

# *Vrn-H1* and *Vrn-H2* allelic diversity in barley may explain specific adaptation to the Mediterranean environments

Aula Dei Experimental Station, CSIC  
Zaragoza, Spain

# Background - 1

- Vernalization response, 3 genes *Sh*, *Sh2* and *Sh3* (Takahashi & Yasuda, 1971). Only the first two segregate in Western materials.
- Genes located on 4H, 5H and 1H
- Epistatic interaction between the genes. Only one genotype (*ShShsh2sh2sh3sh3*) shows winter type
- Multiple allelic series at *Sh2*

# Background - 2

- Cloning of *VrnA<sup>m</sup>1* (Yan et al., 2003), *VrnA<sup>m</sup>2* (Yan et al., 2004)
- Orthologous genes in wheat (TaVRT1, Danyluk et al., 2003; WAP1, Trevaskis et al., 2003) and barley (von Zitzewitz et al., 2005)
- Both genes are transcription factors: *VRN1* is a MADS-box, similar to *AP1*; *VRN2* is a zinc finger and contains a CCT domain, similar to *CO*
- *Sh* = *Vrn-H2*, *Sh2* = *Vrn-H1*

# Interaction *VrnH1*/*VrnH2*

*VrnH2* is a repressor of *VrnH1*. *VrnH1* is activated by cold treatment whereas *VrnH2* by daylength (Yan et al., 2004; Trevaskis et al., 2006)

- Promoter of *VrnH1* (Yan et al., 2003, Beales et al., 2005)
- Intron 1 of *VrnH1* (Fu et al., 2005; von Zitzewitz et al., 2005)

