



# Allelic Imbalance in Candidate Genes in barley

**Identification**      GCP Project Number: #17

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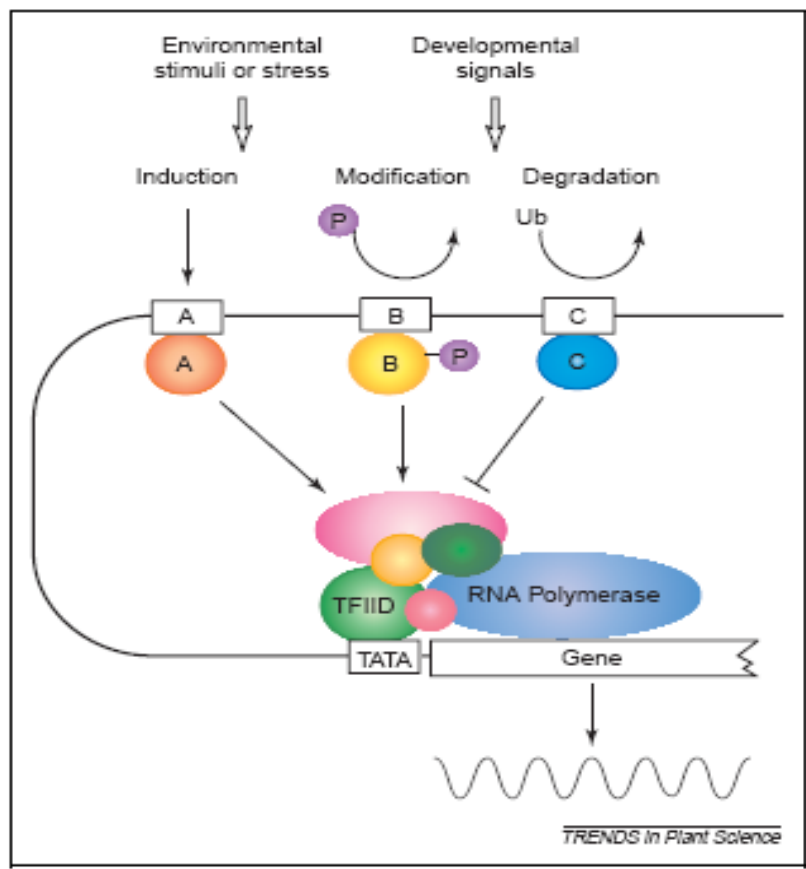
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# Detection of cis-regulatory variation in barley

- Detection of cis-regulatory variation in barley
- Analyse changes in cis dependent expression in response to abiotic stress



Cis-acting elements play a central role in gene expression cascades under abiotic stress

(Yamaguchi-Shinozaki and Shinozaki 2005)

A, B, C

Cis- regulatory elements

A, B, C

Transcription factors

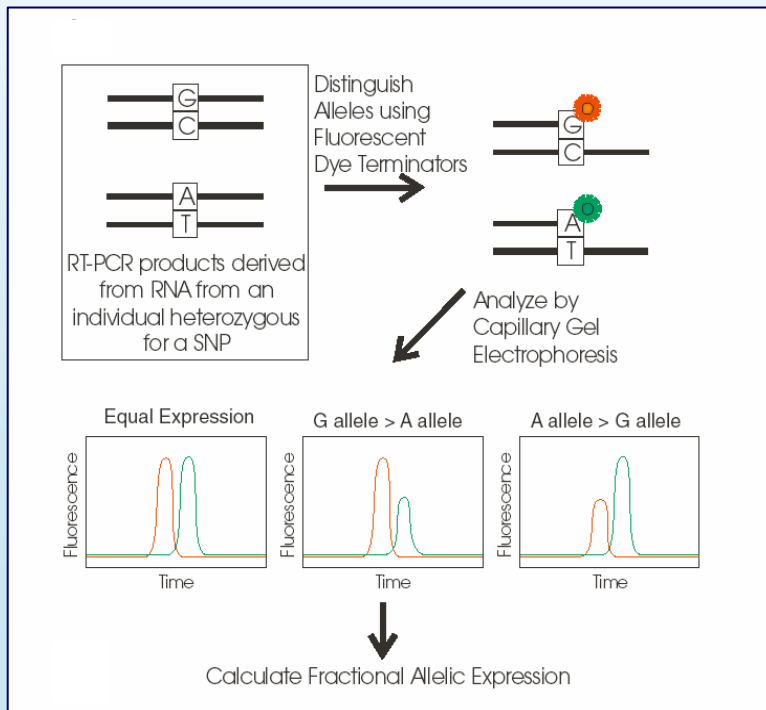
# HOW TO DETECT *CIS*-ACTING REGULATORY VARIATION (i.e. MEASURE ALLELE-SPECIFIC EXPRESSION LEVELS F1- Hybrids )



