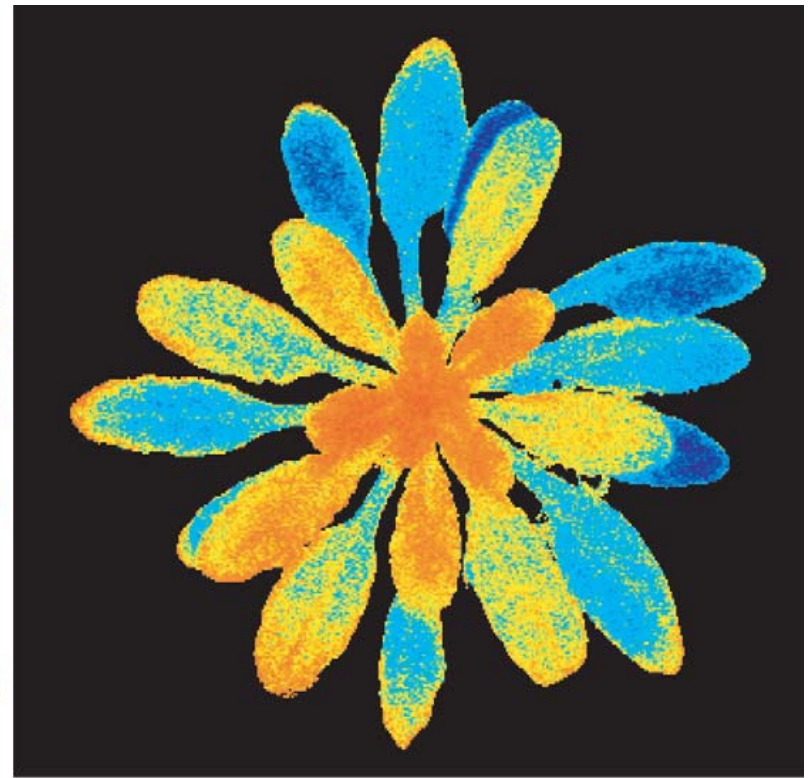
The Plant Accelerator®



Images of the chlorophyll fluorescence parameter F_q/F_m for *Arabidopsis thaliana*

Well watered

drought



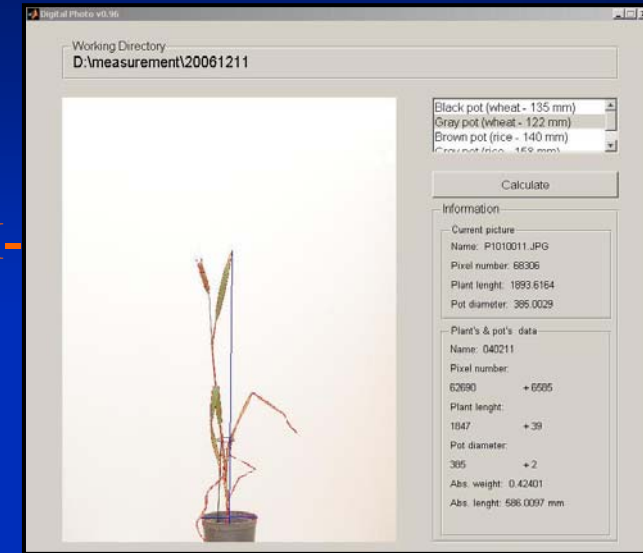
F_q'/F_m' scale: 0.2 0.3 0.4 0.5



Morison et al. Phil. Trans. R. Soc. B (2008) 363, 639–658

COMPLEX STRESS DIAGNOSTIC SYSTEM

LOW COST SEMI-AUTOMATIC PLATFORM SZEGED

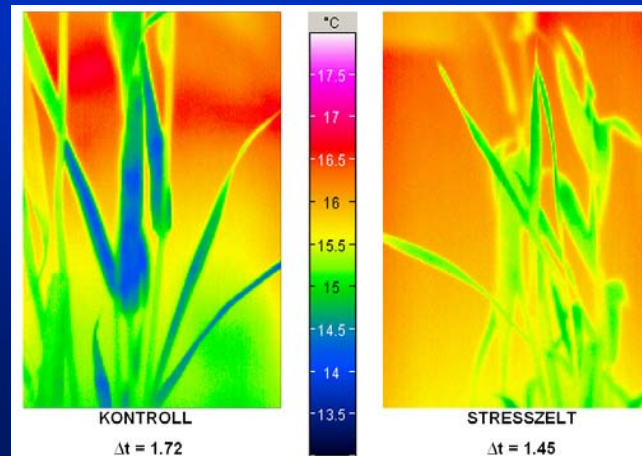


Chlorophyll fluorescence Fv/Fm

Thermal imaging of plants



The efficiency of leaf evaporation was assessed by measuring leaf temperature relative to the surrounding air using a sensitive VarioSCAN 3021 ST thermocamera.



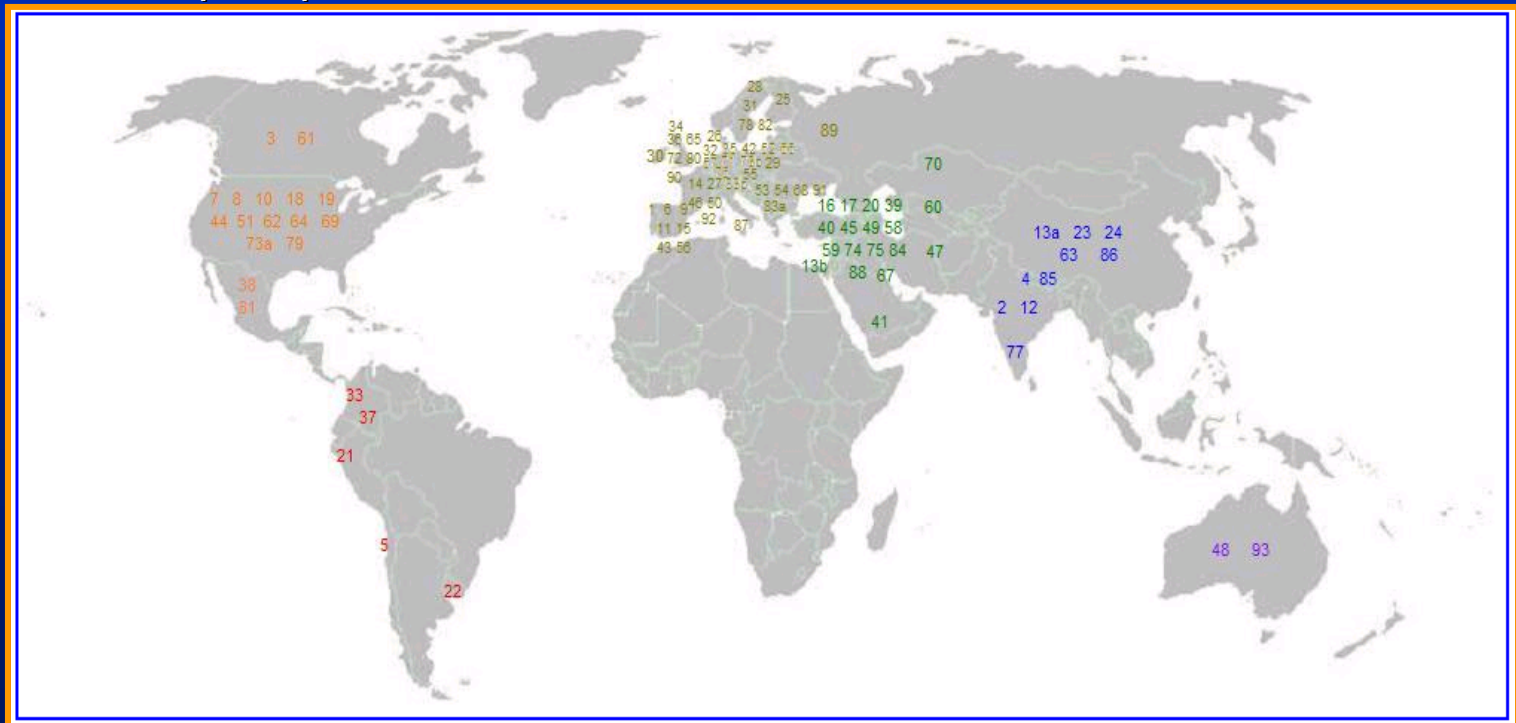
Establishment of a genotype collection to test the allelic variation

A set of 96 genotypes of barley:

- ❖ cultivars
 - ❖ landraces
- Collected worldwide

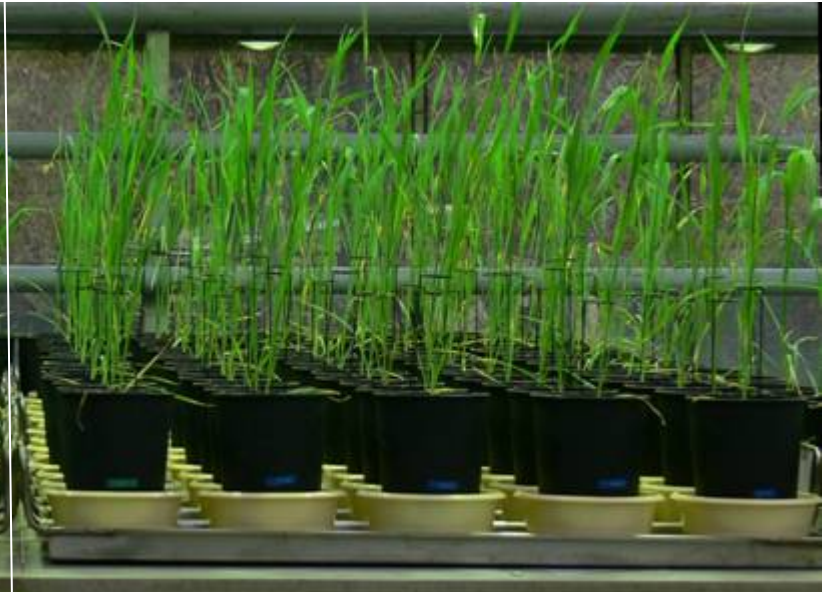


Selection of drought tolerant and sensitive genotypes based on literature and available passport data.



