



Allele Mining Based On Non-Coding Regulatory SNPs in barley germplasm

Identification GCP Project Number: #17

Principle Investigator: **Michael Baum**, ICARDA

Collaborators:

K. Stamati, W. Powell, NIAB, UK.

P. Langridge, Mark Tester, Australian Centre for Plant Functional Genomics Pty Ltd, PMB 1 Glen Osmond SA 5064 Australia

J. K. Eglinton, School of Agriculture and Wine, University of Adelaide, Waite Campus, PMB 1, Glen Osmond SA 5064 Australia

M. Morgante, Dipartimento di Scienze Agrarie ed Ambientali Università di Udine Via delle Scienze 208 I-33100 Udine ITALY

S. Ceccarelli, S. Grando, S.M. Udupa ICARDA, P.O.Box 5466, Aleppo, SYRIA,
W. Choumane, Faculty of Agriculture, Tishreen University, P.O.Box 2099, Lattakia, Syria.



Allele Mining Using Allelic Imbalance Assay

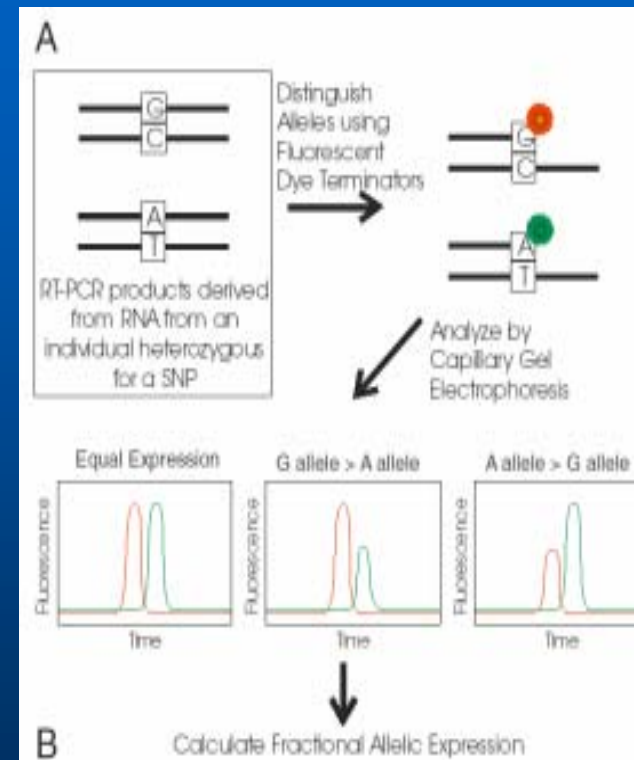
Objectives:

To measure allele-specific expression and
To detect cis-acting regulatory variation

How?

Fluorescent Single-Base Extension (SBE) of a locus-specific RT-PCR product, **using an allele-specific primer adjacent to the marker SNP of interest**, will allow detection of the variant base on a fluorescence detection platform, such as the ABI sequencer.

The ratio of the two alleles is inferred by comparison with known mixtures used as reference standards.





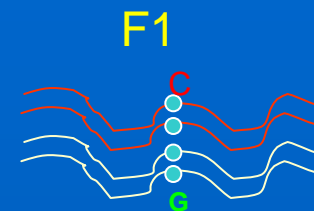
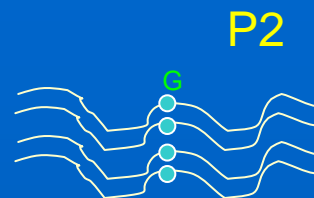
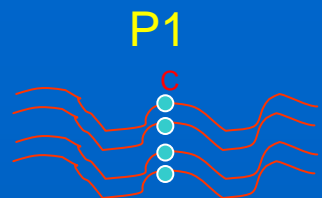
Allele Mining Using Allelic Imbalance Assay

- **Advantage of the use of hybrids:** Avoid environmental and cellular differences and internally normalised relative expression
- **Strength of the approach:** The method identifies variation in expression levels due to cis-acting regulatory variation without directly identifying or requiring knowledge of specific regulatory variants



ICARDA

Extract DNA from parents, and F1s including reciprocals



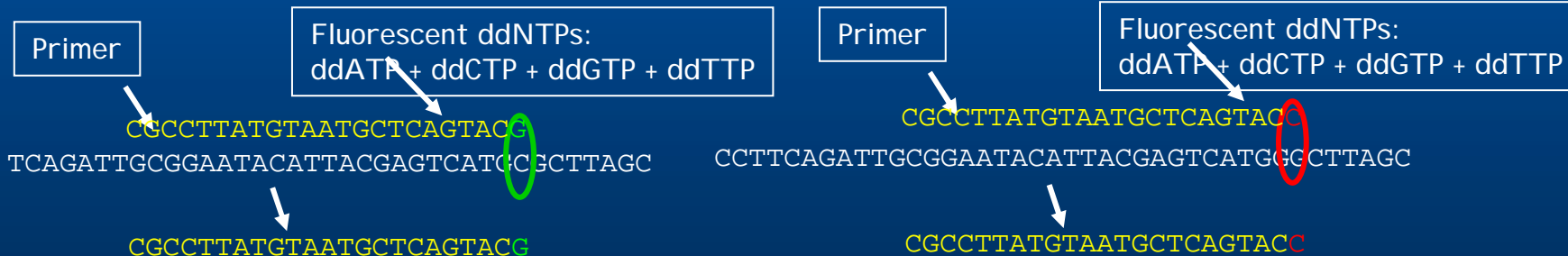
Mix template DNA of P1 and P2 in 25:75, 50:50 and 75:25