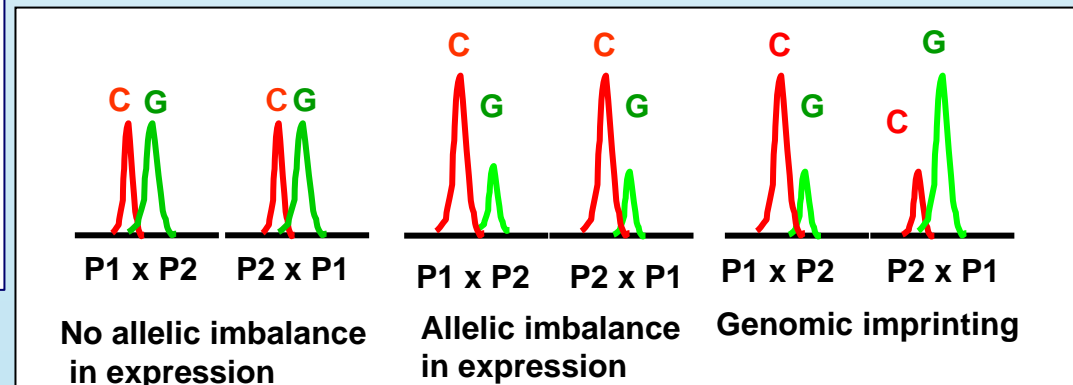
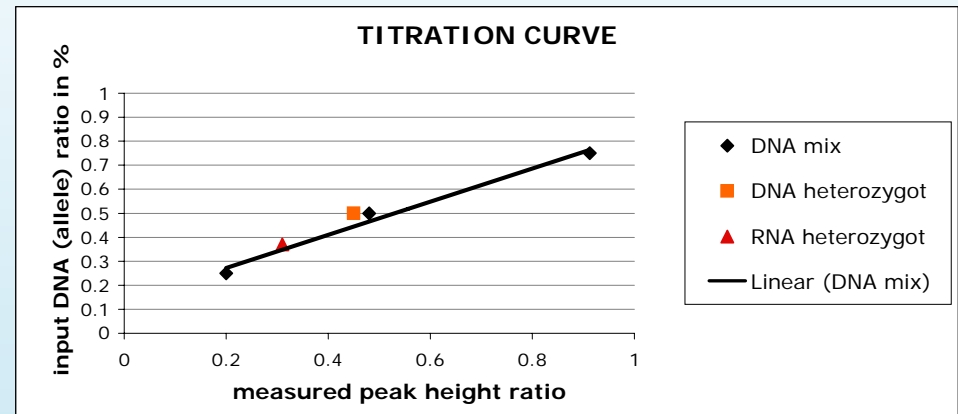
## • F1 HYBRIDS

- avoid trans and environmental differences
- internal normalization (relative expression)

## • RT-PCR SNP ASSAY



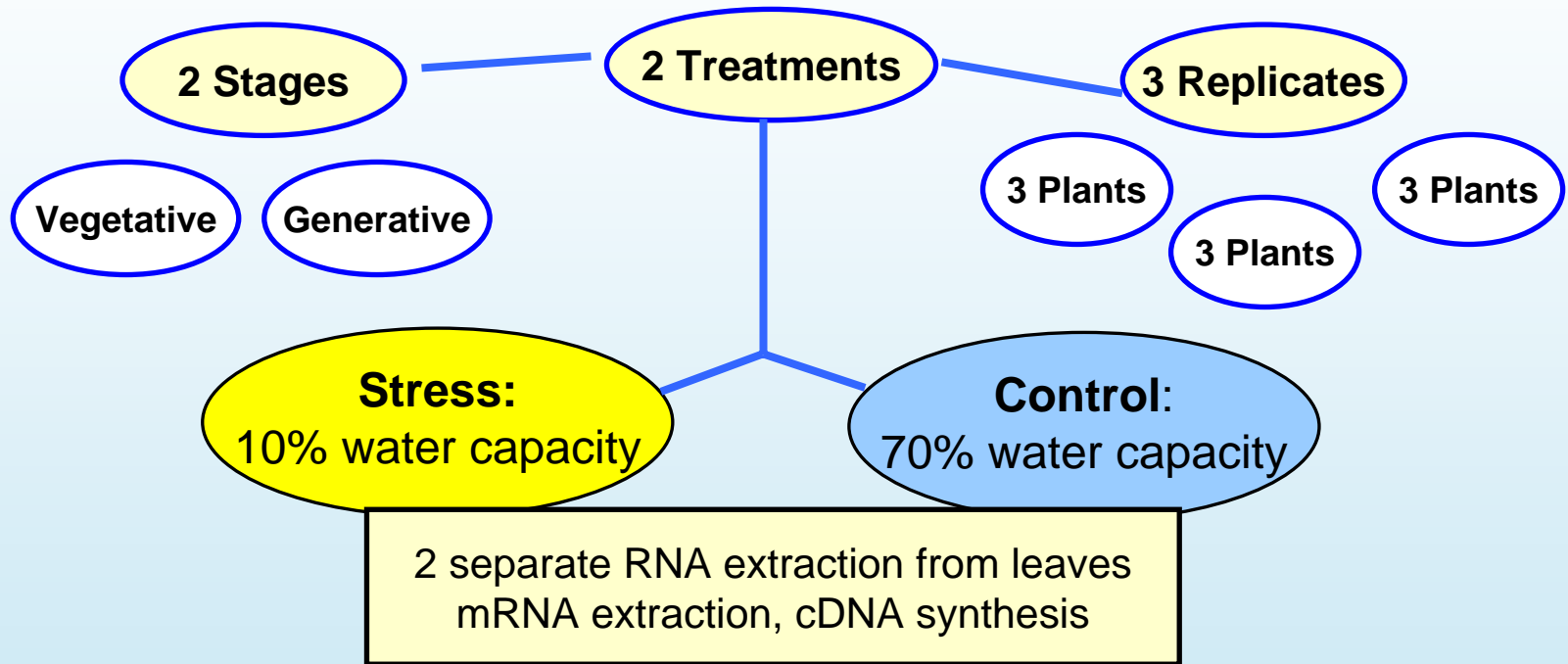
Modified from Yan *et al.* Science, 297 (2002)