20% soil water

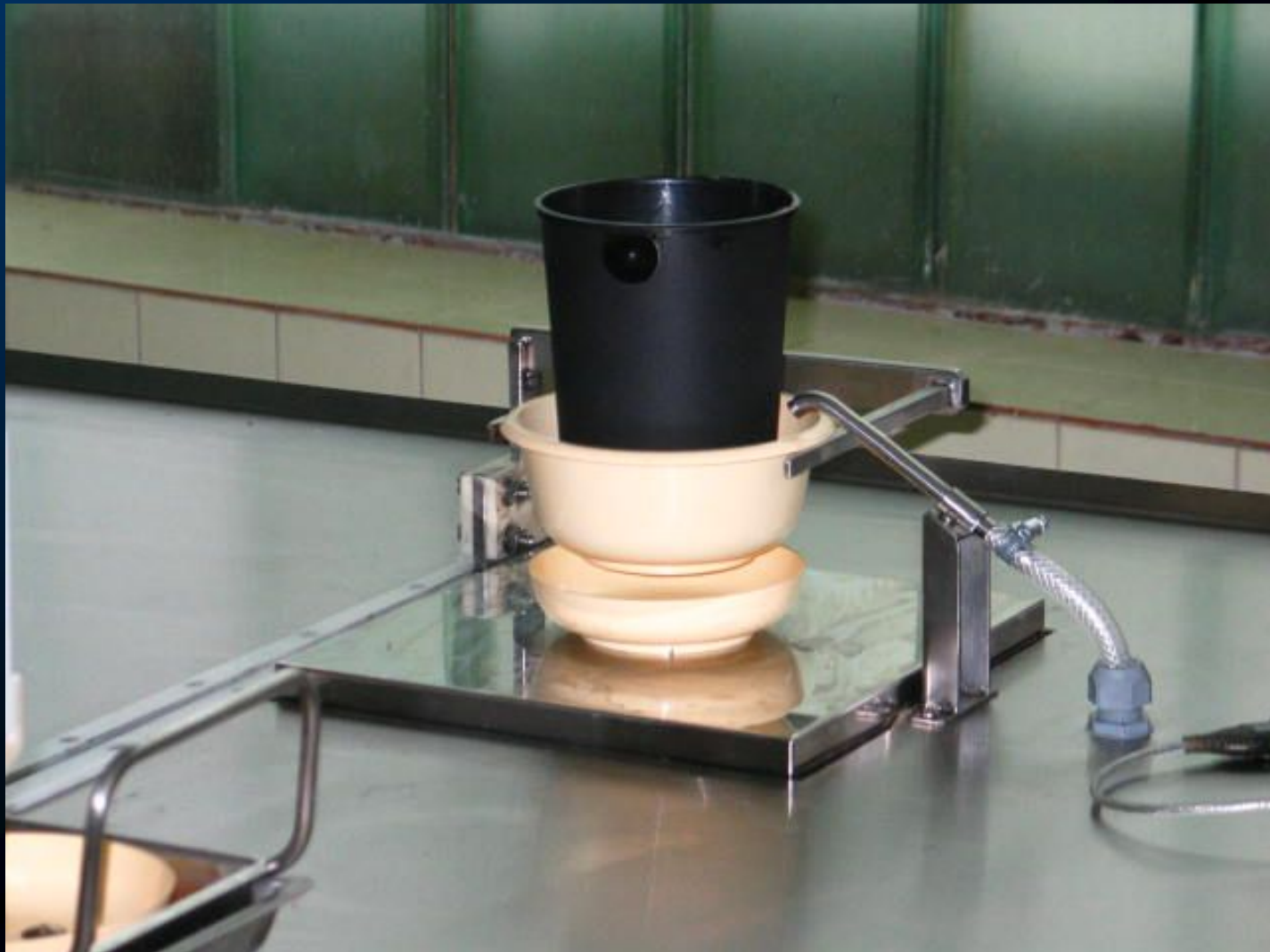
60% soil water



Complex stress diagnostic system



COMPUTER-CONTROLLED WATER SUPPLY



Digital photography



Imaging system



**Capelle Desprez-
control**



**Capelle Desprez-
drought**

DIGITAL PHOTOGRAPHY

Original RGB picture



The „green” pixels of the picture

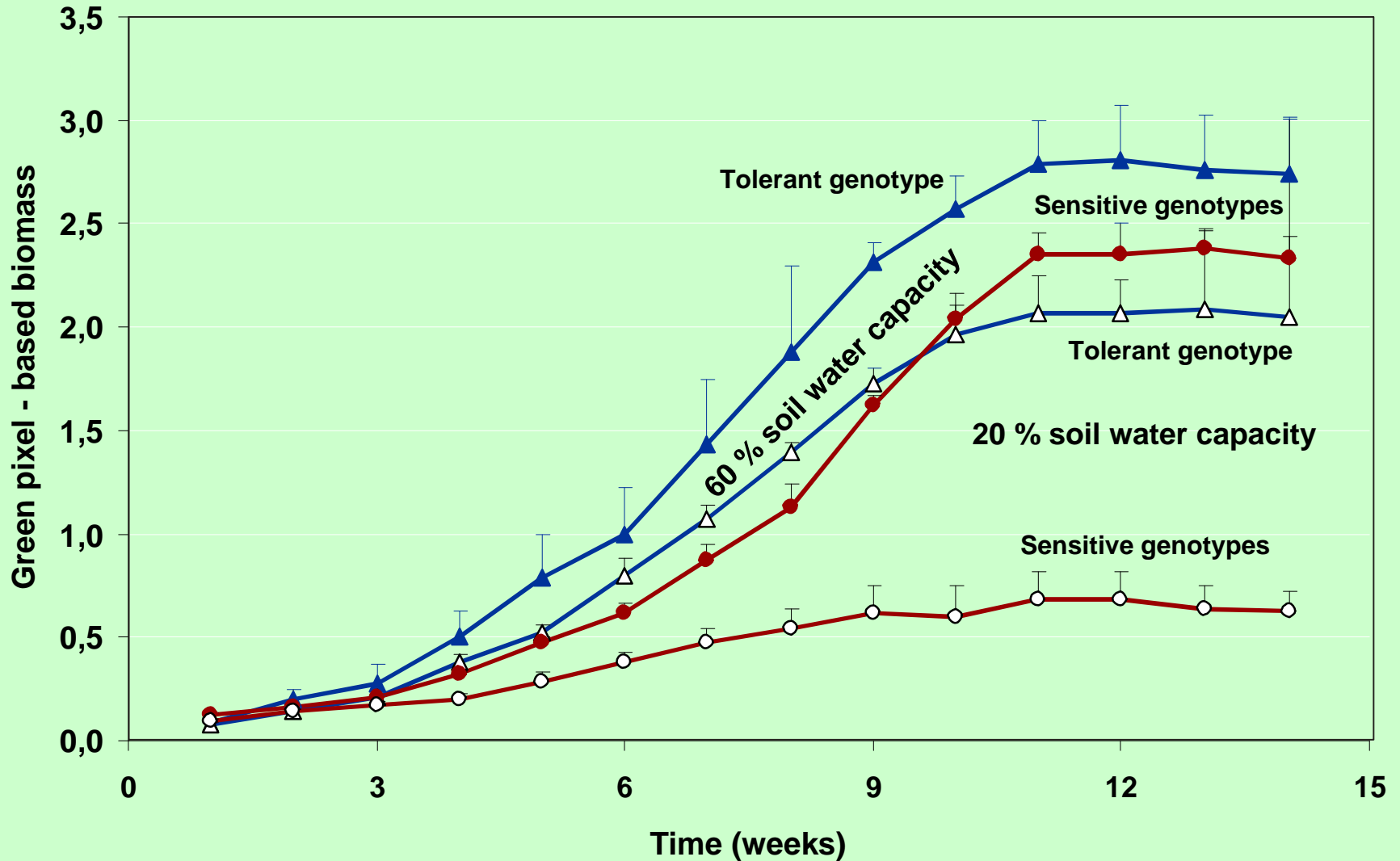


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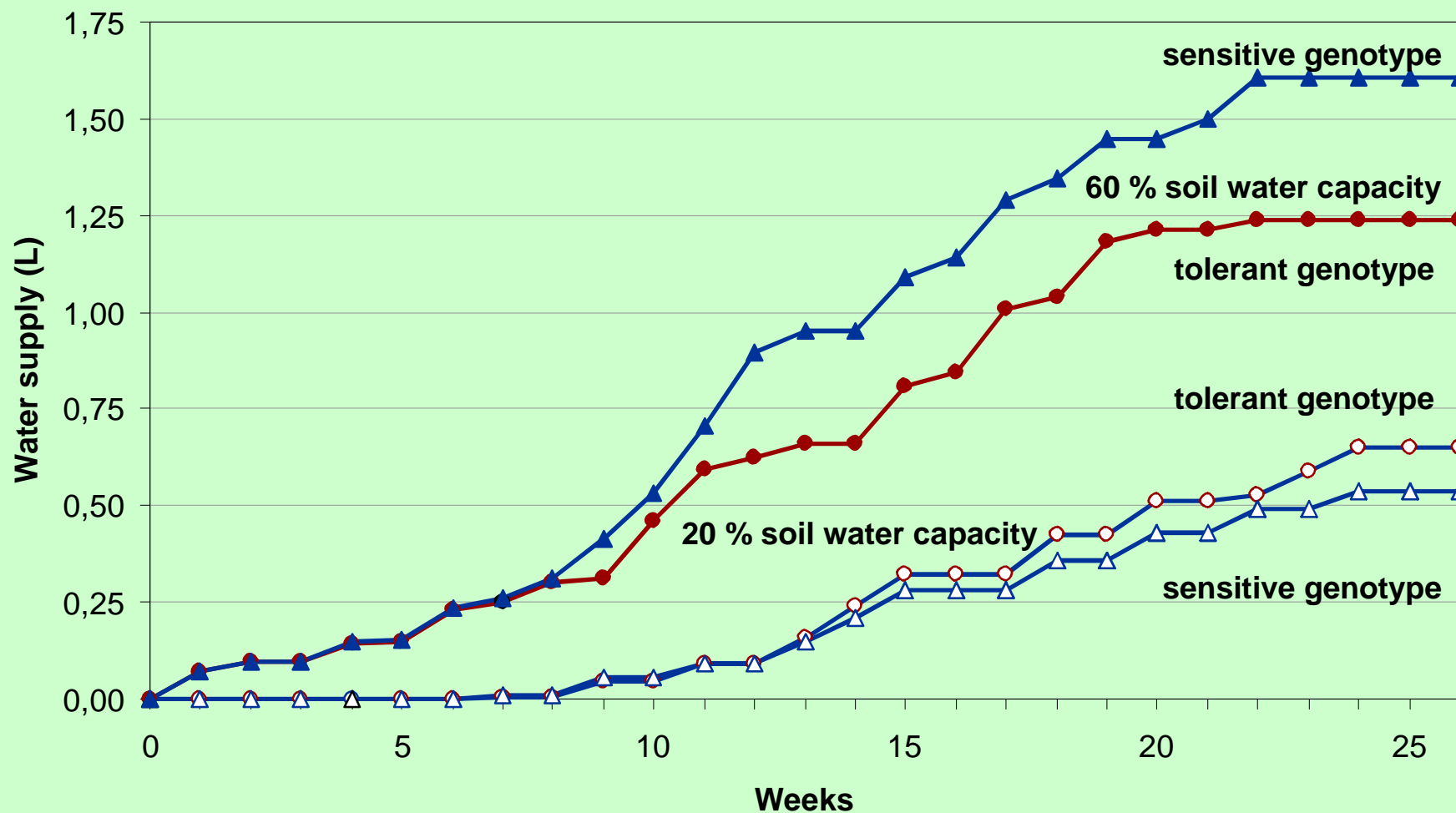
Pixel number: 189009

Height: 64.4 cm

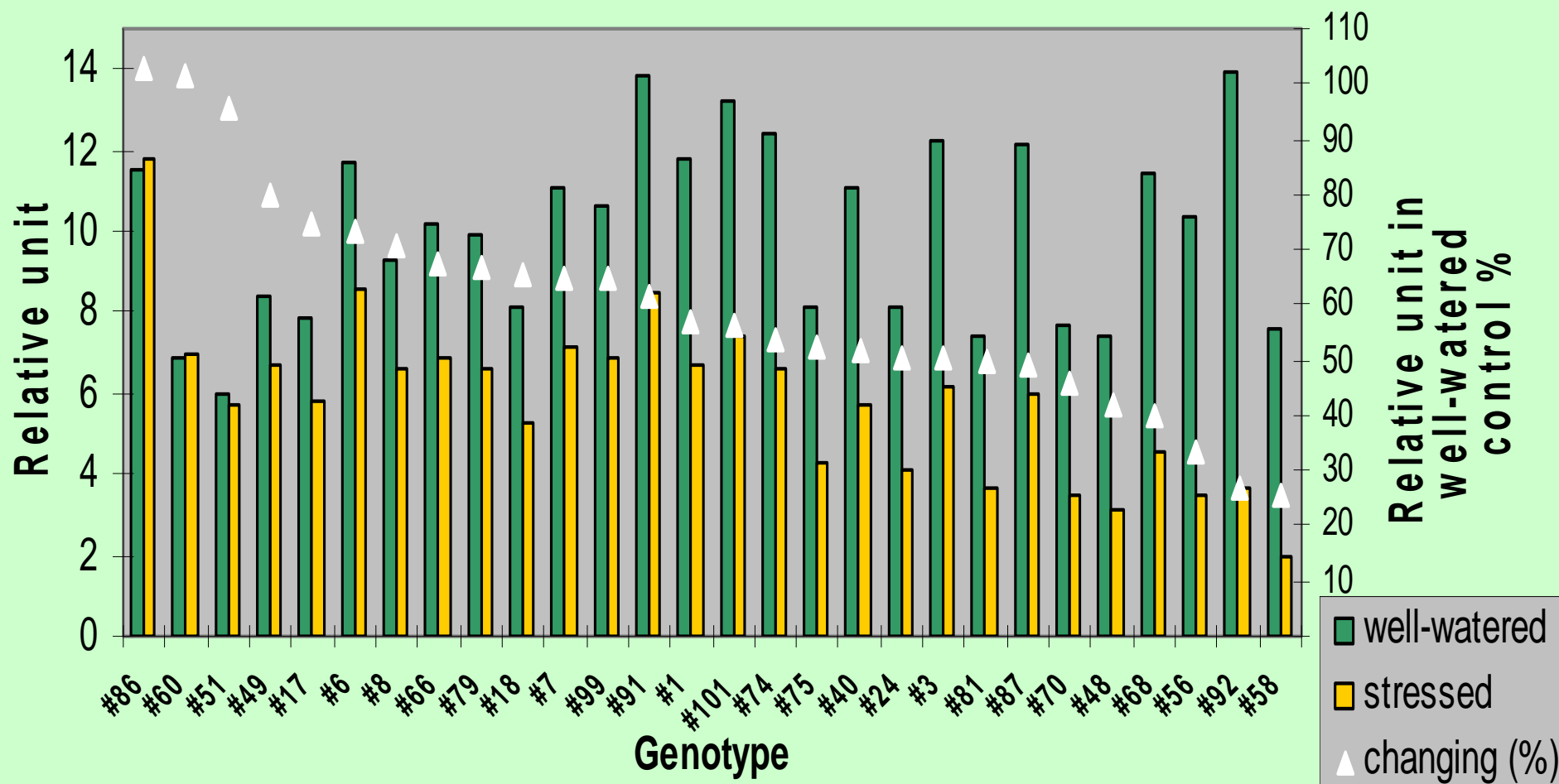
GREEN BIOMASS ACCUMULATION DURING DEVELOPMENT DEPENDS ON THE SOIL WATER STATUS AND THE GENOTYPE