Titration curve

PCR Amplification of desired gene using mixed templates and F1s

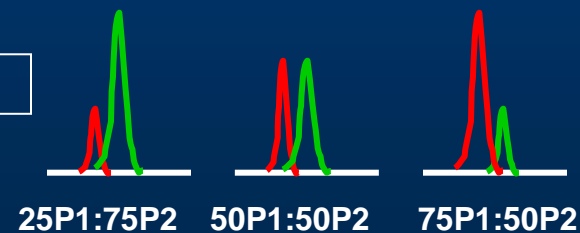
Remove dNTPs and Primers by SAP and ExoI

Single-base extension using SNaPshot kit



Remove unincorporated ddNTPs using SAP or CIP

Electrophorese samples on ABI3100 and GeneScan analysis





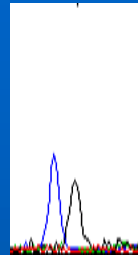
Titration Curve (continued...)

In reality.....

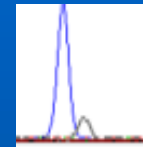
DNA (template) →



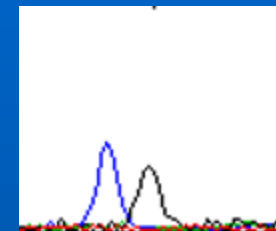
P1:P2
25:75



P1:P2
50:50

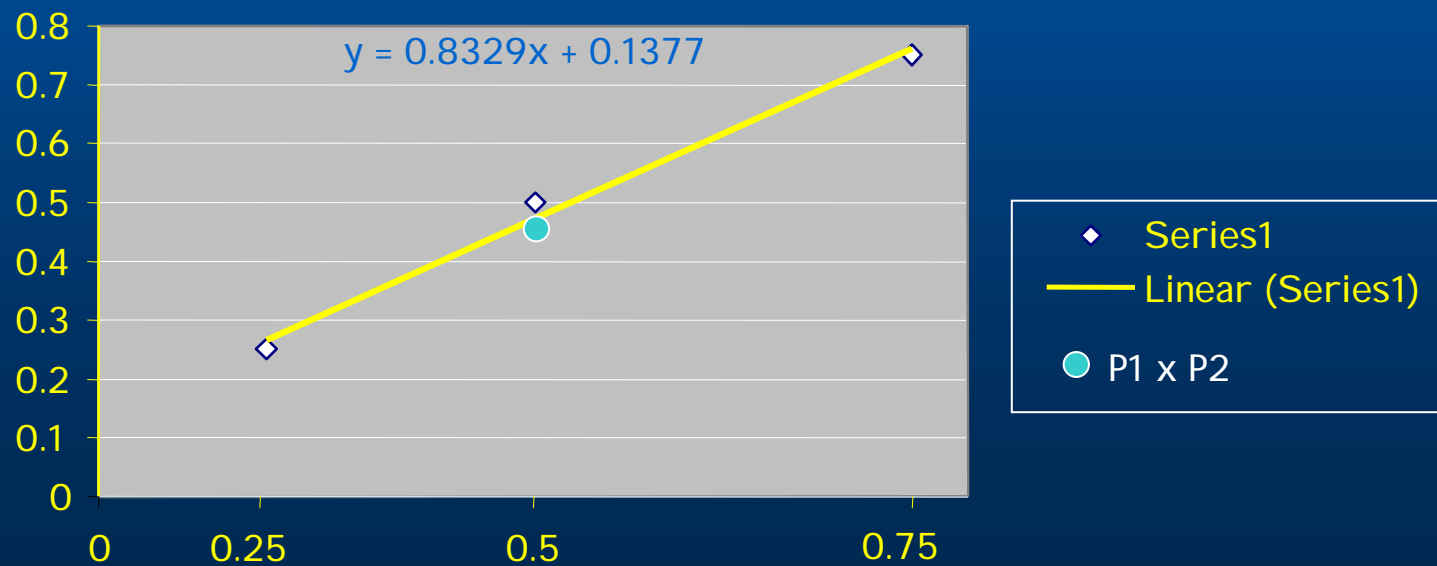


P1:P2
75:25



P1 x P2

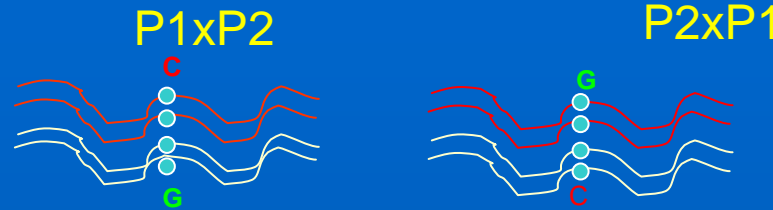
Titration curve



Allelic imbalance in expression levels



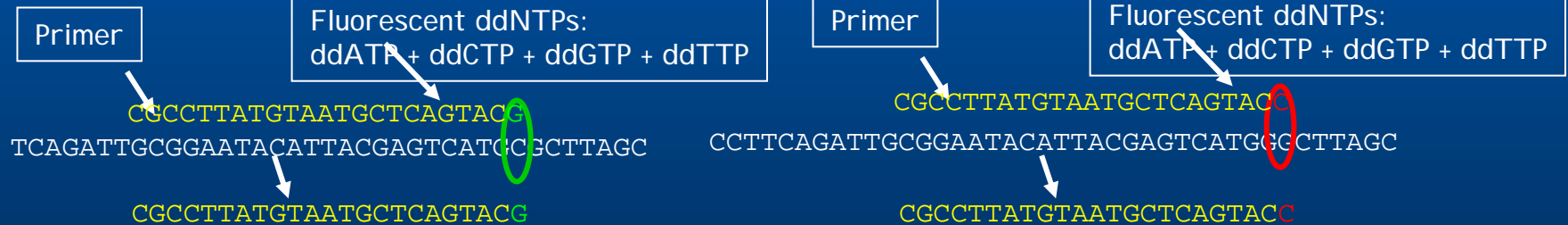
Extract mRNA from F1s including reciprocals