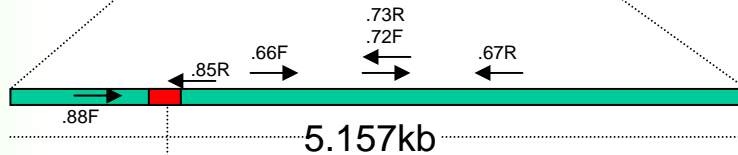
# Intron 1

Spring: Morex

5.586kb

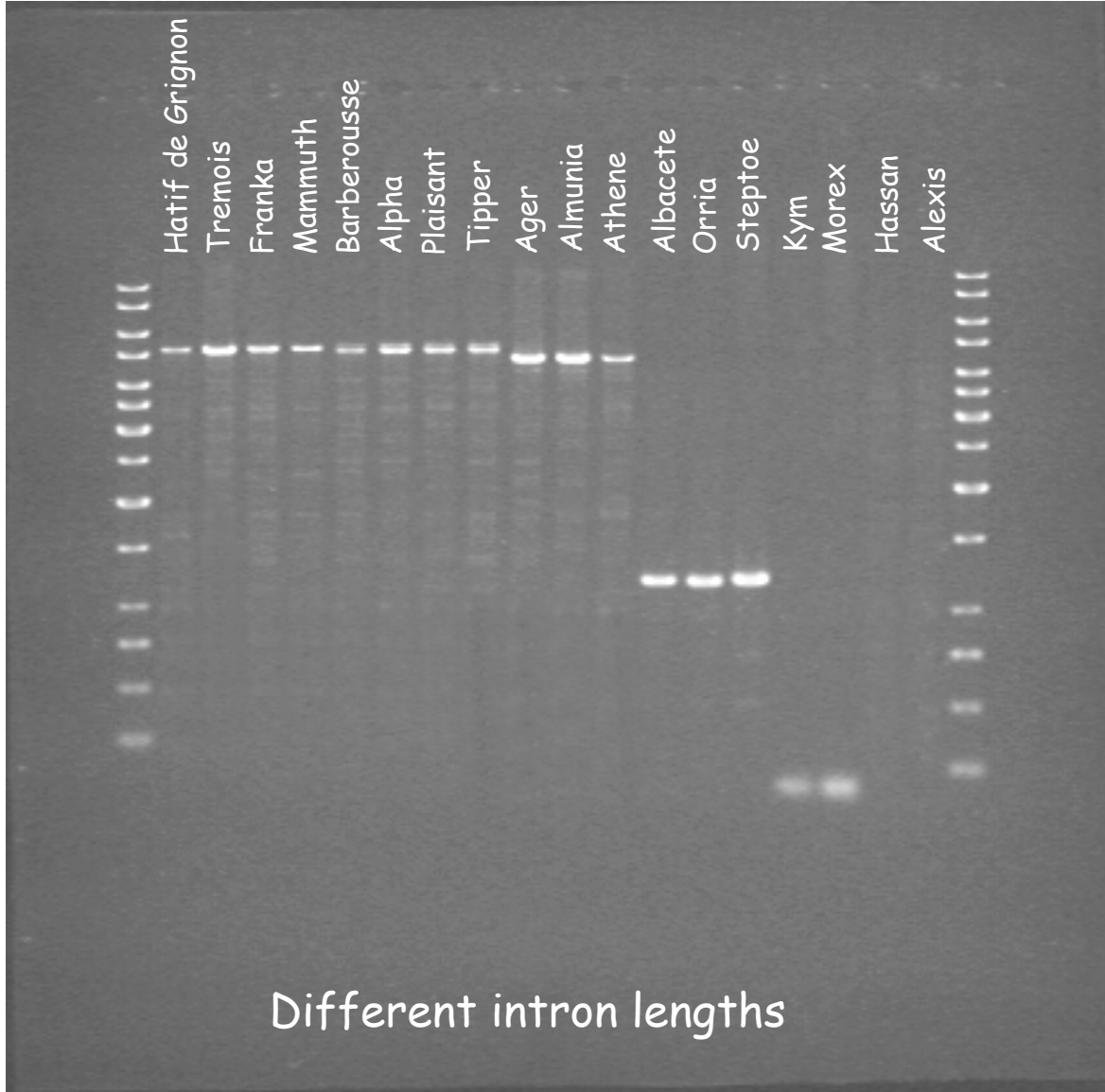


Winter: Strider and Dicktoo



Regulatory  
element  
436-bp

*Allelic diversity within functional markers*



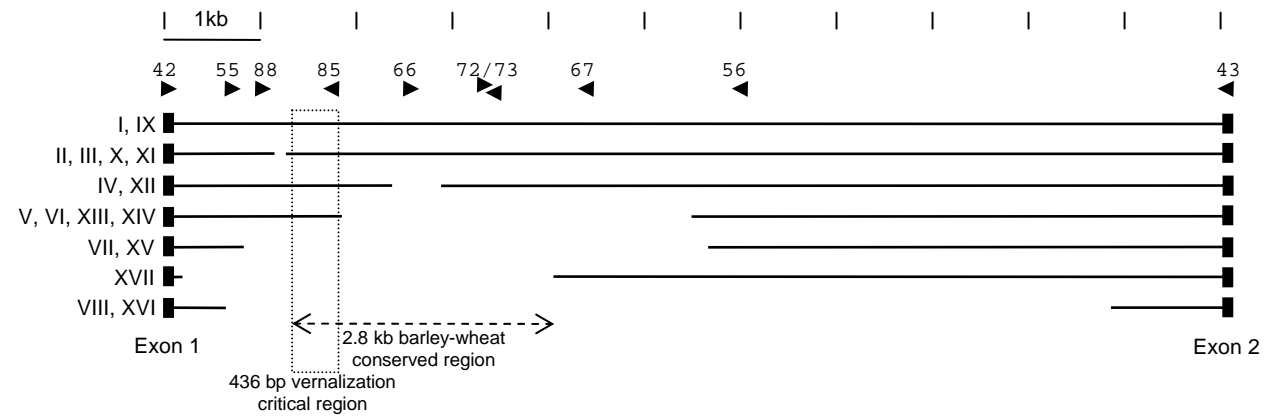
- *VrnH1*: 9 alleles within intron 1
  - 5250, 5200, 5150, 4850, 1200, 150 bp, 1900 bp, null allele
  - Critical vernalization region of 436 bp
- *VrnH2*: 2 alleles, presence or absence of HvZCCT cluster
  - Several SNPs within HvZCCTb