GENOTYPE- AND WATER SUPPLY-DEPENDENT WATER USE OF BARLEY PLANTS

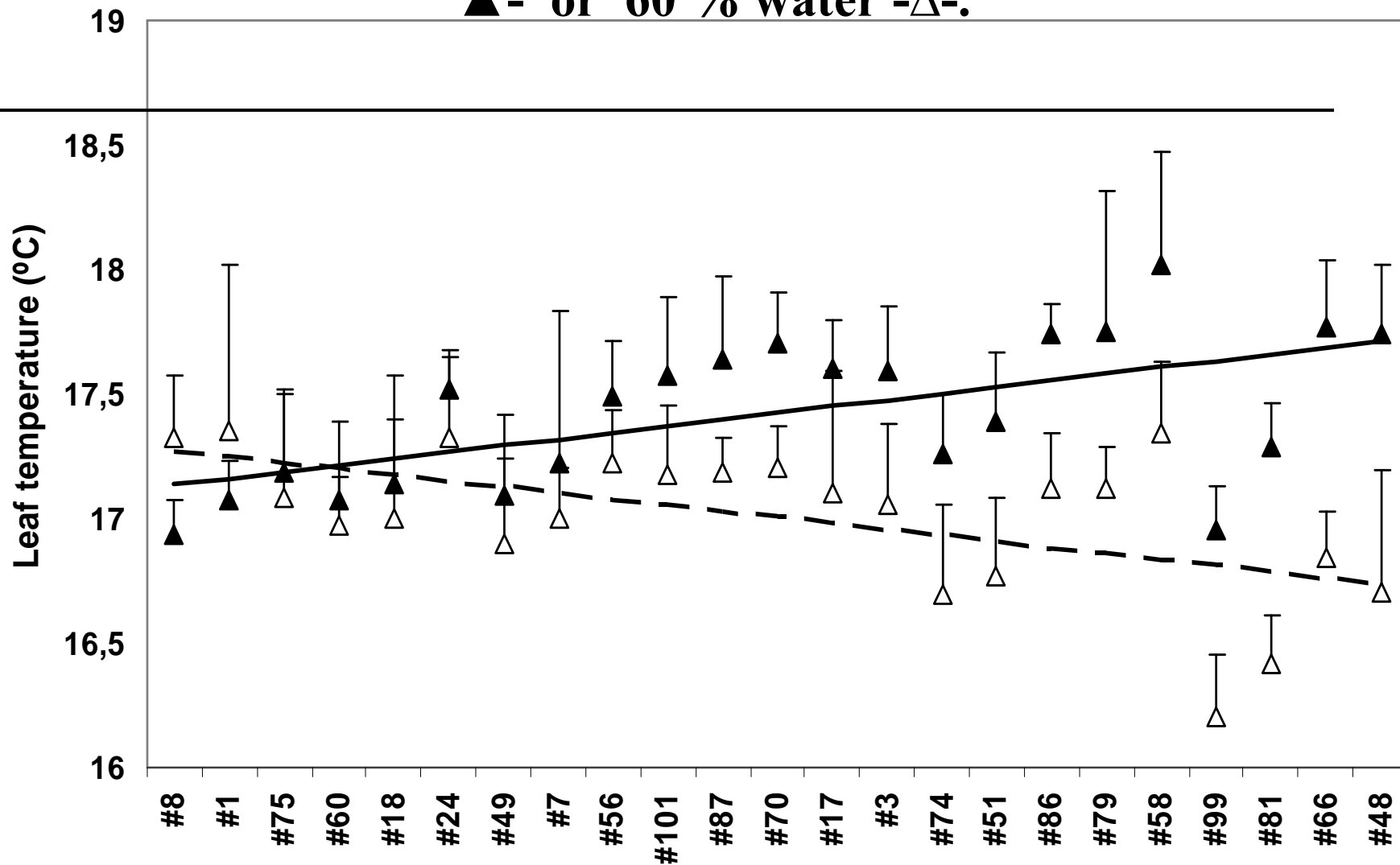


Biomass at flowering



Thermal imaging of leaf temperature as an indicator for evaporation by barley plants grown in soil with 20 % water -

▲ - or 60 % water - △ -.



AIR TEMPERATURE = 18.56 °C

Genotypes

DEPENDENCE OF WATER CONSUMPTION, BIOMASS ACCUMULATION AND LEAF TEMPERATURE ON THE INTERACTION BETWEEN GENOTYPE AND ENVIRONMENT DETERMINED BY WATER SUPPLY.

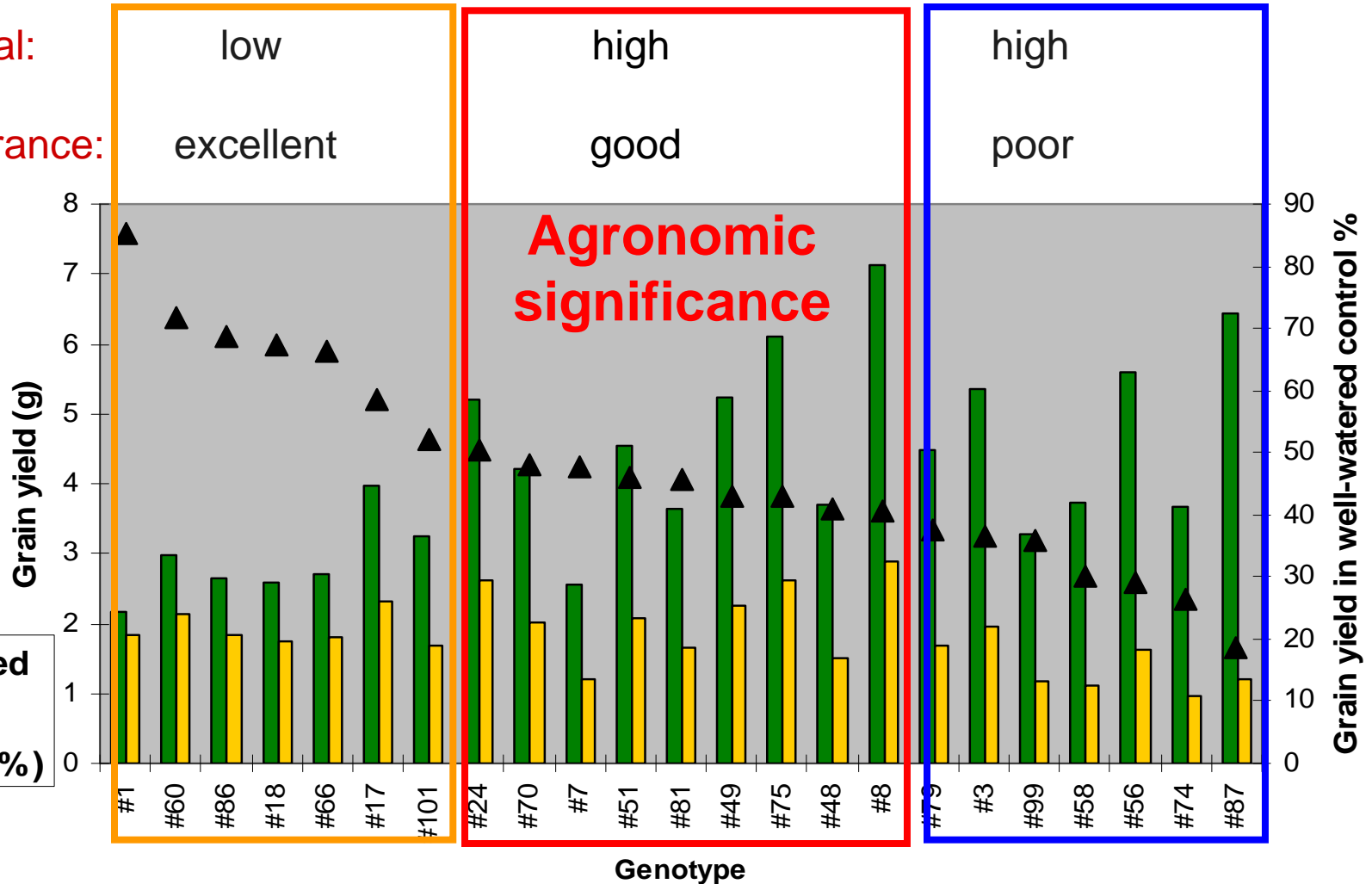
		Water consumption (L)	Biomass (relative unit)	Leaf temperature differencies (°C)
Sensitive genotypes	60% soil watercapacity	1.90 (0.24)*	1.52 (0.24)*	-1,21 (0.17)*
	20% soil watercapacity	0.41 (0.19)*	0.39 (0.16)*	-0.53 (0.21)*
Tolerant genotype	60% soil water capacity	1.63 (0.22)*	2.66 (0.25)*	-2.35 (0.18)*
	20% soil water capacity	0.91 (0.06)*	1.37 (0.11)*	-1.60 (0.26)*

Effects of drought stress on the grain yield

/Complex Stress Diagnostic System/

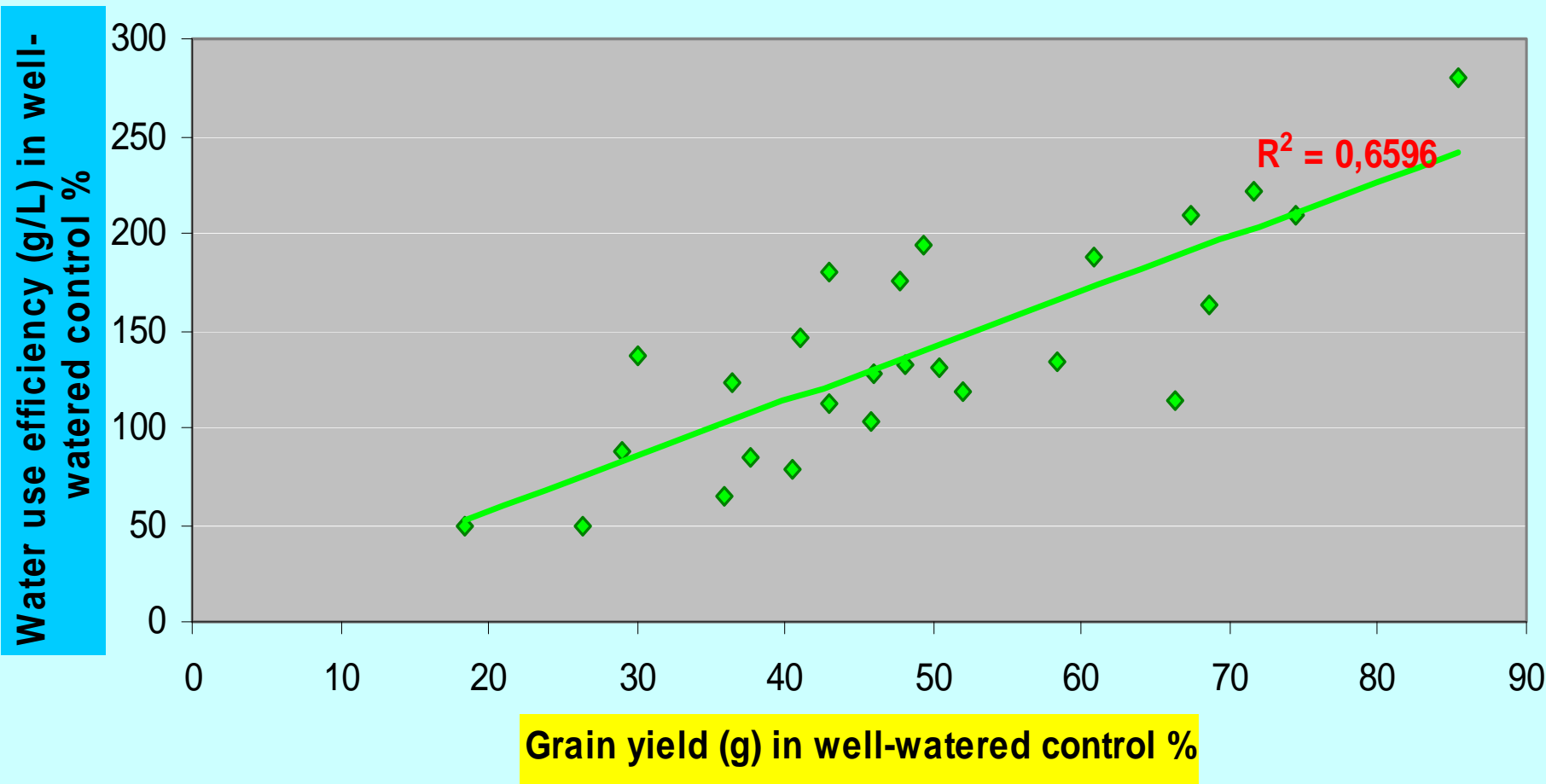
Yield potential:

Drought tolerance:



Correlation between water use efficiency and grain yield

/Complex Stress Diagnostic System/



EcoTILLING technology as a polymorphism discovery tool.

A variant of *Targeting Induced
Local Lesions IN Genomes.*

- ✓ *high throughput,*
- ✓ *low cost technique for*
- ✓ *rapid discovery of
polymorphisms*

in natural populations.