RT-PCR Amplification of desired gene in F1s including reciprocals

Remove dNTPs and Primers by SAP and ExoI

Single-base extension using SNaPshot kit

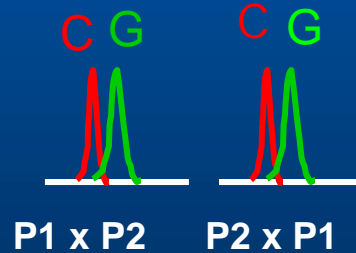


Remove unincorporated ddNTPs using SAP or CIP

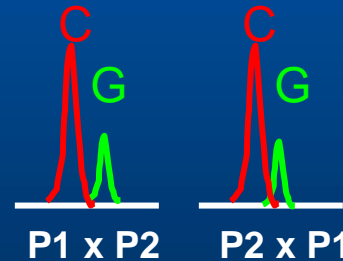
Electrophorese samples on ABI3100 and GeneScan analysis

Allelic imbalance in expression levels (continued..)

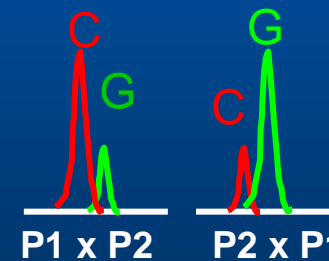
Electrophoresis samples on
ABI3100 and GeneScan
analysis



**No allelic imbalance
in expression**



**Allelic imbalance in
expression**



Genomic imprinting

e.g. Dhn12



Allele Mining Using Allelic Imbalance Assay

- Currently optimising testing of different genes for allelic imbalance at NIAB, Udine, ICARDA
- Testing different tissues (leaf, roots)
- Testing “drought” treated tissues



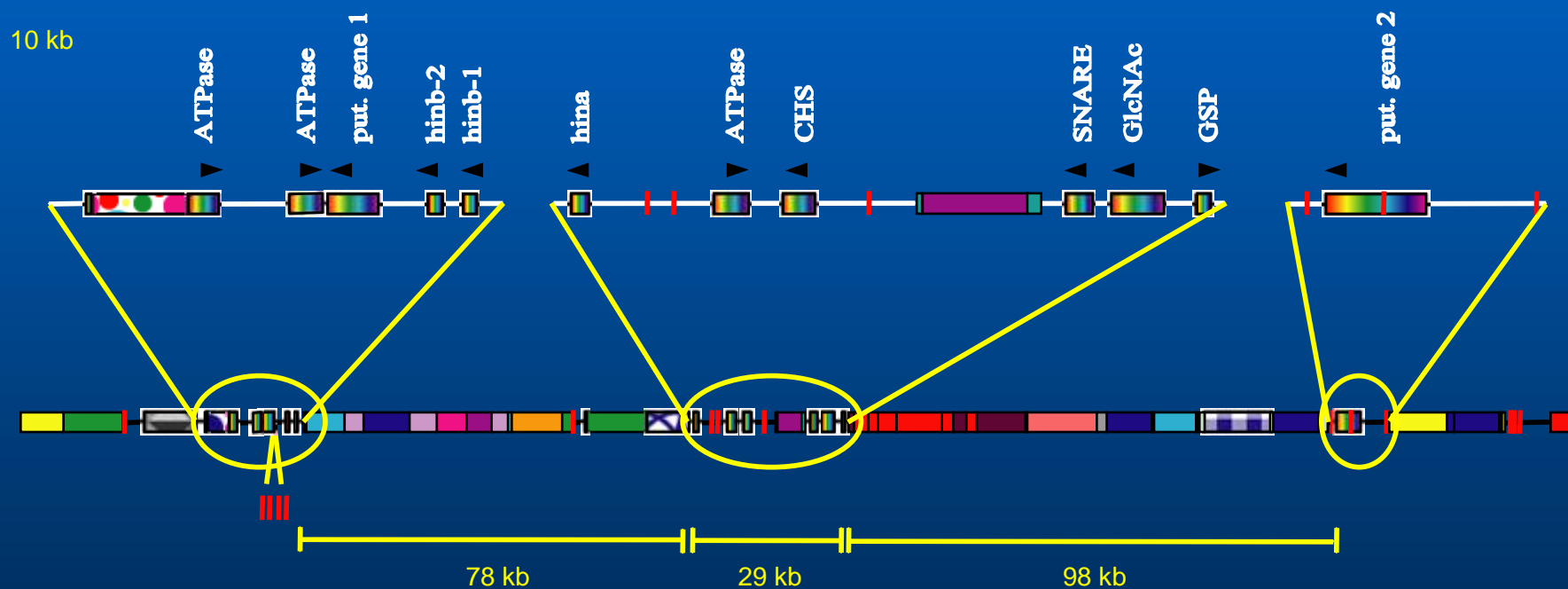
EST-based validated coding SNPs (OWB)