## Allelic diversity within functional markers

Haplotype	<i>Vrn-H1</i> <i>HvBM5A</i>	<i>Vrn-H2</i> <i>HvZCCT</i>	
I	5250 n	1400	winter
II	5150 n	1400	winter
III	5250 n	1400	winter
IV	4850 n	1400	winter
V	1200 n	1400	intermediate
VI	1200 n	1400	intermediate
VII	150 n	1400	spring
VIII	n 1900	1400	spring
IX	5250 n	null	facultative
X	5200 n	null	spring
XI	5150 n	null	facultative
XII	4850 n	null	spring
XIII	1200 n	null	spring
XIV	1200 n	null	spring
XV	150 n	null	spring
XVI	n 1900	null	spring
XVII	n n	null	spring



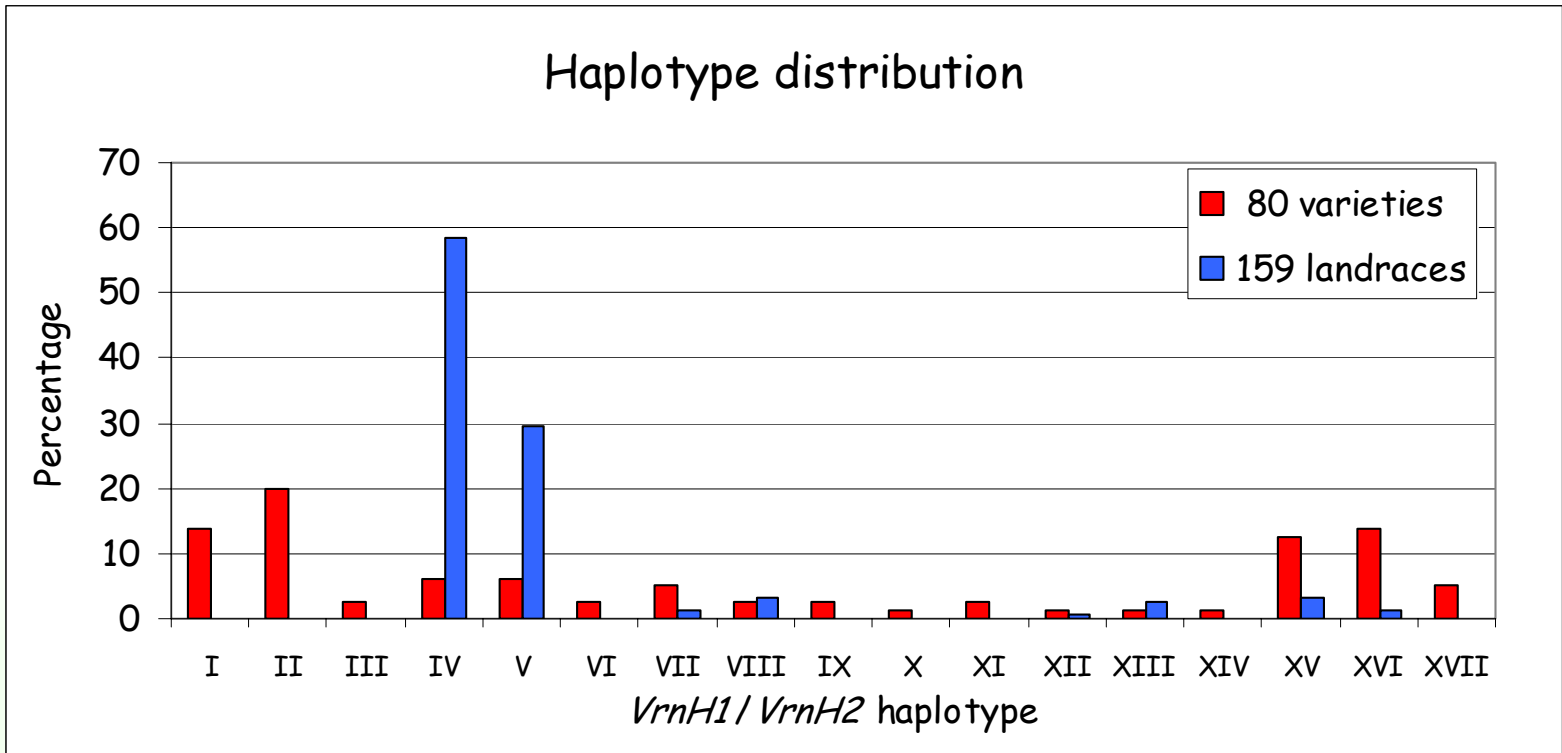
# Allelic diversity within functional markers