Efficient detection of:

- *single nucleotide
polymorphisms(SNPs)*
- *small insertions and deletions
(INDELs)*

EcoTILLING reaction

1. PCR amplification of target regions:

Fluorescently labeled nucleotides

primer design:

available *genomic* or *mRNA* sequences

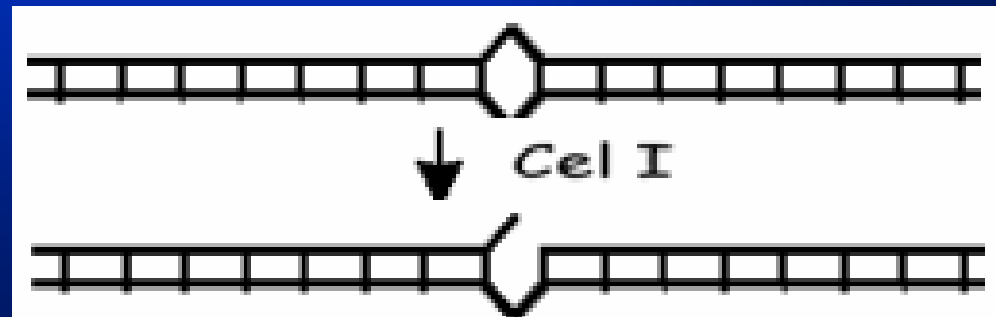
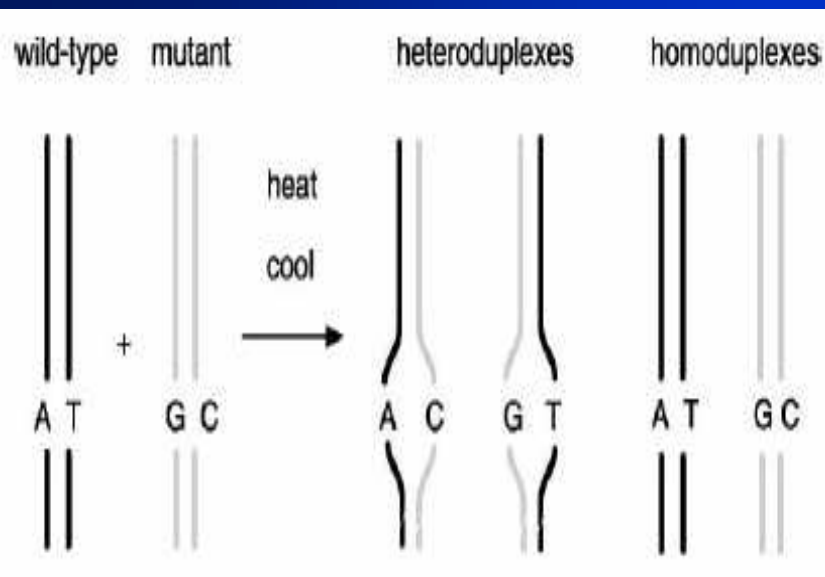
optimal amplicon size:
~1000 bp (500 – 1500)

2. Heteroduplex digestion:

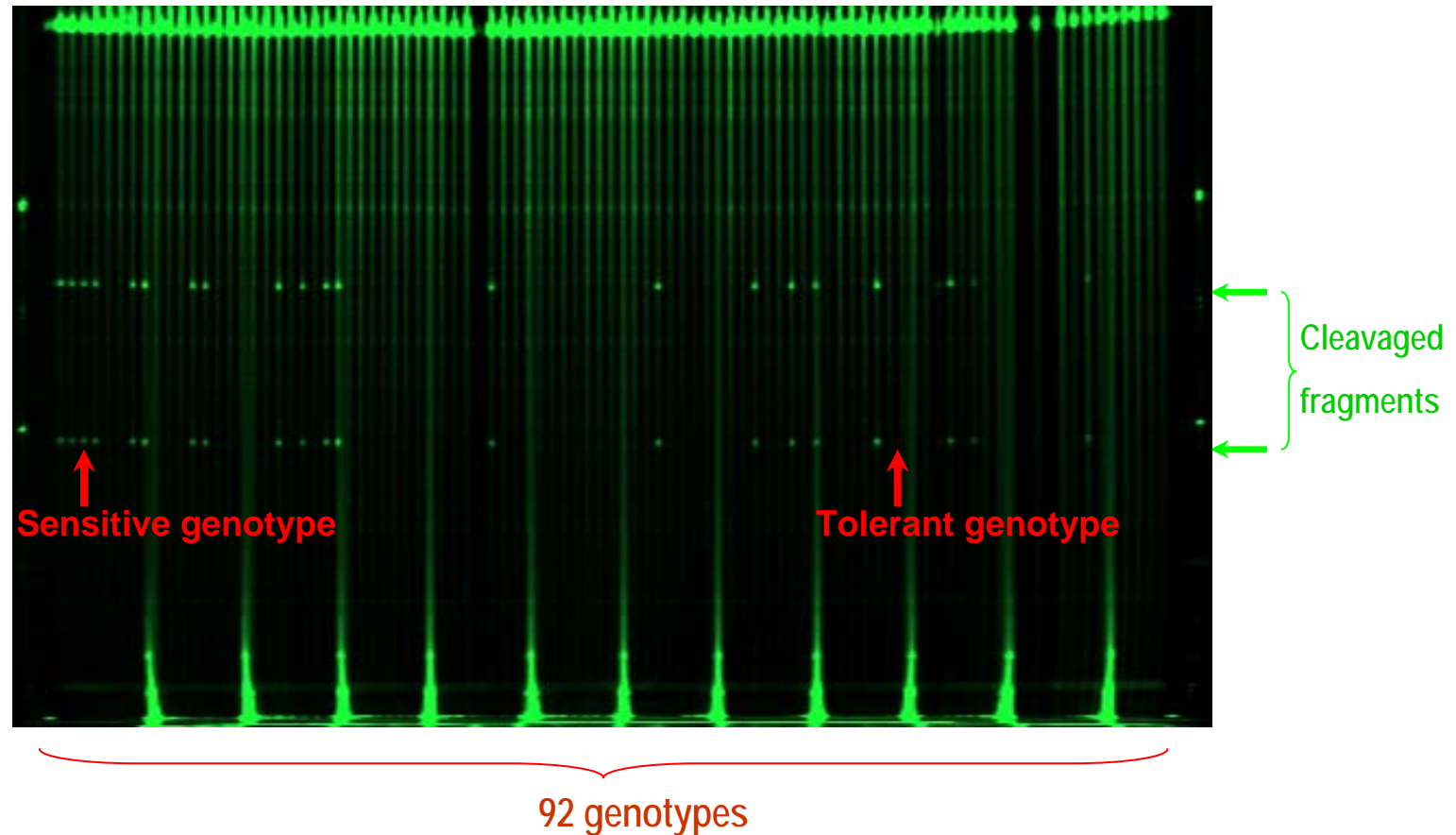
1. Heteroduplex formation
2. Nuclease digestion of DNA mismatches :

Single-strand specific nucleases (mismatch cleavage activity)

- *CEL1* (isolated from celery)
- *Endo-1* (Serialgenetics)



EcoTILLING screen of barley Dehydration Responsive Element Binding Protein gene

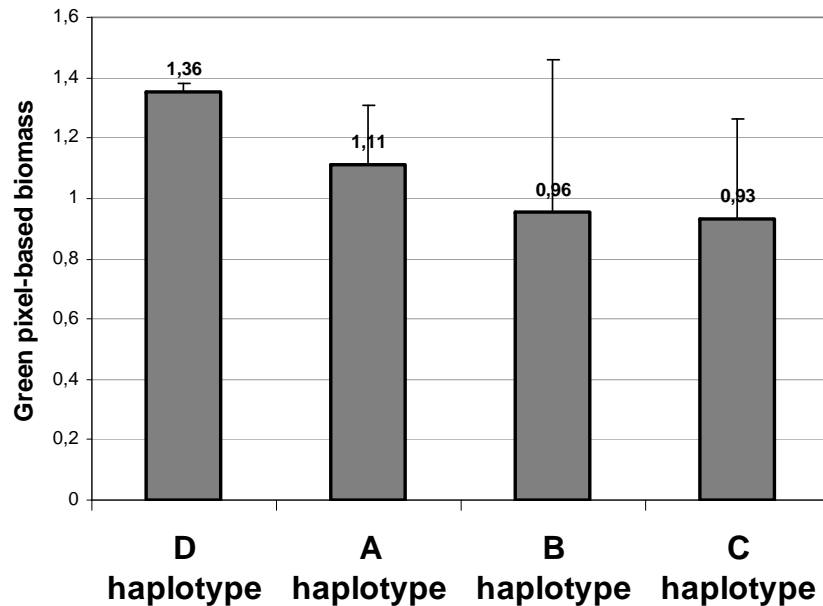


Multiple alignment for gene HvDREB1 plus/minus 30 bp around position 372

Haplotype id	Haplotype sequence
HvDREB1-4B	ATCTCCTCCTTCTCTTGTCTCAAATGCGCC A ACAGCTGCGCTGCATCGGTCTGATGCCAAG
HvDREB1-5A	ATCTCCTCCTTCTCTTGTCTCAAATGCGCC G ACAGCTGCGCTGCATCGGTCTGATGCCAAG
HvDREB1	ATCTCCTCCTTCTCTTGTCTCAAATGCGCC G ACAGCTGCGCTGCATCGGTCTGATGCCAAG

Sensitive genotype
Tolerant genotype

The haplotype D of the barley group 3 LEA protein HvA1 gene can positively influence of the green pixel-based shoot surface area



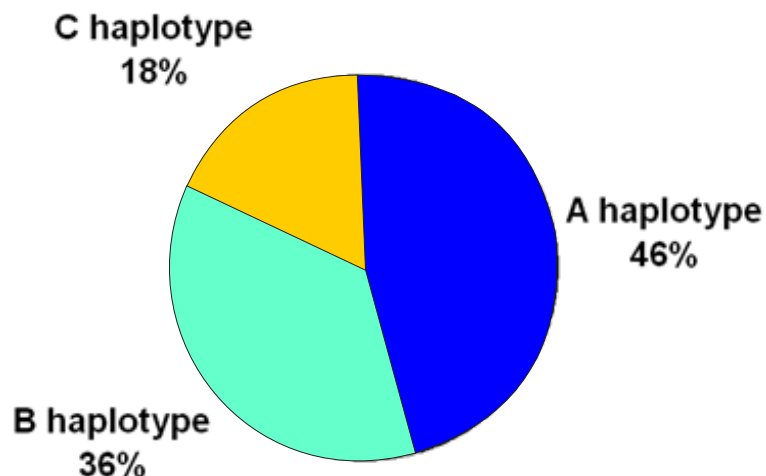
Average of green pixel-based shoot surface area data belonging to the four haplotypes

Nucleotide sequence of 4 unique haplotypes of HvA1 gene with the following characteristics

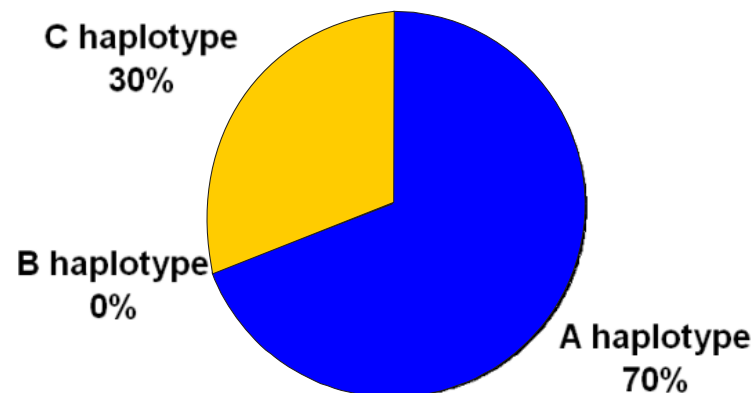
	1.S	2.S	3.S	4.S	5.S	6.S	7.S	8.S	1.D	9.S	10.S	11.S	12.S	2.I
Database sequence	G	A	G	C	C	G	C	G	33bp	G	G	G	G	---
A haplotype	G	A	G	C	C	G	C	G	33bp	G	G	G	G	---
B haplotype	G	A	G	C	T	G	C	G	33bp	G	G	A	A	---
C haplotype	C	A	G	T	T	A	G	C	33bp	G	G	G	A	---
D haplotype	C	A	C	T	T	A	G	C	33bp	C	G	G	A	CCA
Base pair mutation	G/C/A	A/G	G/C	C/T	C/T	G/A	C/G	G/C	33 bp/-	G/C	G/A	G/A	G/A	-/CCA
Exon/intron	I	I	I	I	I	I	E	E	E	E	E	E	E	E
Amino acid mutation	Q/D	Q/D	11aa	Q/H	A/T	T/T	A/T	-/T

POTENTIAL LINK BETWEEN THE GRAIN YIELD PRODUCTION AND HAPLOTYPE FREQUENCY HORDEUM VULGARE DEHYDRATION-RESPONSIVE FACTOR-1 (HVDRF1) GENE BY COMPARISON OF TOLERANT AND SENSITIVE GENOTYPE GROUPS.