SCRI locus	Code	Blastx hit	Acc. No.	SNP type
SNP1239	scsnp00460	sucrose synthase	S24966	A/T
SNP703	scsnp00703	glyoxylase I	BAA36759.1	C/G
SNP957	scsnp22290	unknown	NP_567356.1	A/G
SNP1993	scsnp01216	aquaporin	P42767	A/G
SNP490	scsnp02972	transcriptional coactivator-like protein	NP_191427.1	A/G
SNP2297	scsnp03343	calcium binding protein	AAK92225.1	A/G
SNP824	scsnp00940	fructose 1,6-bisphosphate aldolase	CAD12665.1	C/G
SNP2733	scsnp00940	fructose 1,6-bisphosphate aldolase	CAD12665.1	C/G
SNP3167	scsnp23255	unknown	AAK20044.1	A/C
SNP85	scsnp01648	glutamine synthetase	AAK18848.1	A/G
SNP2332	scsnp21116	potassium transporter	CAD21000.1	A/G
SNP3218	scsnp04260	cinnamyl alcohol dehydrogenase	AAL99535.1	A/G
SNP86	scsnp06144	glucose 6-phosphate isomerase	T09153	C/T
SNP2719	scsnp00177	UDP-glucose pyrophosphorylase	Q43772	C/T
SNP2901	scsnp02109	actin-depolymerizing factor 3	Q41764	C/T



Fully sequenced 300 kb region of the barley genome containing genes involved in grain texture

Caldwell, Langridge and Powell 2004





Identification of Drought induced Genes in Adapted Germplasm

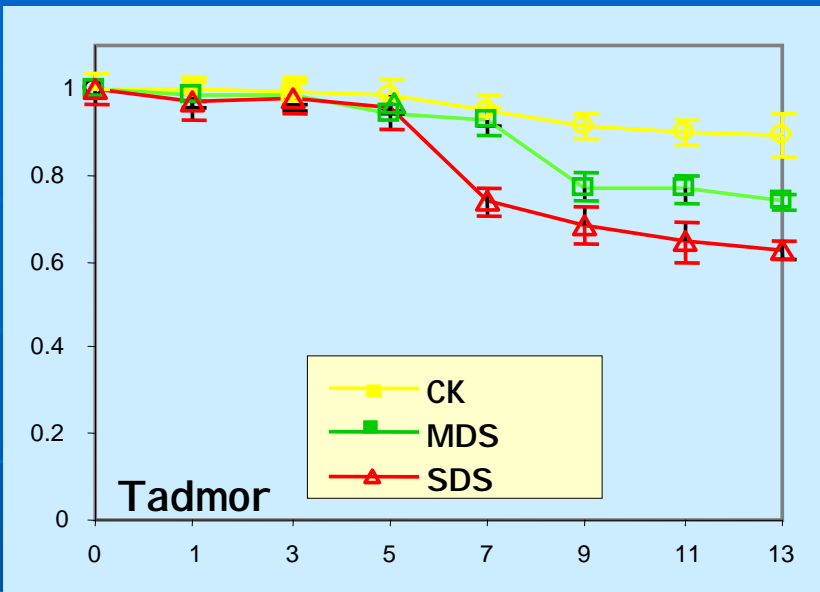
BMZ funded collaborative Collaborative project ICARDA/IPK
Gatersleben



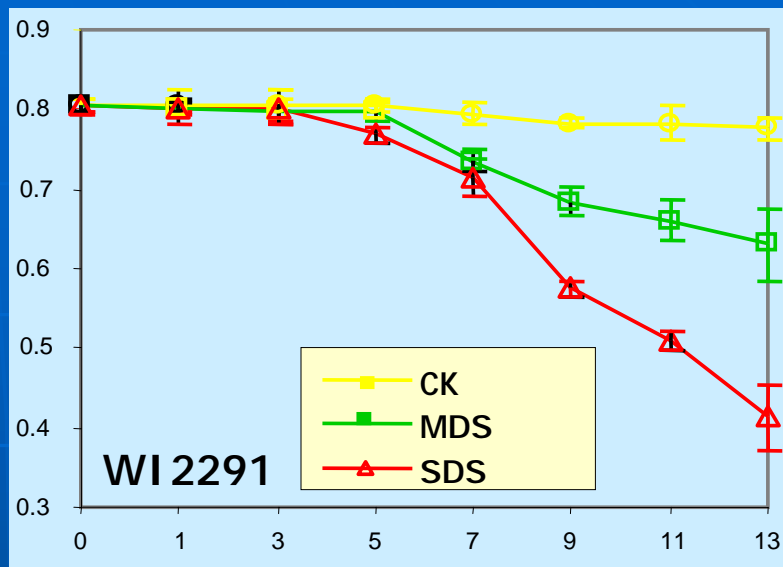
Tadmor

WI 2291

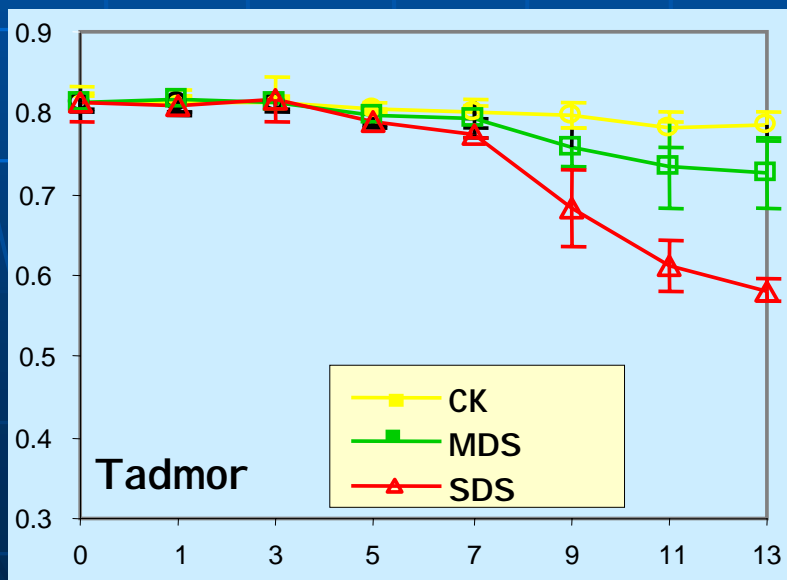
Chlorophyll fluorescence (Fv/Fm)