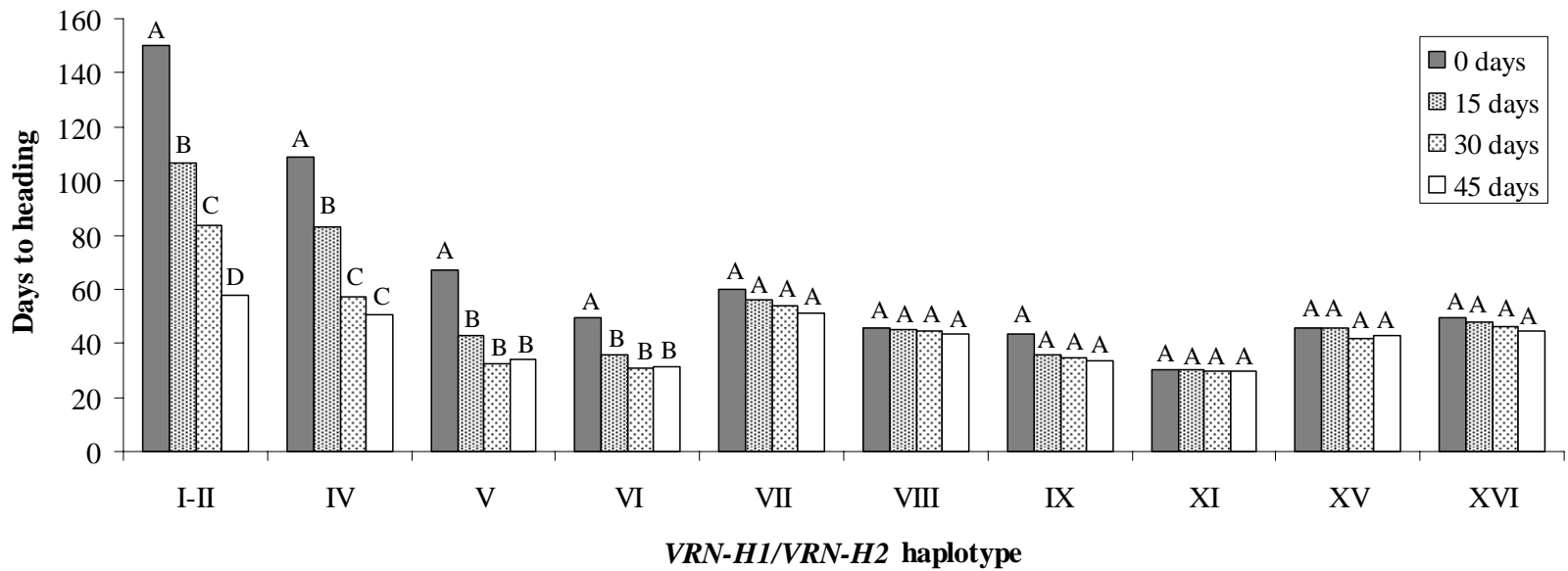


- I/IX - Kompolti korai, Dicktoo
- II - Igri, Nure
- IV - Ager
- V - Albacete, Pané
- VI - Orria