# Measuring the impact of drought stress on allelic expression in F1s



**F1/RF1: Tadmor/Sloop Tadmor/WI3408 Sloop/WI3408**  
**Hsp41-1/Arta Hsp41-1/Alexis**



## **Selection of Genes (Drought):**

Literature (i.e. Rostocks et al. 2005)

QTL analysis

ADOC project

Microarray experiment- BMZ project

**70** genes sequenced, **30** revealed SNPs in at least one of the five reciprocal crosses

(where possible two SNPs per gene were targeted for confirmation)

# Genes analysed in the five different crosses – Allelic Imbalance



Genes	Tadmor/Sloop	Tadmor/WI3408	Sloop/WI3408	Hsp41-1/Arta	Hsp41-1/Alexis	Total	AI
ABC00149	x	x		x		3	
ABC0314	x	x		x	x	4	
ABC0422	x		x		x	3	2
ABC0600	x		x		x	3	2
ABC871	x	x	x	x	x	5	5
ABC0949	x	x			x	3	2
ABC0953	x		x	x	x	4	
ABC1249	x	x		x	x	4	
ABC01741	x	x			x	3	2
ABC2112	x	x				2	1
ABC2113	x	x		x	x	4	4
ABC2329	x	x	x		x	4	1
ABC2333	x	x				2	2

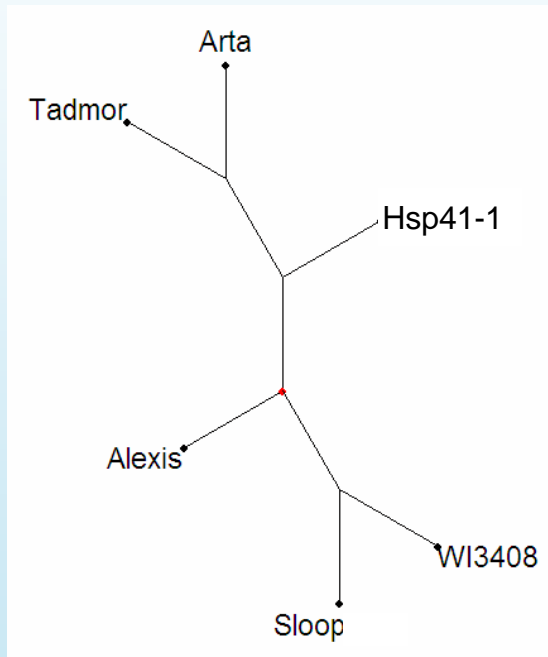
•17 out of 30 genes (57%) showed allelic imbalance in at least one cross

ABC5236	x	x				2	
ABC5604	x		x	x	x	4	1
ABC5702		x				1	
ABC06144	x	x				2	
Contig8246_at		x		x		2	
Contig2924_s_at				x	x	2	
Contig7787_at	x	x				2	2
Contig10029_at	x	x		x		3	2
Contig3499_at	x	x		x	x	4	3
Contig15719_at	x		x		x	3	1
AJ300144	x		x	x		3	
M55448	x	x	x			3	3
<b>Total</b>	<b>27</b>	<b>19</b>	<b>11</b>	<b>12</b>	<b>15</b>	<b>84</b>	
<b>AI</b>	<b>13</b>	<b>8</b>	<b>3</b>	<b>3</b>	<b>9</b>		<b>36</b>
<b>AI (%)</b>	<b>0.48</b>	<b>0.42</b>	<b>0.27</b>	<b>0.25</b>	<b>0.60</b>		<b>0.43</b>