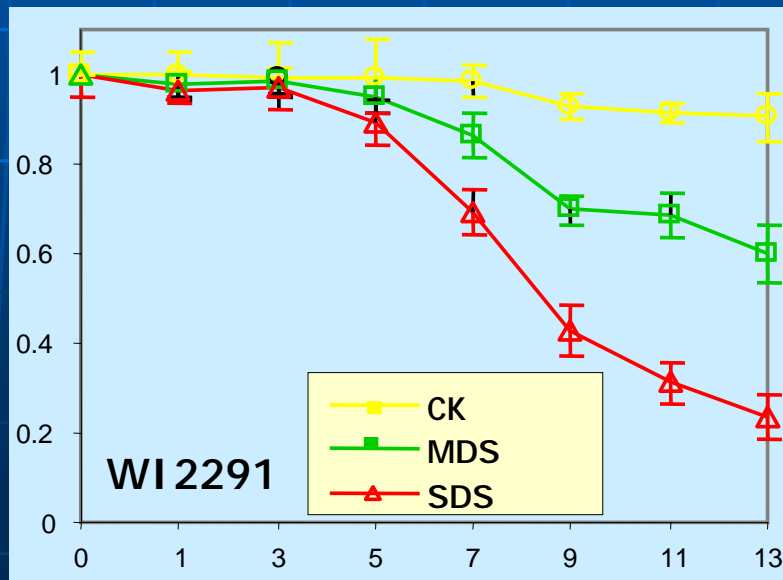
Chlorophyll fluorescence (Fv/Fm)

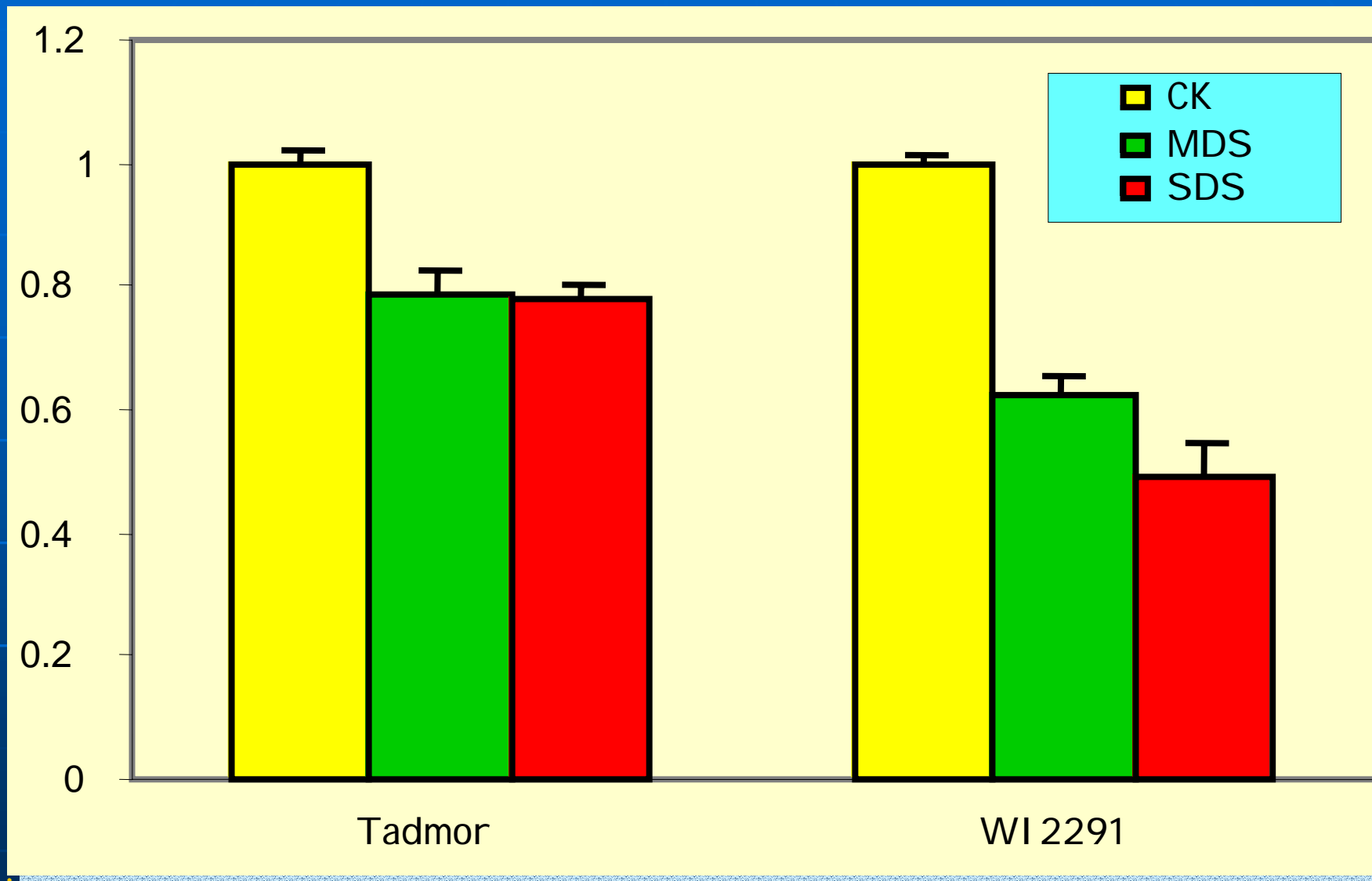


Chlorophyll Relative Content (%)