Tolerant genotypes



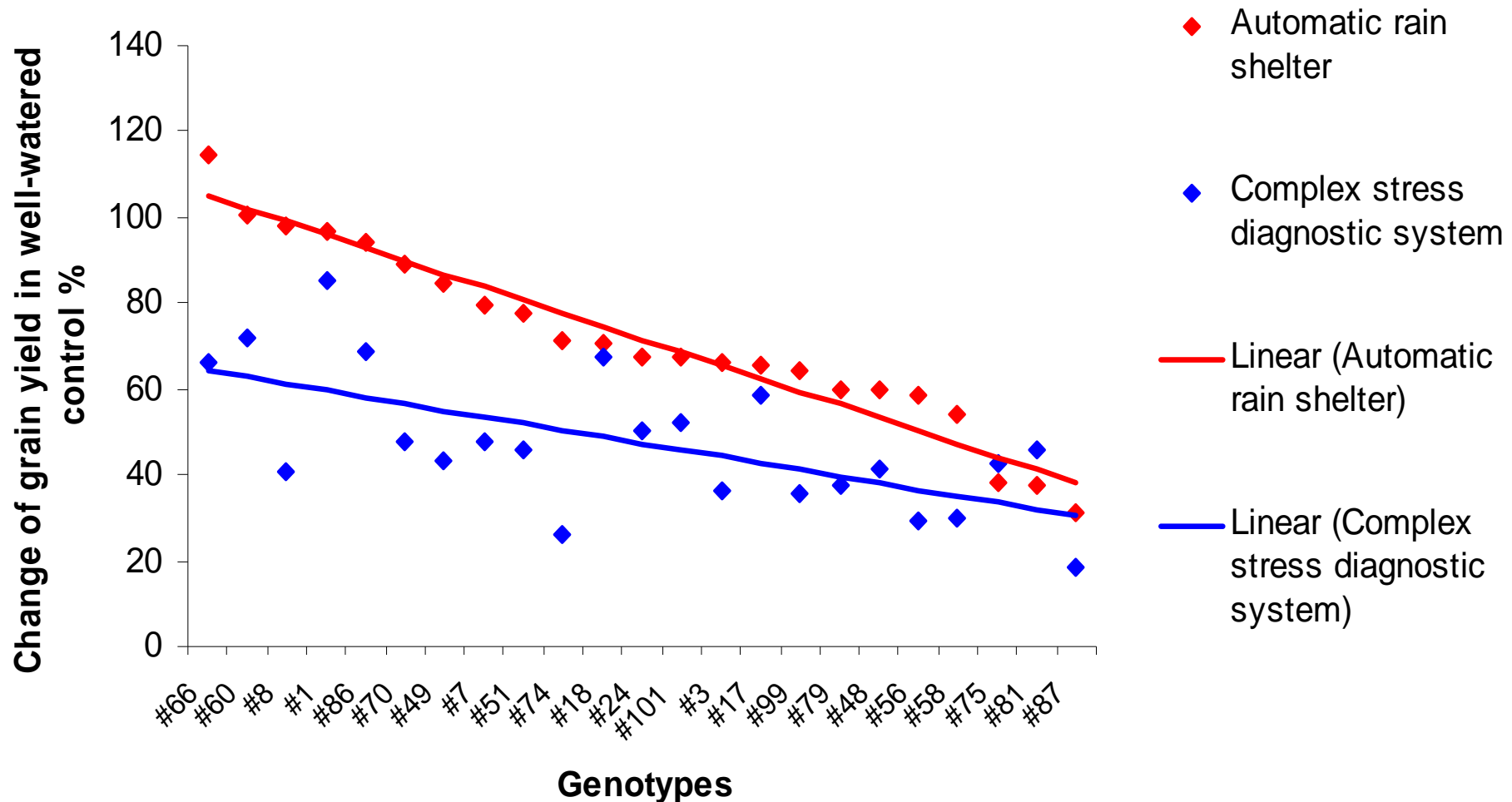
Sensitive genotypes



	8.S	9.S	10.S	11.S	12.S	13.S	14.S	4.I	15.S	16.S
Database sequence	A	A	G	G	G	G	C	15bp	C	G
A haplotype	A	A	G	G	T	A	C	15bp	.	G
B haplotype	A	G	G	G	T	G	A	15bp	G	G
C haplotype	A	A	G	G	T	G	C	.	C	G
Base pair mutation	A/G	A/G	G/A	G/T	G/T	G/A	C/A	CCCGAG CCAACA CTG/.	C/G/-	G/A
Exon/intron	E	E	E	E	E	E	E	E	E	E
Amino acid mutation	D/G	Q/R	V/V	A/S	R/L	M/I	A/D	ARANT/--- --/AGANT	V/I	.



Ranking of barley genotypes on the basis of grain yield response in the two phenotyping systems



**Ranking of barley genotypes
on the basis of grain yield
response in the two
phenotyping systems**

Complex stress diagnostic system		Automatic rain shelter	
barley genotype	grain yield in control %	barley genotype	grain yield in control %
#1	85,343228	#66	114,41897
#60	71,630728	#60	100,54286
#86	68,651093	#8	97,920108
#18	67,340591	#1	96,544314
#66	66,224189	#86	94,018103
#17	58,417339	#70	89,336027
#101	52,007412	#49	84,945496
#24	50,326797	#7	79,609809
#70	48,032243	#51	77,808638
#7	47,698662	#74	71,127259
#51	45,985643	#18	70,354343
#81	45,764576	#24	67,483007
#49	42,977421	#101	67,284532
#75	42,913386	#3	66,118218
#48	41,061755	#17	65,404268
#8	40,438079	#99	64,095693
#79	37,64496	#79	60,066049
#3	36,506159	#48	59,513629
#99	35,919365	#56	58,277648
#58	30,053476	#58	54,165057
#56	28,954903	#75	38,031844
#74	26,398696	#81	37,666833
#87	18,447205	#87	31,451973



Conceptional Basis of Phenomics as an Independent Discipline:

Interpretation of high-dimensional phenomic data from different levels of organization:
from morphology through physiological state to molecular scale

External Phenotype

morphology, growth, development



yield potential

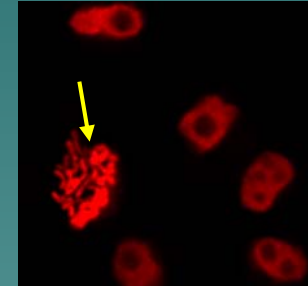


Internal Phenotype

tissue/organ
structure-anatomy

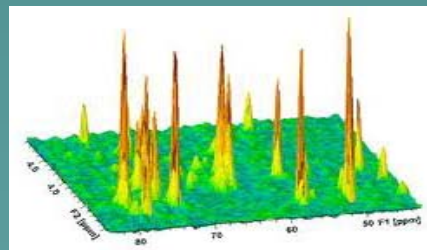


cellular structure-function

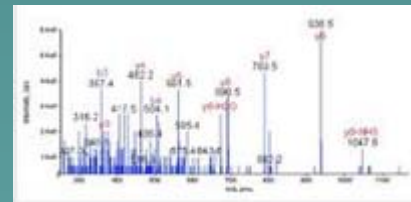


molecular phenotyping

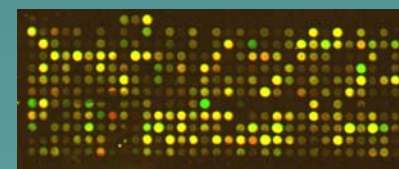
metabolomics



proteomics



transcriptomics



Genetic variants: **germplasm collections**; mutant libraries; mapping populations; **transgenic lines**



Control plants

GM plants

**Rice, rapeseed and maize GM plants
with drought tolerance in the fields (Pennisi, 2008)**

GÉNTÉCHNOLÓGIÁVAL NEMESÍTETT HIBRID ASZÁLYTŰRÉSE

GM hibrid



Termés a 2012. évi amerikai aszályban

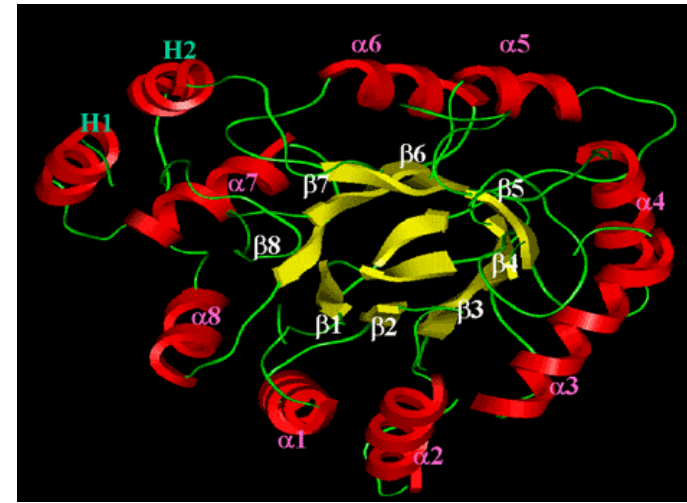


Versenytárs hibrid

foto: Reeves W.R.

About the aldose reductase superfamily in general:

- Wide range of substrate specificity
- Highly conserved structure (NADH or NADPH binding region, catalytic tetrad)
- Occurrence: from bacteria to *Homo sapiens*



Vol. 217, No. 3, 1995

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

polyol pathway

detoxification of reactive aldehydes

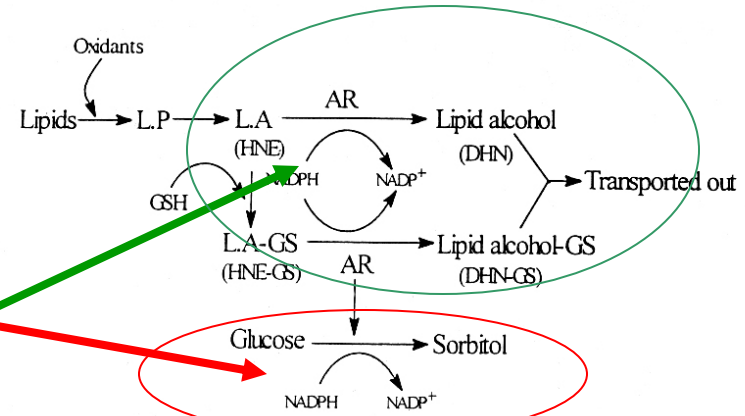
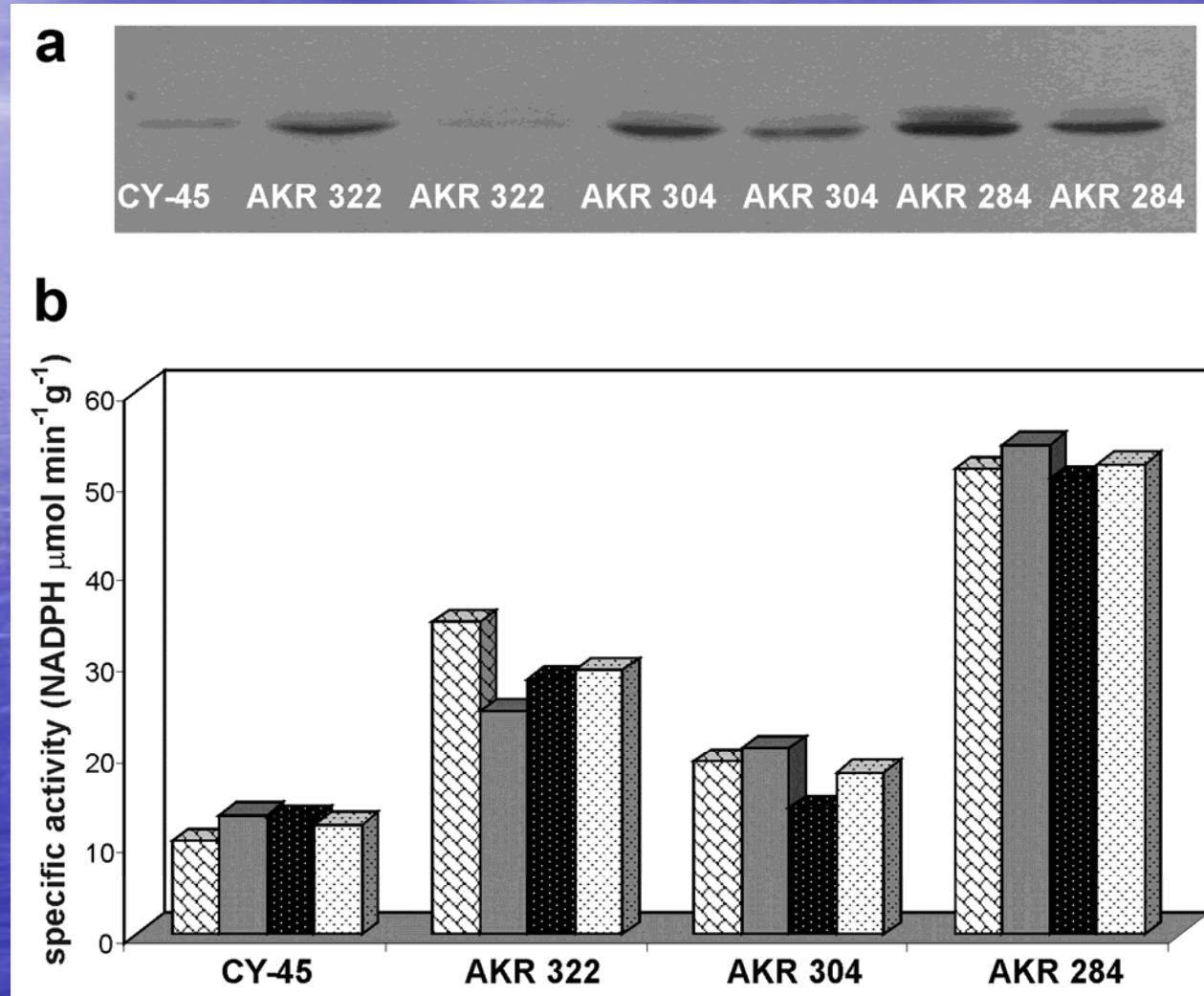
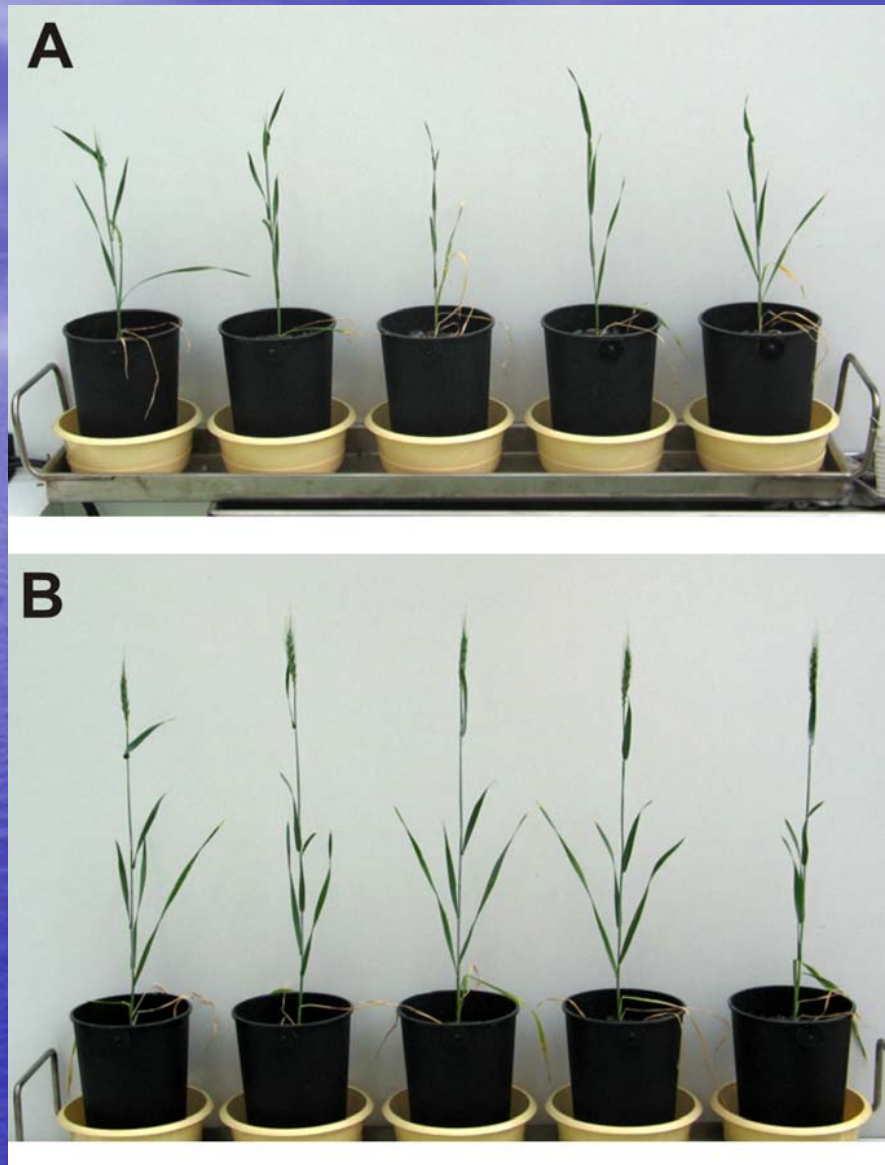