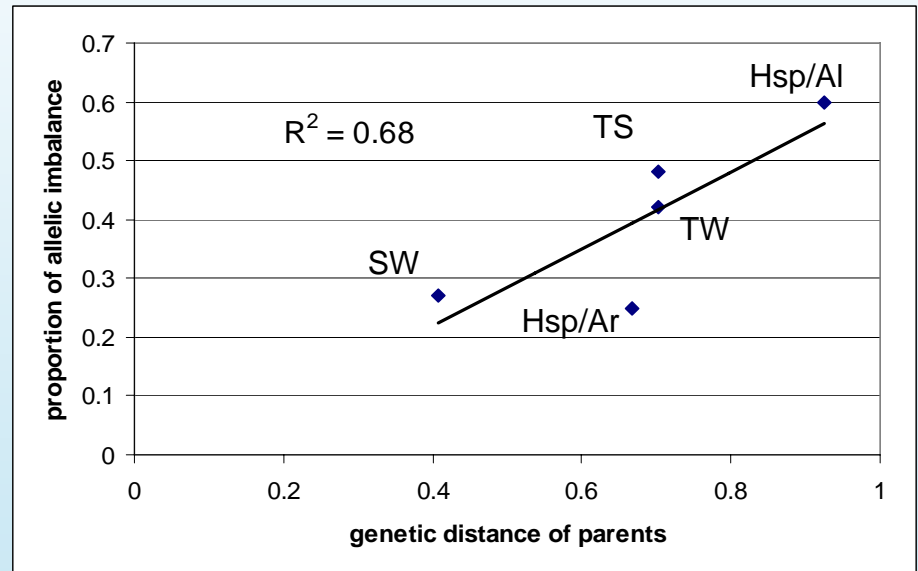
# Correlation between proportion of imbalanced genes and genetic distance between parents



## Neighbour joining tree based on SSRs (GCP genotyping kit)

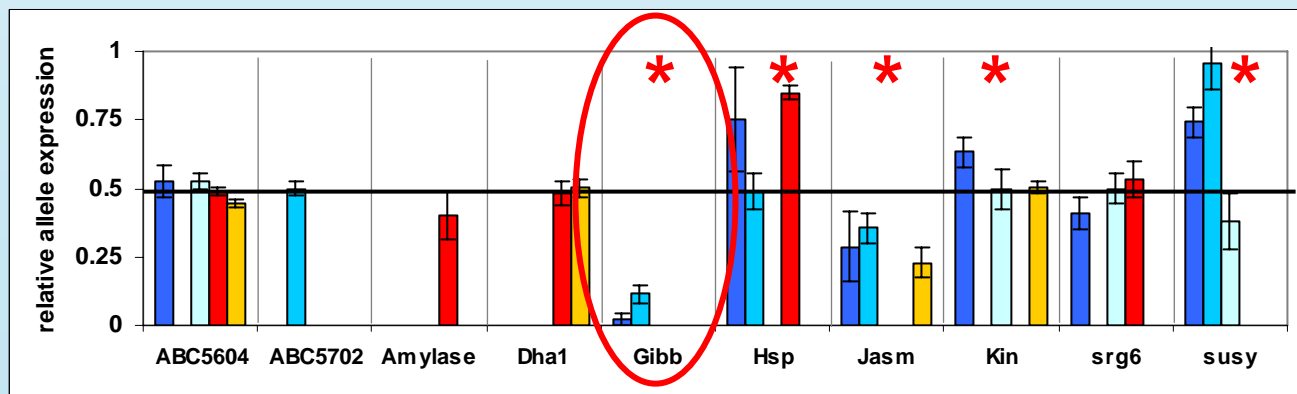
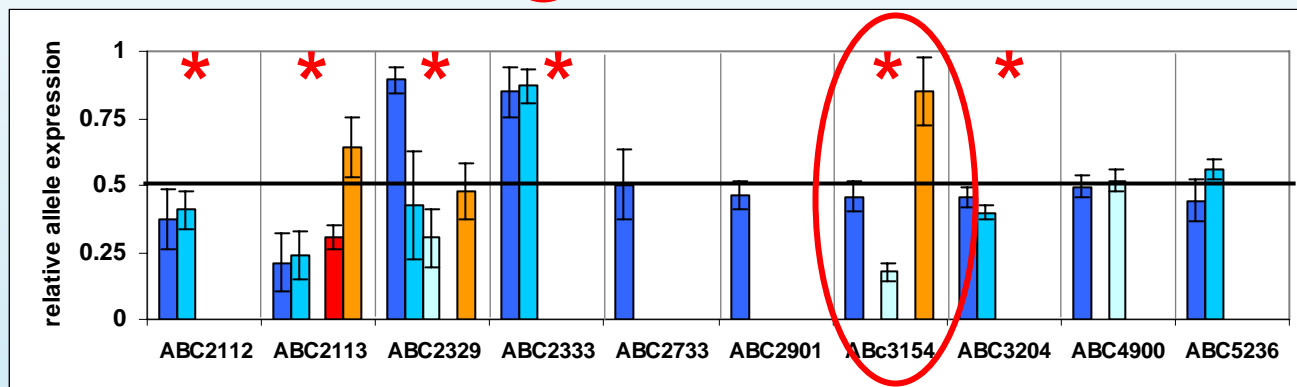
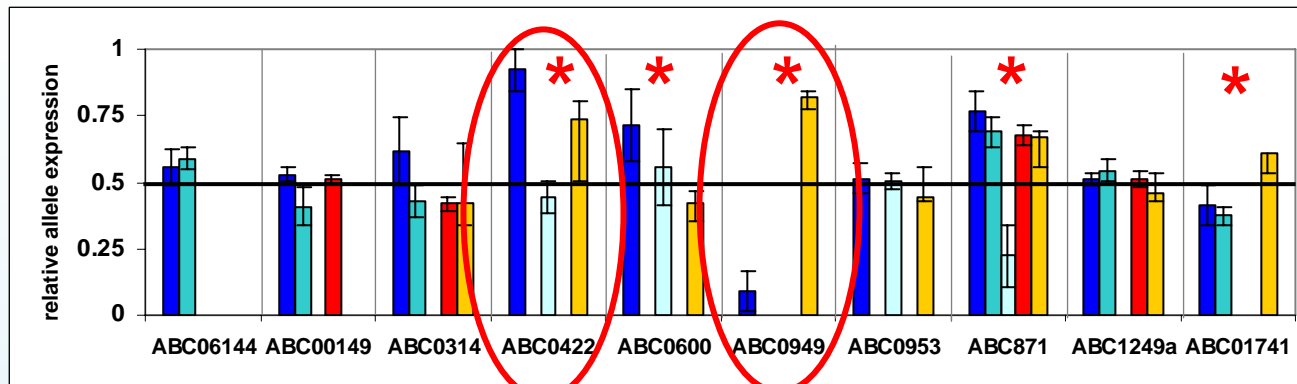