Chlorophyll Relative Content (%)



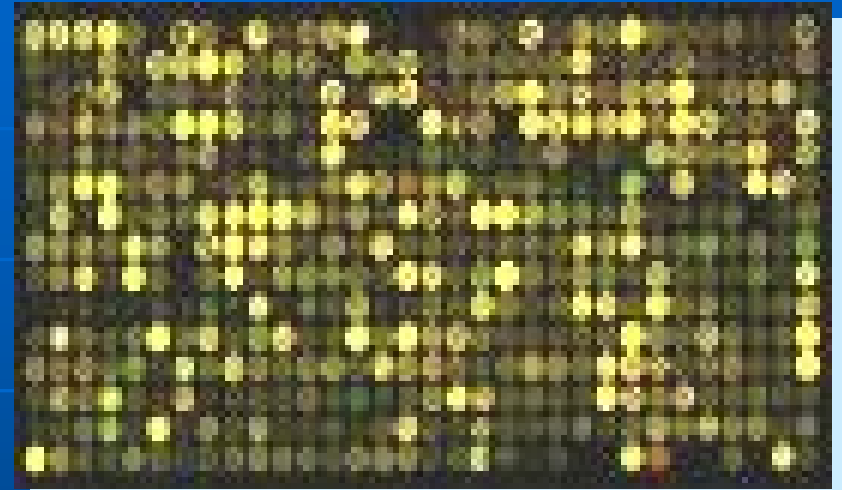


Grain yield of Tadmor and WI 2291 under the three moisture regimes in the soil.

Identification of drought candidate genes with Affymetric barley genome chip -22K

- 77 genes significantly regulated at severe drought stress during heading stage in both varieties could putatively be only responsive genes to drought stress. e.g.

dehydration-responsive protein rd22, early drought induced protein, heat shock protein-like, dehydrin 3, protein kinase HvPKABA1, protein kinase SPK-3, etc



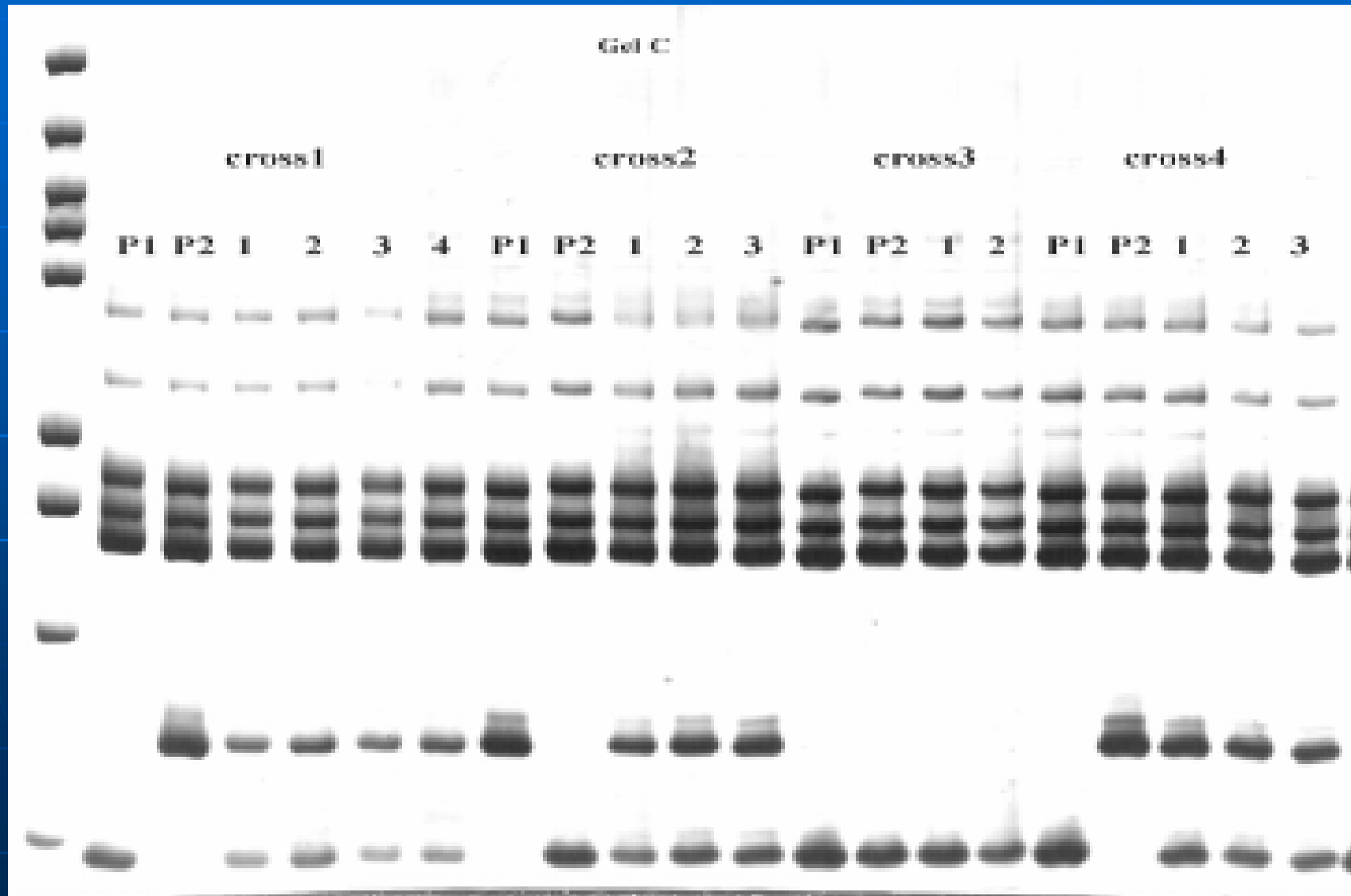
- 372 genes differentially expressed between Tadmor and WI 2291 under drought stress putatively are the specific/responsible genes for drought tolerance in Tadmor during heading stage.
e.g. serine/threonine kinase-like protein, hepatoma-derived growth factor, Inositol-3- phosphate synthase, microsomal signal peptidase, ribulose-bisphosphate carboxylase activase, pathogenesis related protein-1 and 4, RB1-2, PRB1-4, ABC transporter family protein, organic anion transporter, glutathione S-transferase, etc.