Figure 2. Schematic representation of role of aldose reductase in the detoxification of free radicals and lipid aldehydes. LP = lipid peroxide, LA = lipid aldehyde, AR = aldose reductase.

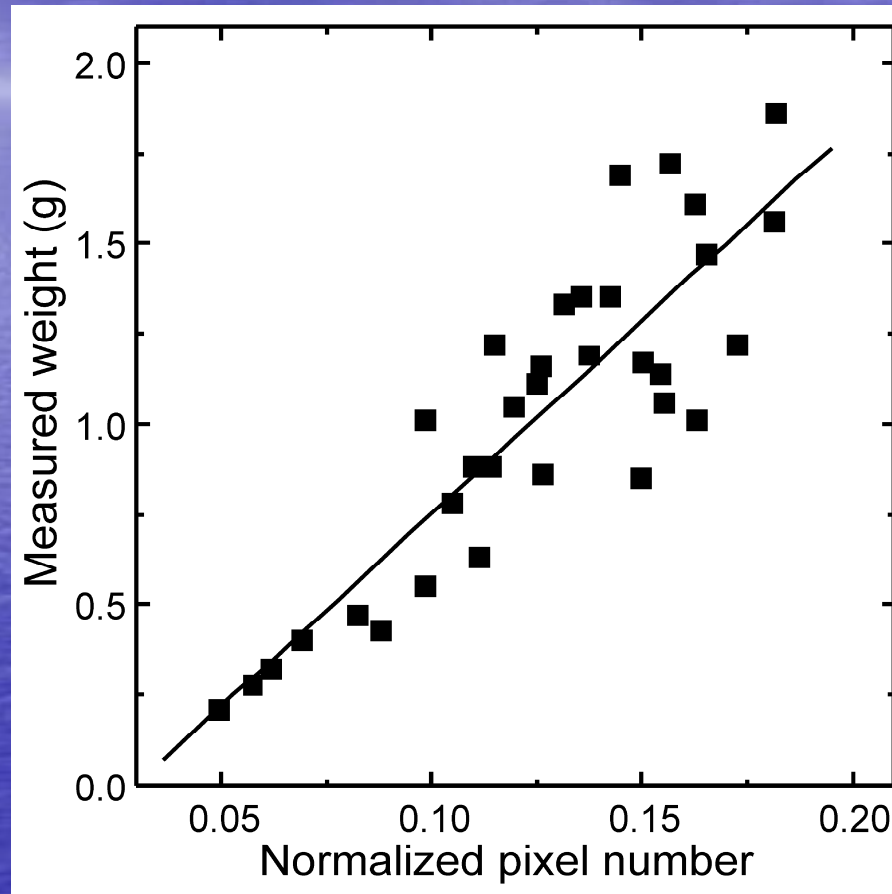
Synthesis of MsALR detoxification enzyme in transgenic wheat lines



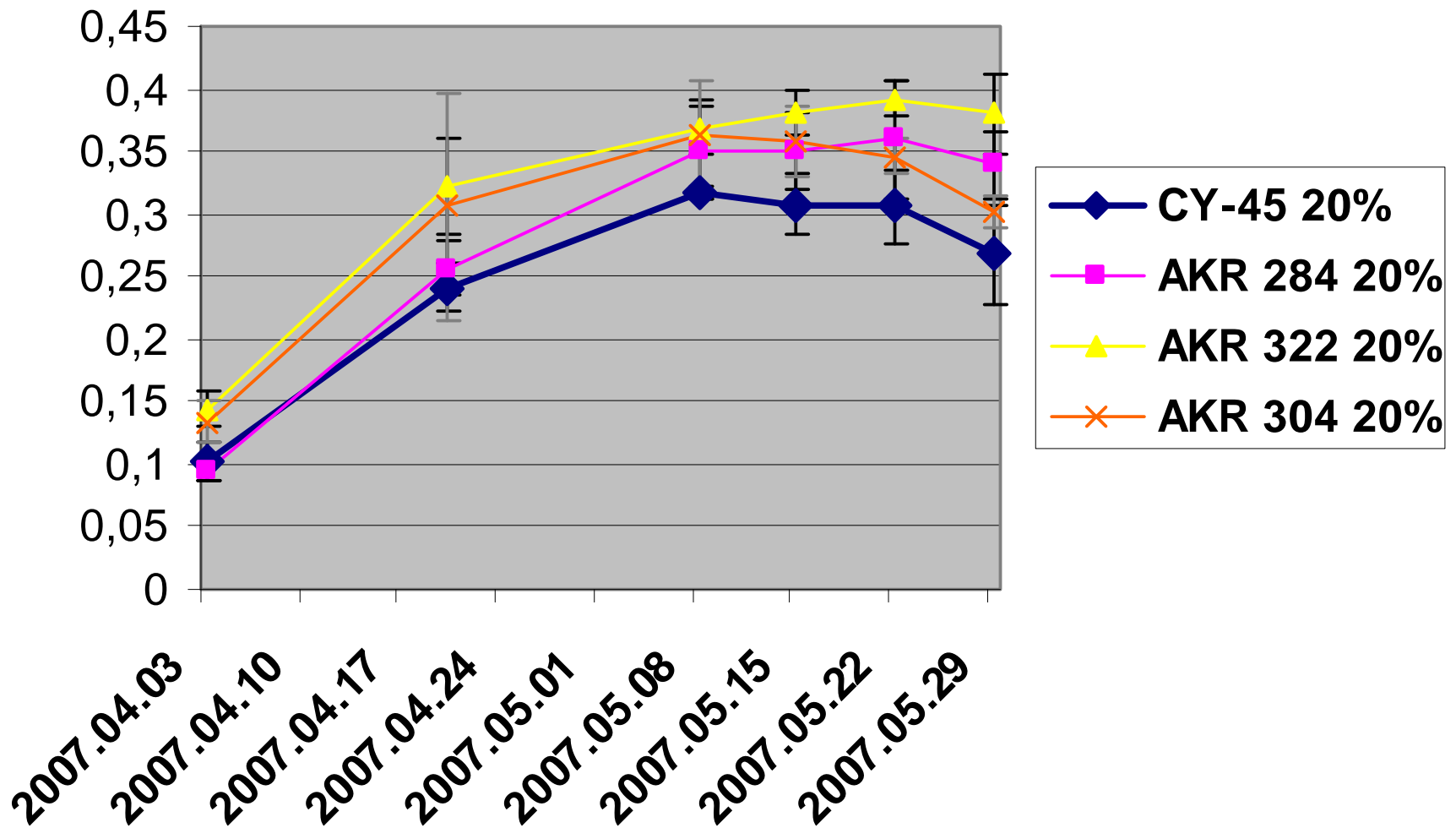


Improved physiological state of transgenic wheat plants (B) expressing the alfalfa aldo-keto reductase detoxifying enzyme

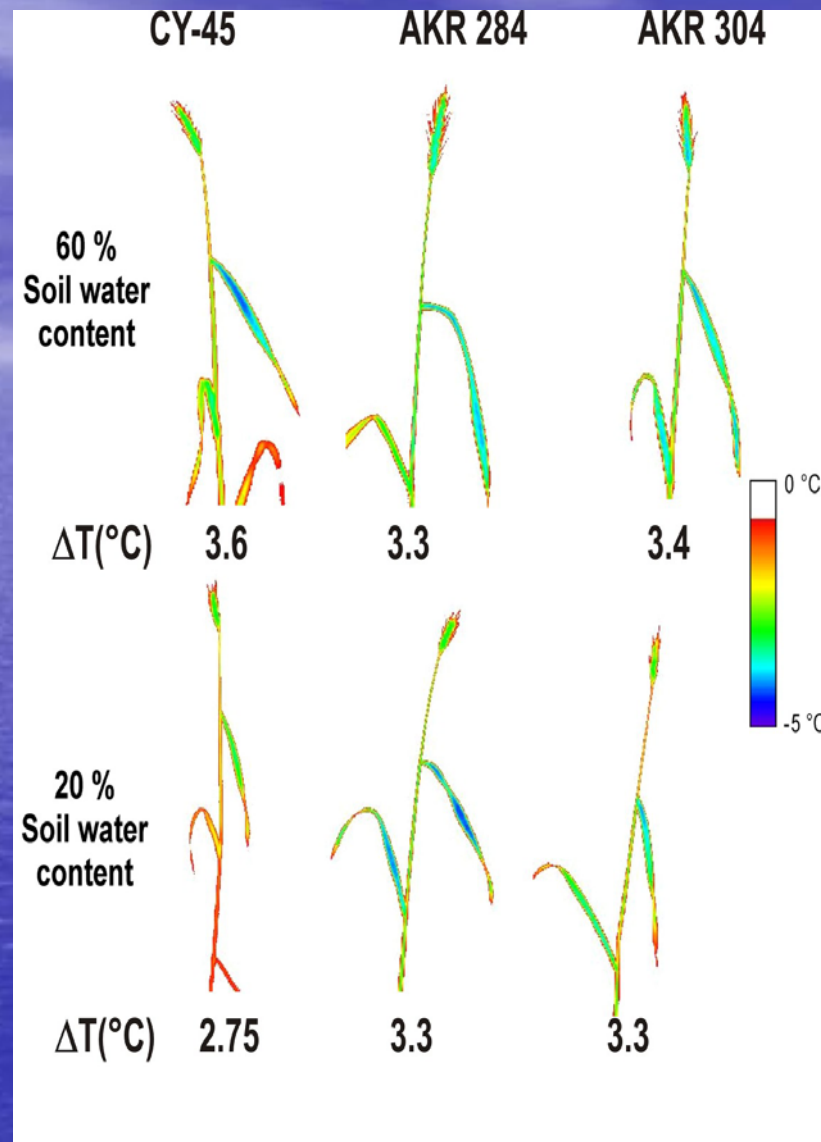
Correlation between fresh weight and green pixel-based shoot surface area in wheat plants