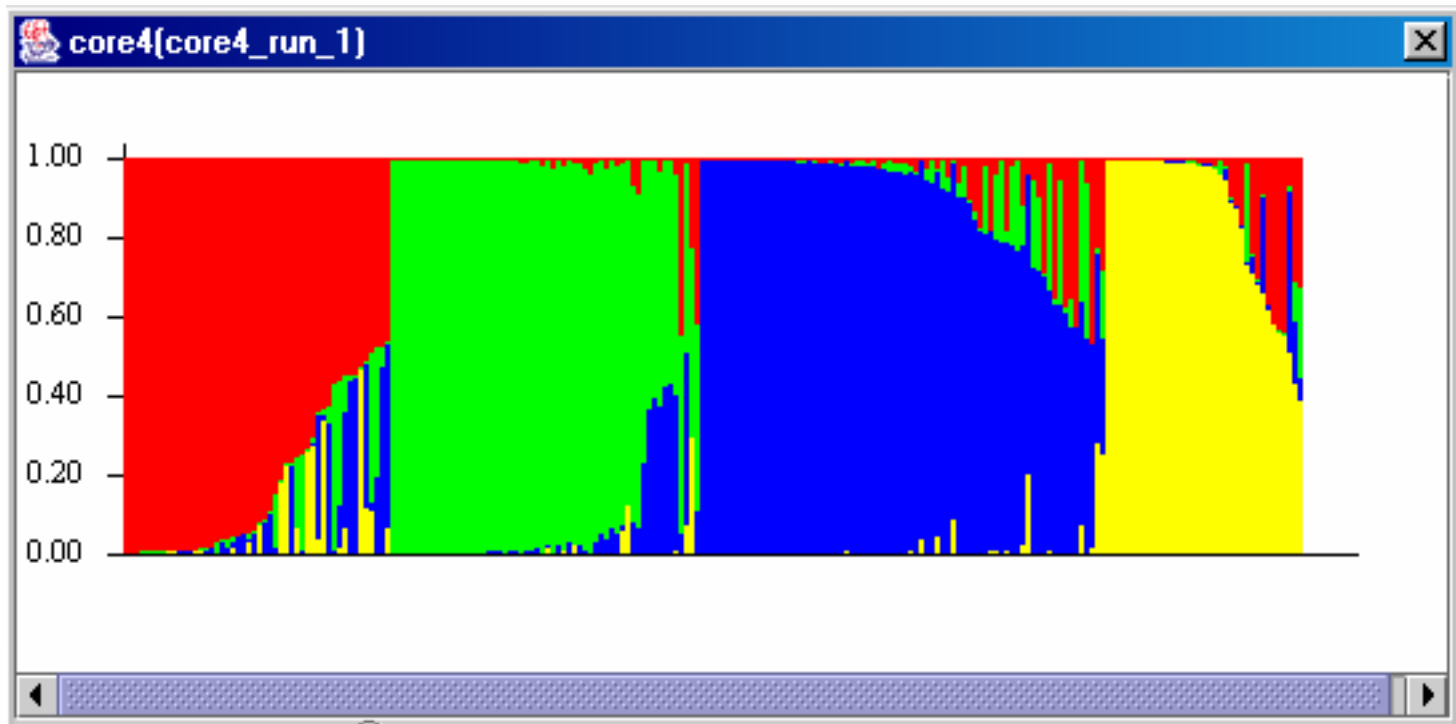
- VII - Morex
- VIII - Triumph
- X - Tremois
- XVII - Maskin