Reciprocal F1 crosses 72 F1 hybrids

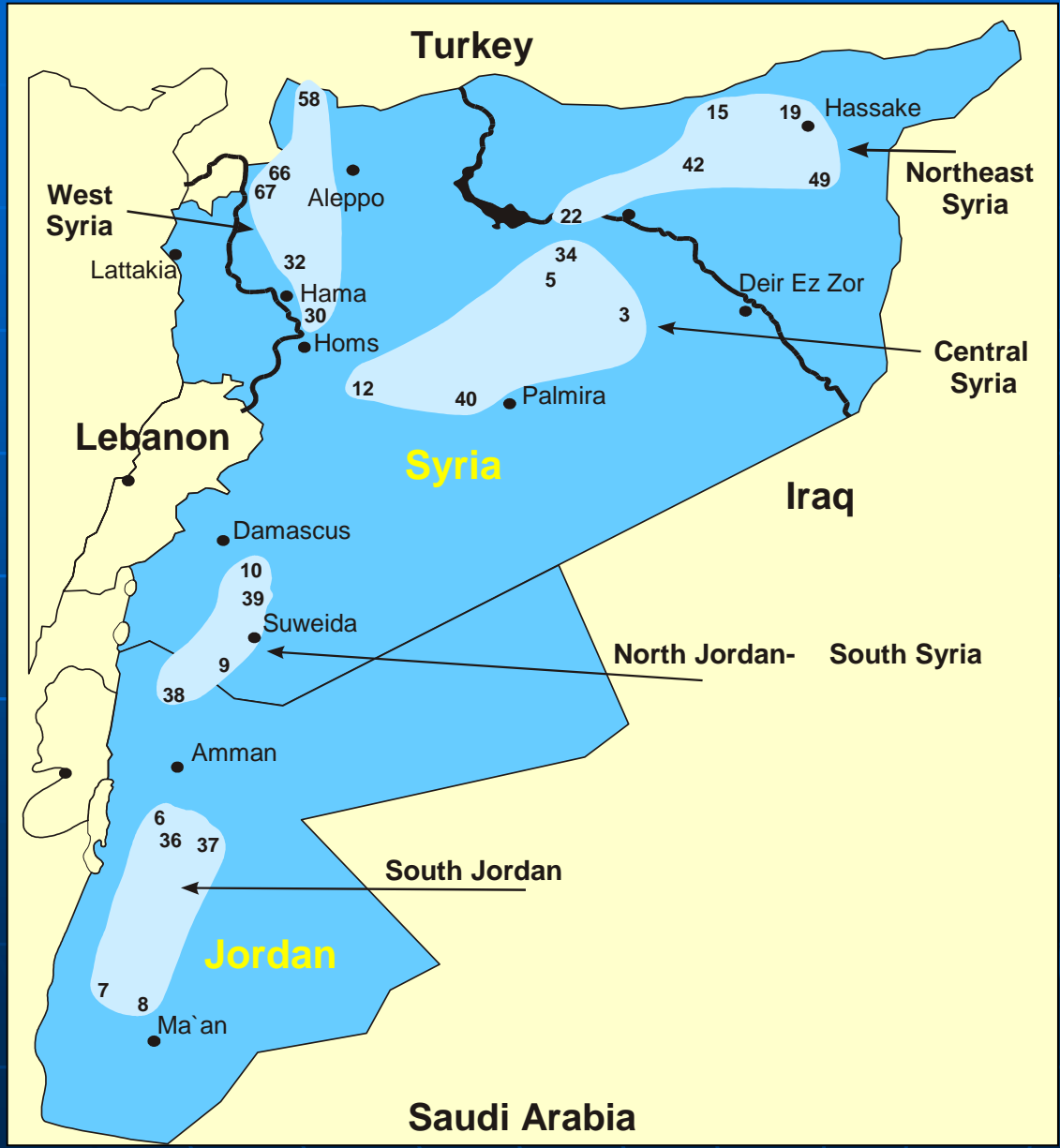
		Tadmor	Alexis	H. spont. 41-1	CPI 71284-48	Arta	Sloop	WI 3408.
		98	99	100	106	101	102	103
Tadmor	98		300	350	106	350	350	400
Alexis	99	105		74	X	220	230	138
H. spont. 41-1	100	427	325		X	372	540	220
CPI 71284-48	106	X	X	X		X	X	X
Arta	101	349	165	110	X		39	66
Sloop	102	400	250	130	X	31		465
WI 3408.	103	200	500	49	X	120	450	

Characterisation of reciprocal F1 with SSRs



Mapping Adaptation of Barley to Drought Environments (MABDE)

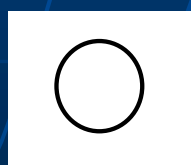
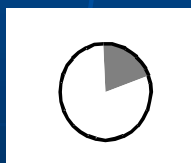
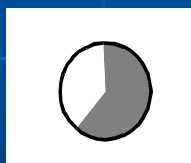
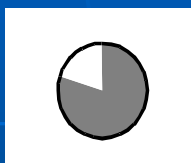
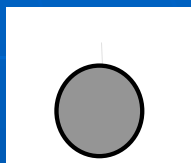
Syrian-Jordan Landrace collection (SJLC)