Green mass

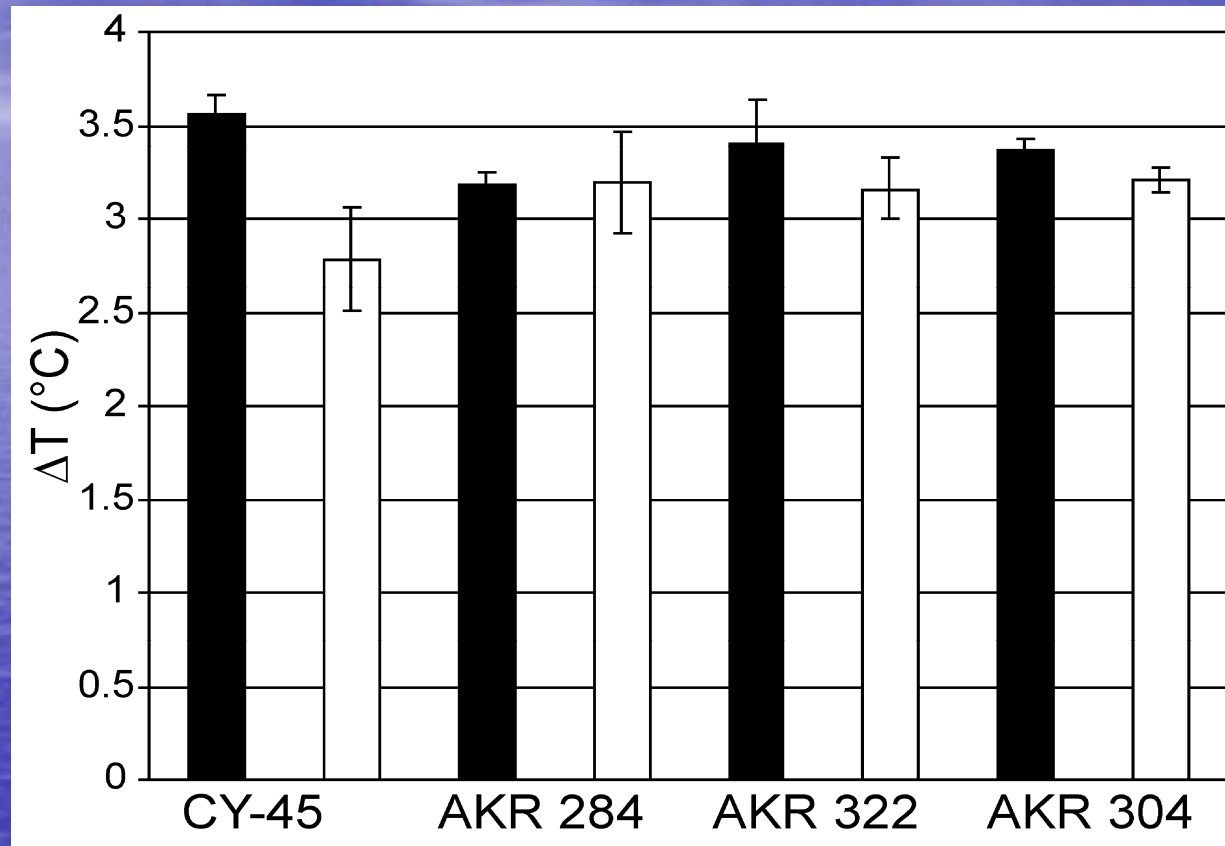


Imaging based green mass phenotyping indicates improved growth parameters for the transgenic wheat lines (AKR 284; 322; 304)
(Fehér et al. manuscript)



Thermal images indicate respiratory function in transgenic plants under low water content

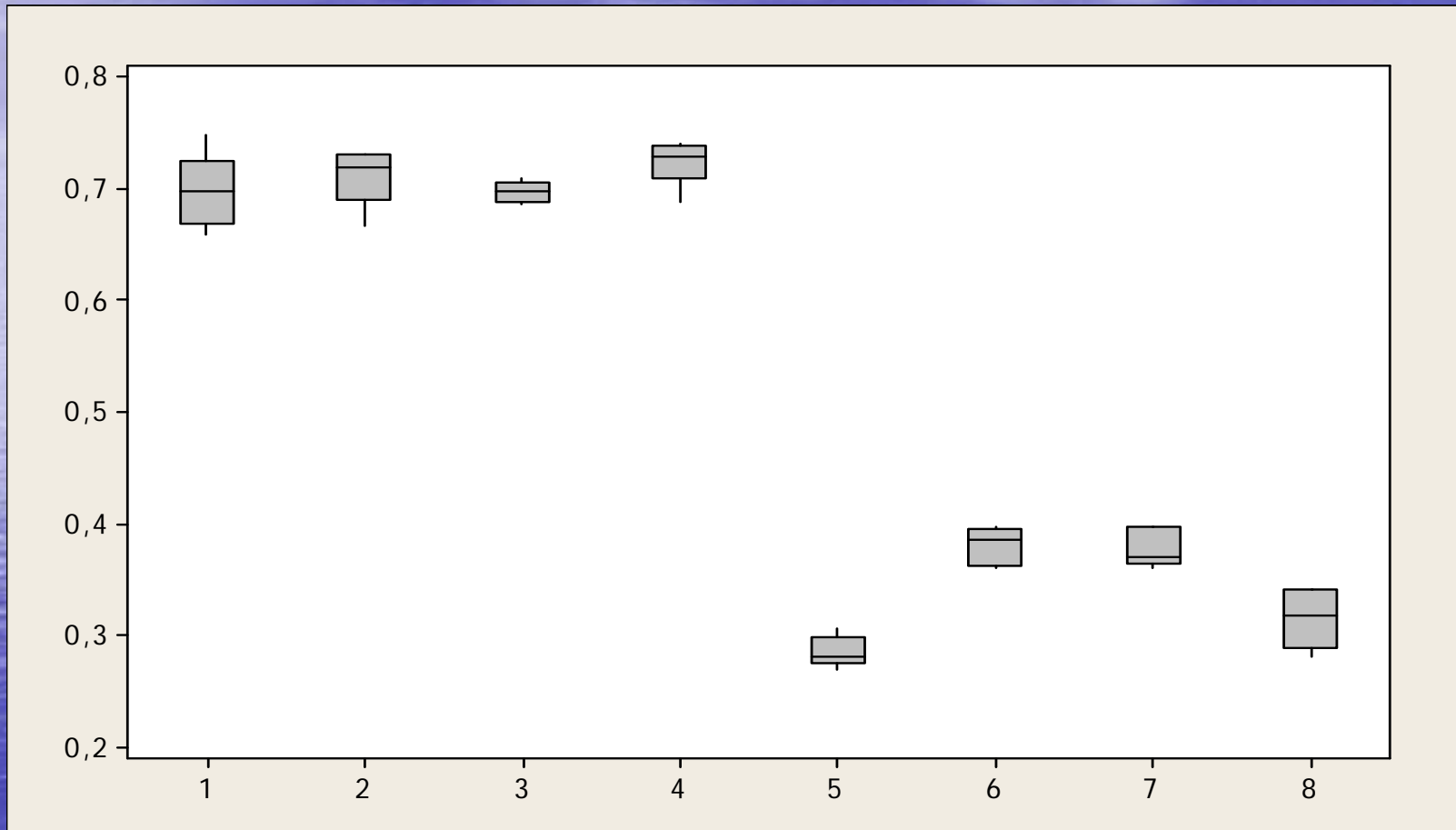
Increased leaf evaporation in transgenic plants as indicated by cooler thermal status of leaves.



■ 60% soil water capacity

□ 20% soil water capacity

GRAIN YIELD (g) PRODUCED BY PLANTS UNDER OPTIMAL (1-4) AND SUBOPTIMAL (5-8) WATER SUPPLY

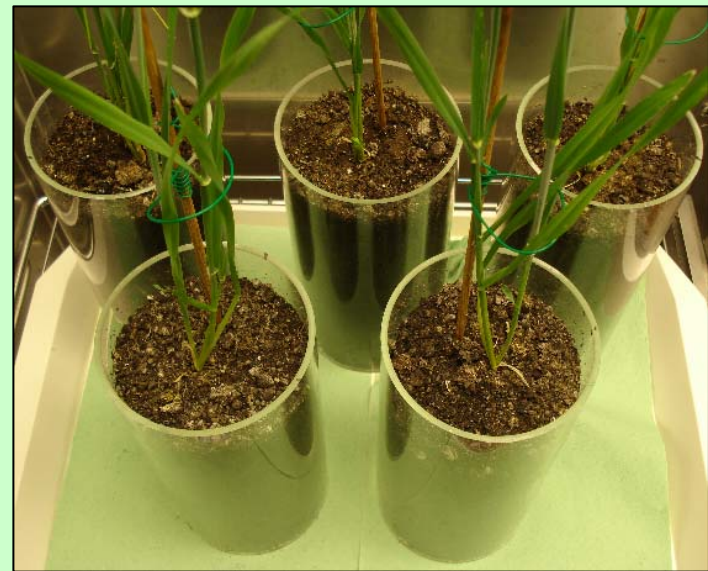


Symbol of genotypes:

CY-45 control: 1; 5, AKR284: 2; 6, AKR304: 3; 7, AKR322: 4 ; 8.

RHIZOCOLUMN FROM PLEXI

for monitoring cereal root development



RHIZOCOLUMN FROM PLEXI

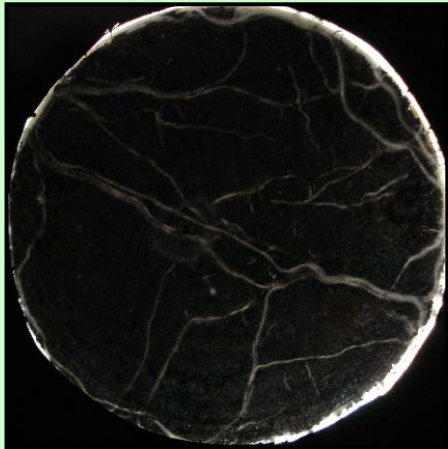
for monitoring cereal root development

Control
(60 % soil water capacity)

side

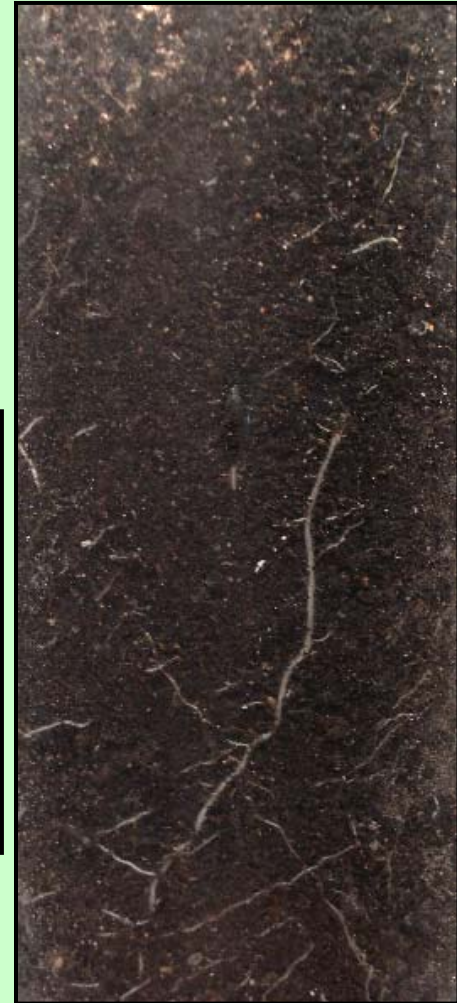


bottom

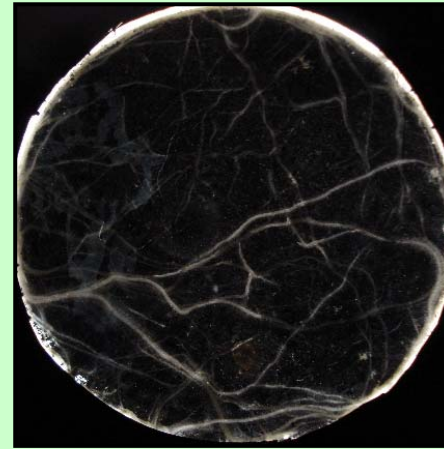


Stressed
(20 % soil water capacity)