## Correlation between proportion of imbalanced genes and genetic distance between parents (SSRs)



Polymorphisms in the cis-regulatory sequences accumulate proportional to mutations in the linked coding region

# Average relative allelic expression per cross (F1 RF1)

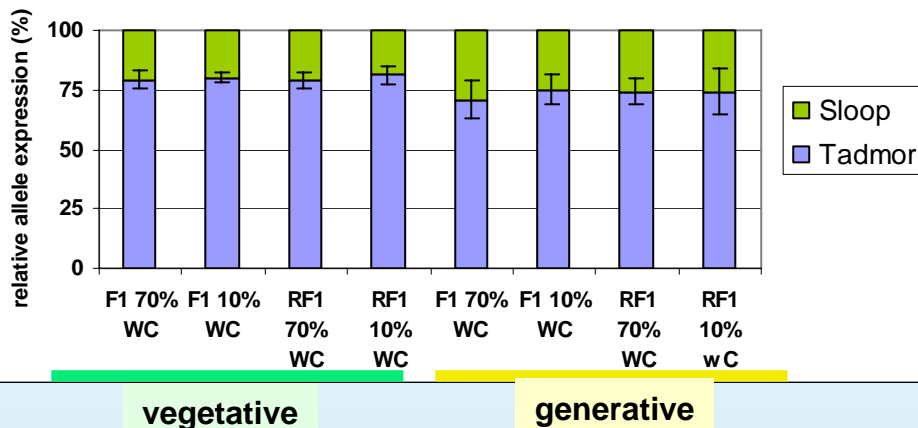


# Example 1: Gamma Hordein precursor with strong allelic imbalance In three different reciprocal crosses



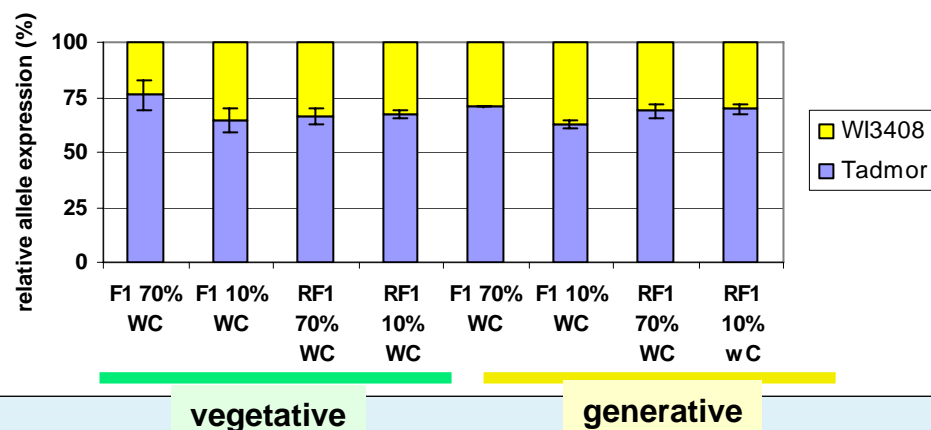
### Tadmor/Sloop

Relative allele expression: Gamma-Hordein in Tadmor/Sloop



### Tadmor/WI3408

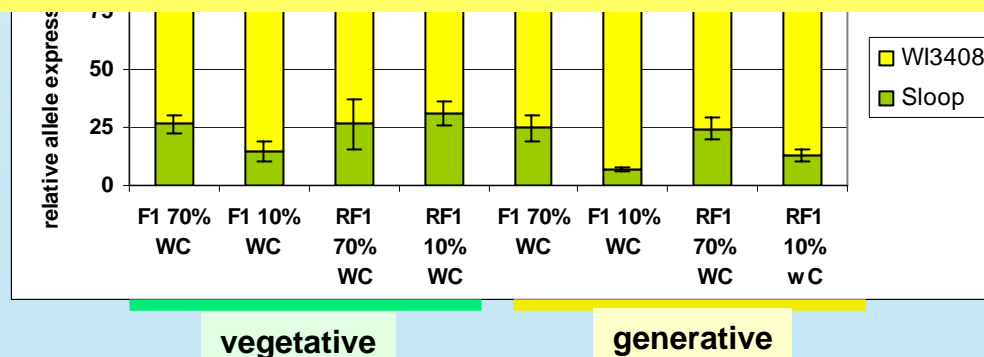
Relative allele expression: Gamma-Hordein in Tadmor/WI3408



### Sloop/WI3408

Three different cis-regulatory elements are present in the three parents.  
Allelic ratios show non-additive effects of cis-elements