More humid



Haplotype





Mapping Haplotypes, Linkage Blocks, high and low expressing transcripts

- Identify conserved linkage blocks with adaptation to drought
- Map genes with high and low expressing transcripts derived from pre- and post-domesticated barley accessions in *H. spontaneum* mapping populations

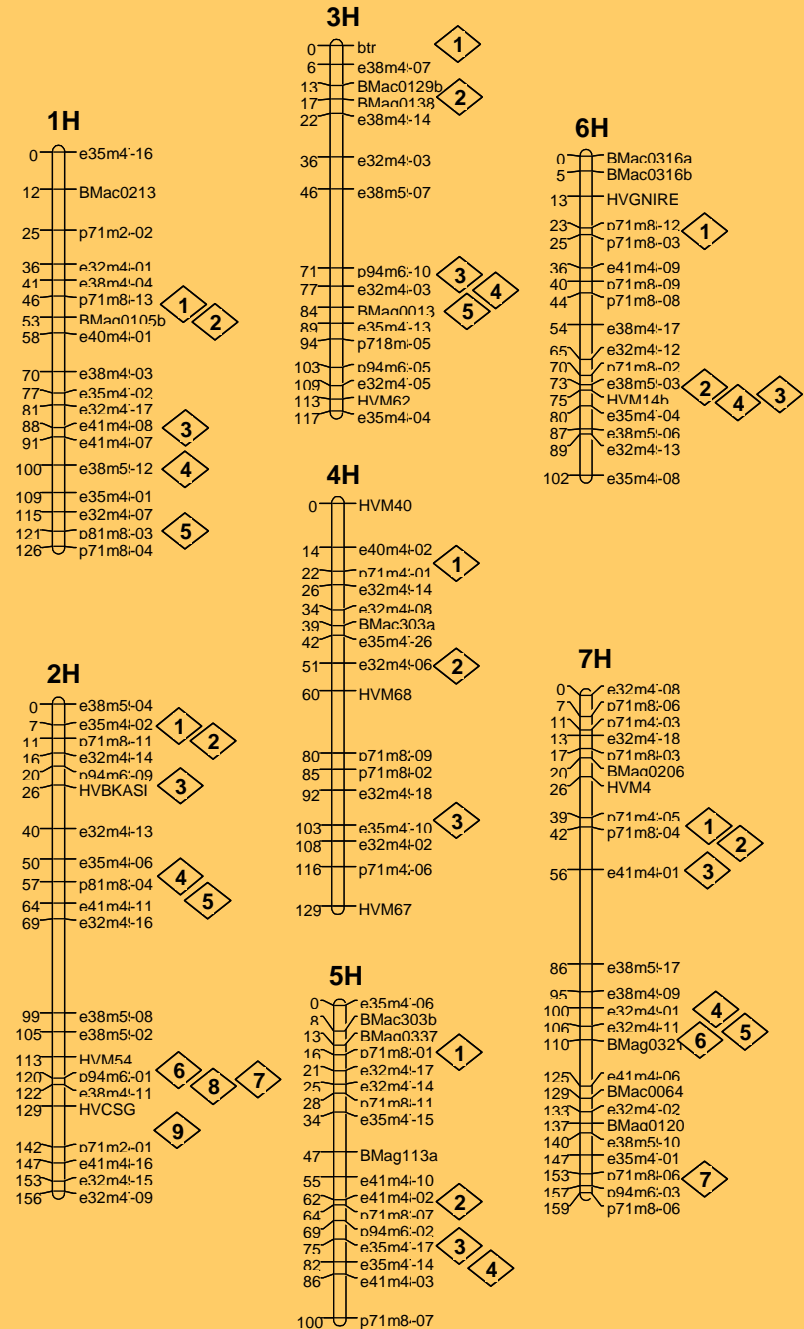


Arta/*H.spontaneum* 41-1

- QTL evaluated for agronomic traits under Mediterranean environments
- Currently mapping of physiological traits
- Advanced backcross population in progress

Barque-73/*Hordeum spontaneum* (CPI71284-48)

- QTL evaluated for agronomic traits
- Advanced backcross population available





Capacity building

1. An annual workshop at the end of the first year has been planned on Allele imbalance and MAS: **meeting at PAG involving all participating institutions**
2. A training course in Allele imbalance and MAS on the second year is programmed to be attended by 10 participants: **training course on DNA marker technology was organised in the first year 20 participants.**
3. The training in Allele imbalance and MAS will involve 2 individual trainees every year: **three ICARDA scientist (M. Baum, S. Udupa, W. Choumane) were trained in the technology at Udine U.,**
Mr. Mohammed El-Kholani, TU, Yemen, Tishreen University,
Ms Lina Housni Ali, Syria, Aleppo University, Department of Plant Protection,
4. 2 NARS scientists are enrolled in Masters course at University Adelaide:
Mr. Berhane Lakew from Ethiopia is enrolled in a PhD course at Southern Cross University, NSW, fully sponsored by MPBCRC
5. The Australian Centre for Plant Functional Genomics will provide two six-month fellowships for students or scientists from developing countries to come to Adelaide to learn the analytical techniques and take part in this program.
6. The University of Adelaide and the University of Udine will actively support and facilitate sabbatical appointments, internships and other types of exchanges to empower national program scientists to actively participate in this program.
7. The University Udine will seek support from the EU to facilitate staff exchanges and visits by national program scientists.



ICARDA

20 participants

DNA marker technology
for Crop Improvement
4-15 September, 2005

leppo, Syria.

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