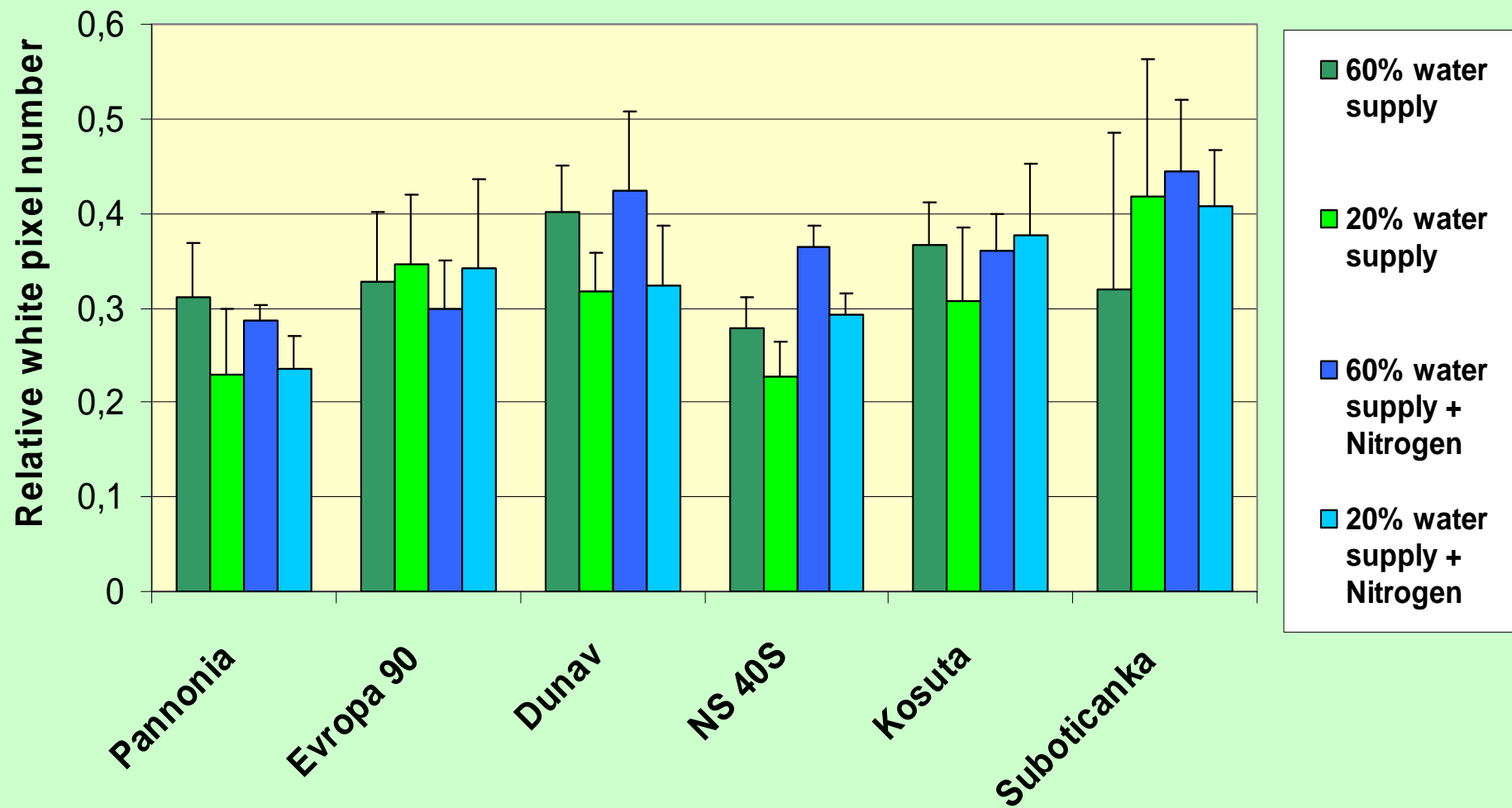
side



bottom



Genotype-dependent root response to water limitation and nitrogen supply: root density at bottom of rhizocolumn

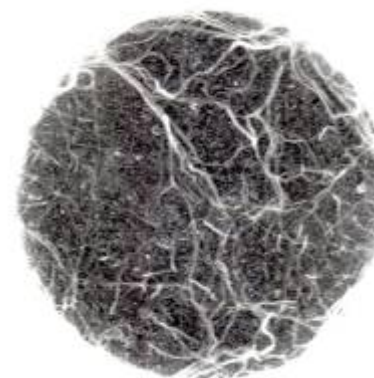


Suboticanka

week 3.



week 4.



week 5.



60% water supply

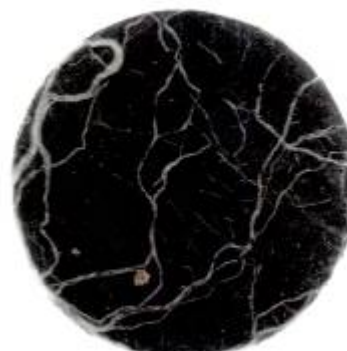
20% water supply

60% water supply
+ Nitrogen

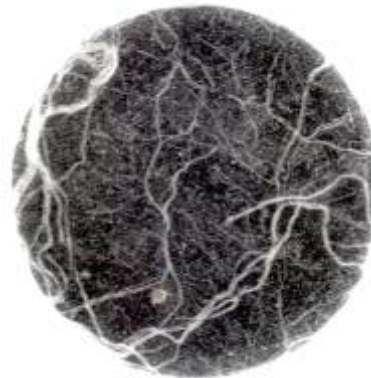
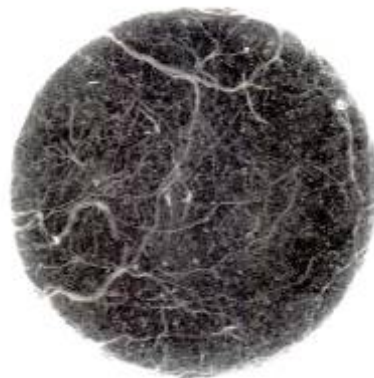
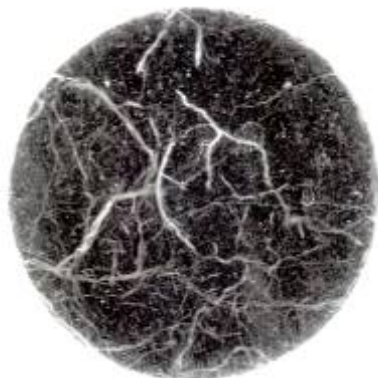
20% water supply
+ Nitrogen

Pannonia

week 3.



week 4.



week 5.



60% water supply

20% water supply

60% water supply
+ Nitrogen

20% water supply
+ Nitrogen

RHIZOBX SYSTEM FOR ROOT ANALYSIS

Siete Cerros



17cm 15cm 12cm 12.5cm

60% 20% 60% 20%
+N +N

NS Avangarda

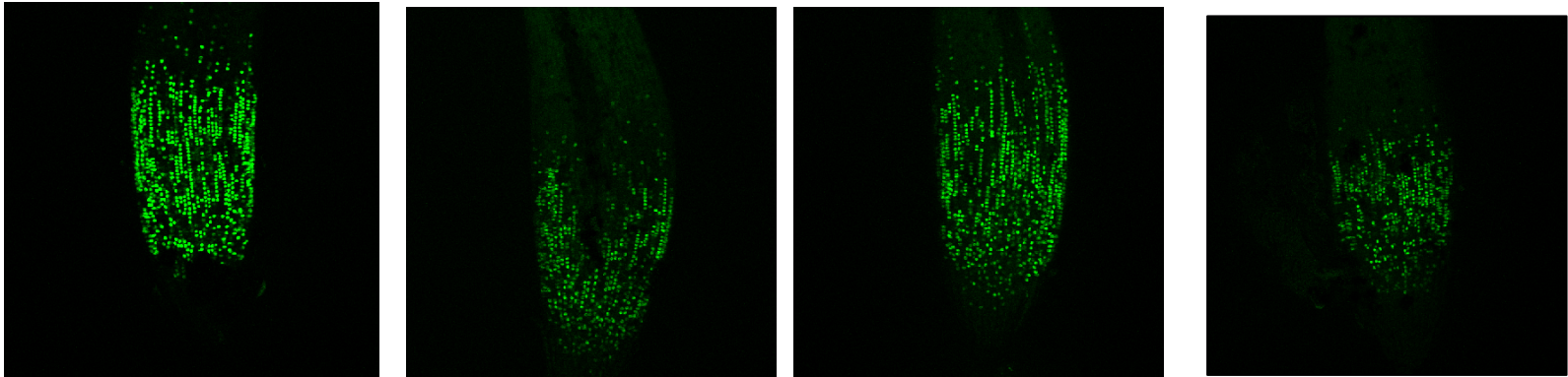


16.5cm 15cm 13.5cm 12.5cm

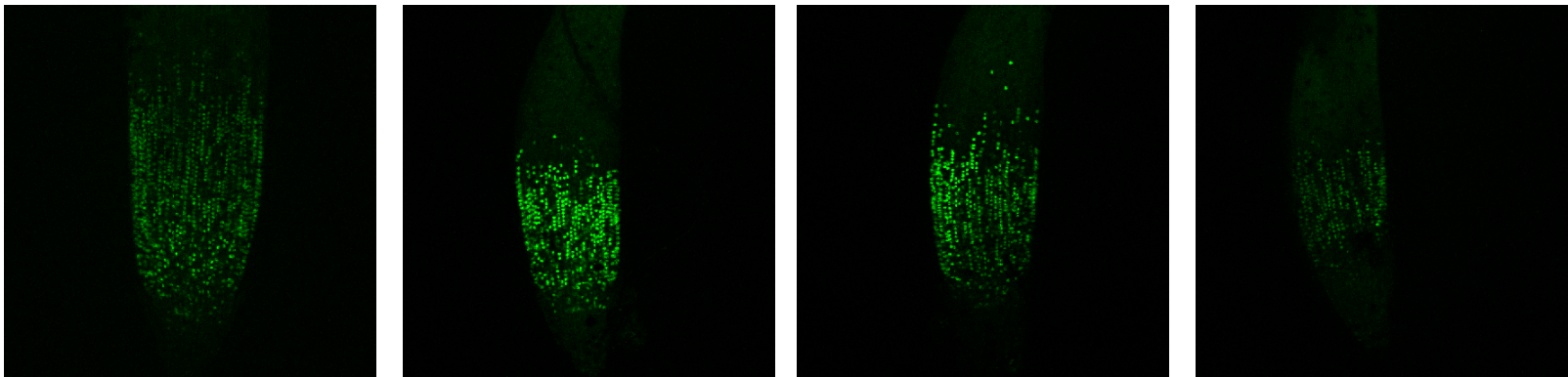
60% 20% 60% 20%
+N +N

The frequency of S-phase cells was detected by 5-ethynil-2-deoxyuridine (EdU) based fluorescent microscopy

Siete Cerros



NS Avangarda



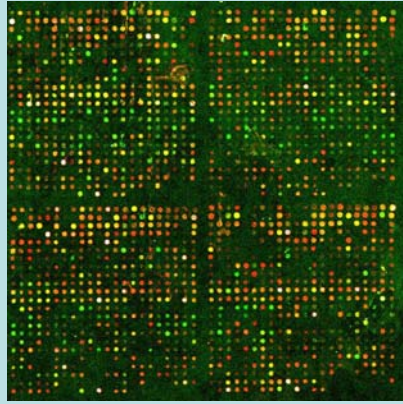
60%

20%

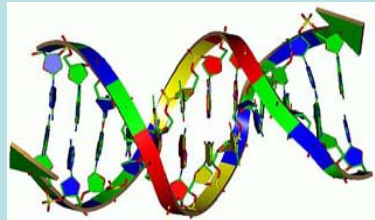
**60%
+N**

**20%
+N**

COMBINATION OF GENE TECHNOLOGY AND PHENOMICS DURING PRODUCTION OF NEW CULTIVARS.



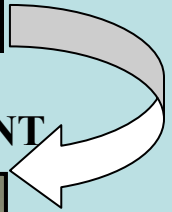
DNA-CHIP: GENE HUNTING



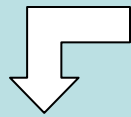
ISOLATED GENE



**GENE INTRODUCTION:
REGENERATION OF GM PLANT**



**PHENOTYPING
IN GREENHOUSE**



20% SOIL WATER CAPACITY 60%



RAIN SHELTER



NEW CULTIVARS IN PRACTICE



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JÁNOS PAUK

THANKS FOR YOUR ATTENTION



CONCLUSIONS

1. By using barley germplasm collection we search for link between allele types of drought-related genes and phenotypes under water stress.
2. The described Complex Stress Diagnostic System providing information about external phenotypes including morphology and physiological functions
3. Establishment of imaging technologies is still in progress by using semi-robotic workstation. Part of the existing capacity is open for collaborative partners
4. We consider as high priority to improve phenotyping technologies for characterization of cellular structures and function e.g. cell division

CONCLUSIONS 1.

1. Drought is one of the major limitation in yield security

2. Yield as the most complex phenotypic trait

Imaging technologies in the Complex Stress Diagnostic System

3. Variation in yield potential under water-scarce

4. Specific allelic variants in selected drought responsive genes

**5. From rhizobox to rhizotubes and rhizotrones for monitoring
Root System Architecture (RSA) of cereal seedlings and plants**

**2. Drought response of barley genotypes reflected is by green
pixel-based biomass**

Soil water availability during a wheat crop cycle