Tadmor:	<b>GCGACCCA</b>
Sloop:	<b>TACGTTAA</b>
WI3408:	<b>GACGTTAG</b>

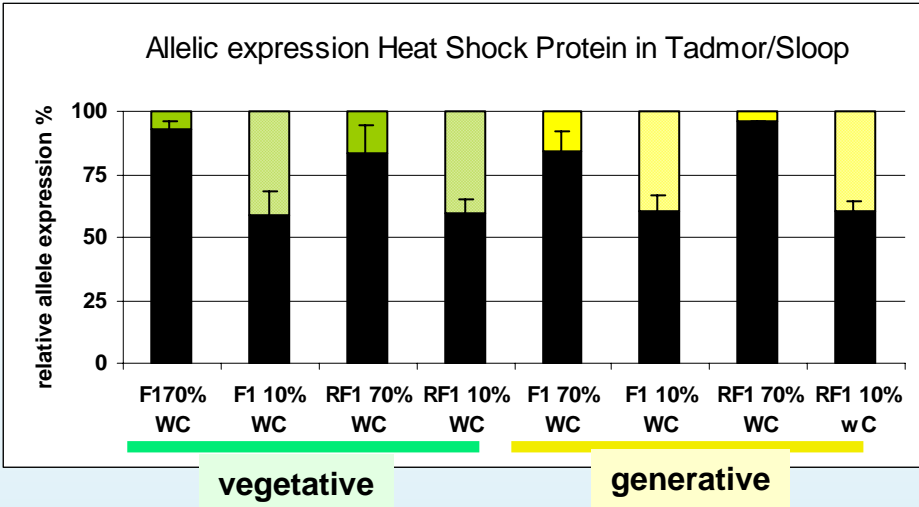




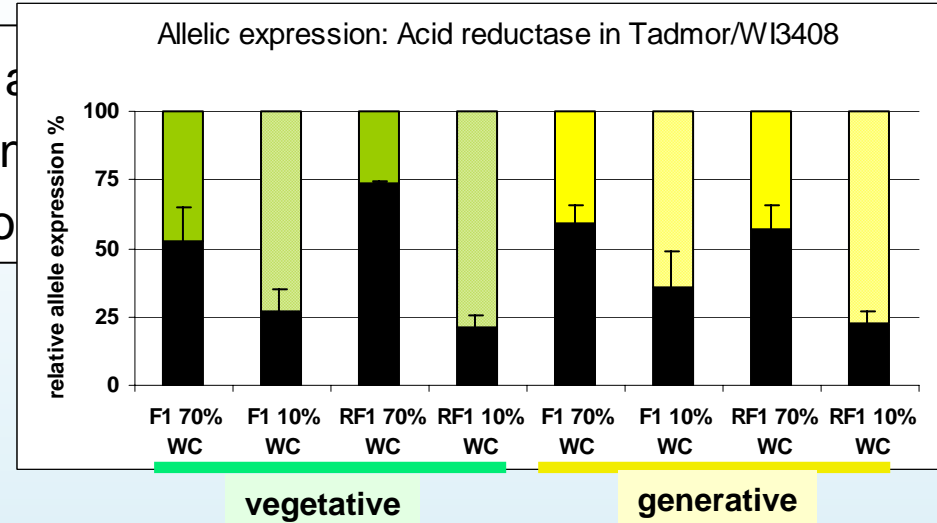


# Example 3: Changes of relative allele expression under Drought Stress

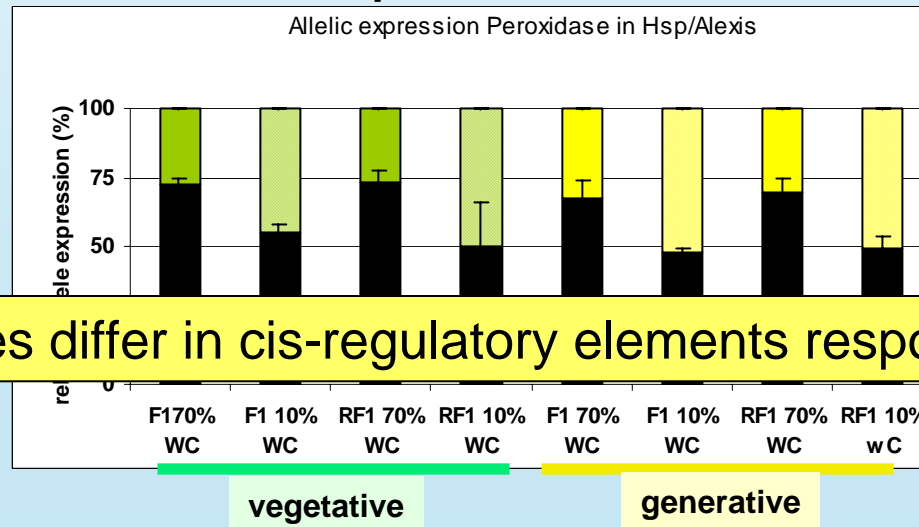
## Tadmor/Sloop



## Tadmor/WI3408



## Hsp/Alexis

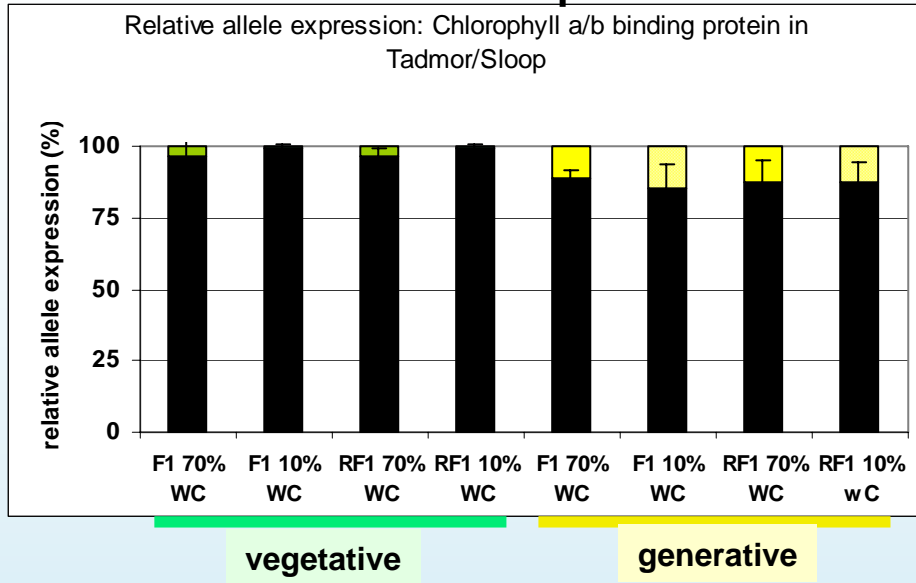


Parents/Genes differ in cis-regulatory elements responsive to drought

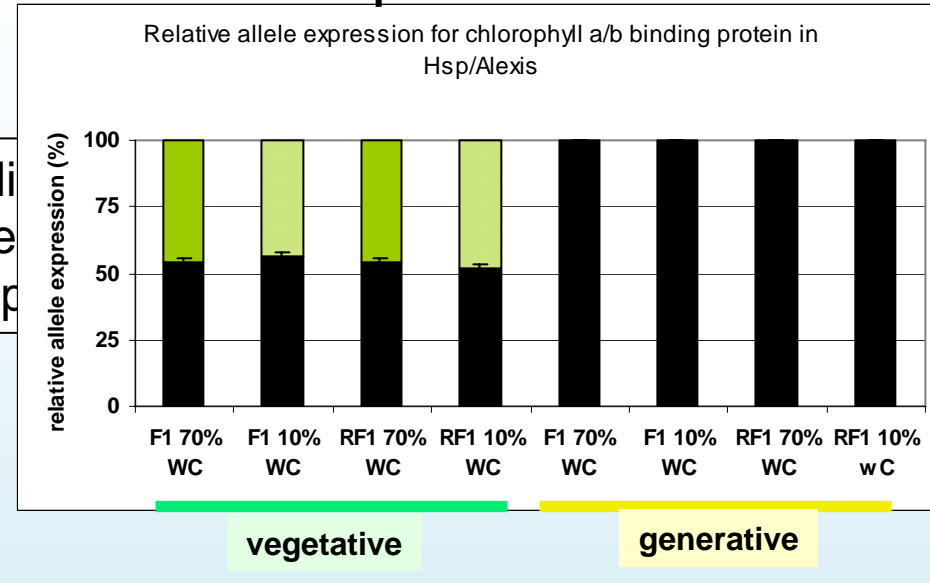


# Example 4: Differences of allele expression between the vegetative and the generative stage

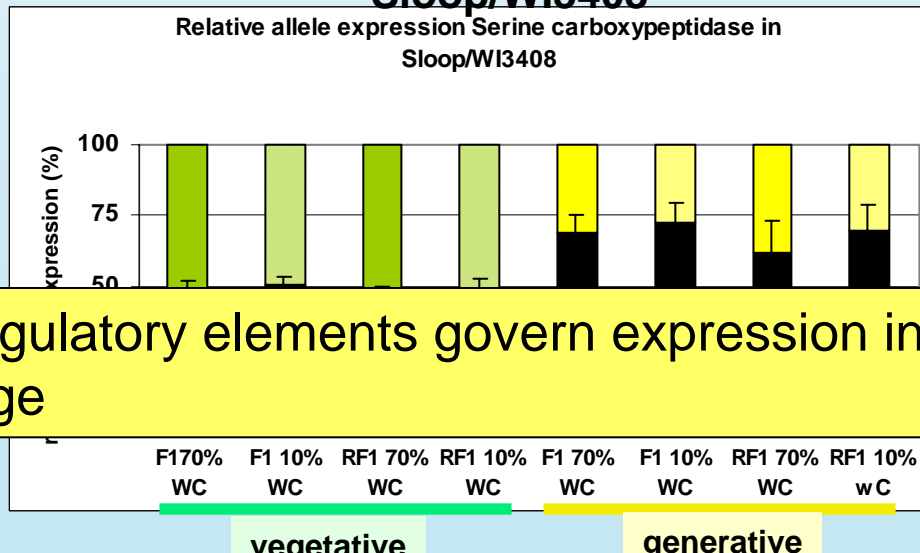
## Tadmor/Sloop



## Hsp/Alexis



## Sloop/WI3408



Different cis-regulatory elements govern expression in the vegetative and generative stage

# Data format

Parents	Cross	Tissue	Treatment	Stage	Rep.	Sample	Gene
Tadmor_Sloop	TS	Leaf	Control	generative	1	1	
Tadmor_Sloop	TS		Drought	vegetative	2	2	
Tadmor_Sloop	ST					3	

Parents of the cross

Cross direction  
F1/RF1

Leaf, Roots

Control  
Drought  
Cold

Developmental  
Stage

RNA extr.  
(2)

Corrected Signal  
Ratios  
 $P1/(P1+P2)$



## Conclusion

- **Cis-acting variation is a common phenomenon in barley (57% of the genes showed AI with up to 9-fold differences in allelic expression -drought)**
- **Two further crosses (AH, OWB) have been tested and preliminary data indicate allelic imbalance between 30 – 80% of the genes tested -cold)**
- **Cis-acting variation is relevant for the stress response, and for response to developmental cues (no genetic imprinting detected).**
- **Sequence diversity: Genetic distance between parents is correlated with the occurrences of allelic imbalance**

## Outlook

- **Mapping of genes (including ADOC genes) in a RIL population is ongoing**
- **Mapping of expression of imbalanced genes in a RIL population to test for cis-acting factors e.g. giant embryo**
- **Analysis of genes with sequence information up and downstream the exon regions for detailed molecular characterization of specific polymorphisms in the regulatory regions**



Thank you!

# Statistical analysis

- **Calculation of relative allele expression:**

$$P1/(P1+P2)$$

- **Test of Allelic Imbalance: 3- factorial ANOVA**  
(for each cross separately)

**Fixed Model:**

$$y_{ijkm} = \mu + S_i + I_j + T_k + SI_{ij} + ST_{ik} + IT_{jk} + SIT_{ijk} + \varepsilon_{mijk}$$

**S = Developmental Stage**

**I = Imprinting, Effect of Reciprocal Cross,**

**T = Treatment**

- **Significance threshold FDR <0.05 (Benjamini-Hochberg)**

- **Test for overall allelic imbalance: significant deviation from 0.5 allele ratio with Dunnett multiple comparison of Lsmeans differences**