*Allelic diversity within functional markers*



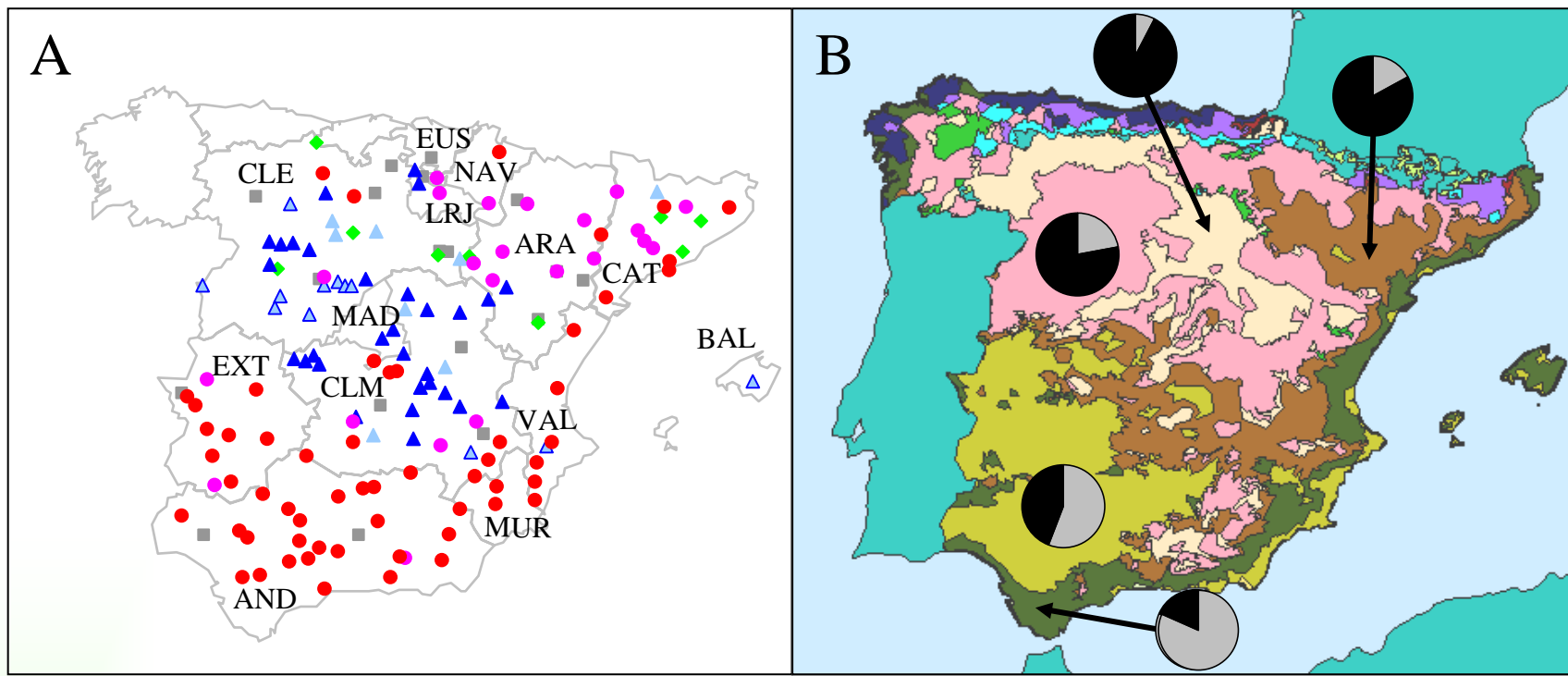


Vernalization response: presence of HvZCCT and at least "minimal vernalization critical region" in HvBM5



- Population 1 (mostly European 6-row)
- Population 2 (Spanish 6-row)
- Population 3 (Spanish 6-row)
- Population 4 (2-row Spanish and non-Spanish)

Core collection plus 53 reference cultivars; 64 microsatellites. Summary plot of Q estimates (proportion of each individual's genome originating from each inferred population)



Subpopulations  
K=4

- I
- ◆ II
- ▲▲ III-1, III-2, III-3
- IV-1, IV-2

Spanish Autonomous Regions

- |                         |                           |
|-------------------------|---------------------------|
| ARA: Aragón             | EXT: Extremadura          |
| AND: Andalucía          | LRJ: La Rioja             |
| BAL: Baleares           | MAD: Madrid               |
| CAT: Cataluña           | MUR: Murcia               |
| CLE: Castilla y León    | NAV: Navarra              |
| CLM: Castilla La Mancha | VAL: Comunidad Valenciana |
| EUS: Euskadi            |                           |

Papadakis Climate Classification

- Temperate Mediterranean
- Fresh Temperate Mediterranean
- Subtropical Mediterranean
- Mediterranean Maritime
- Continental Mediterranean

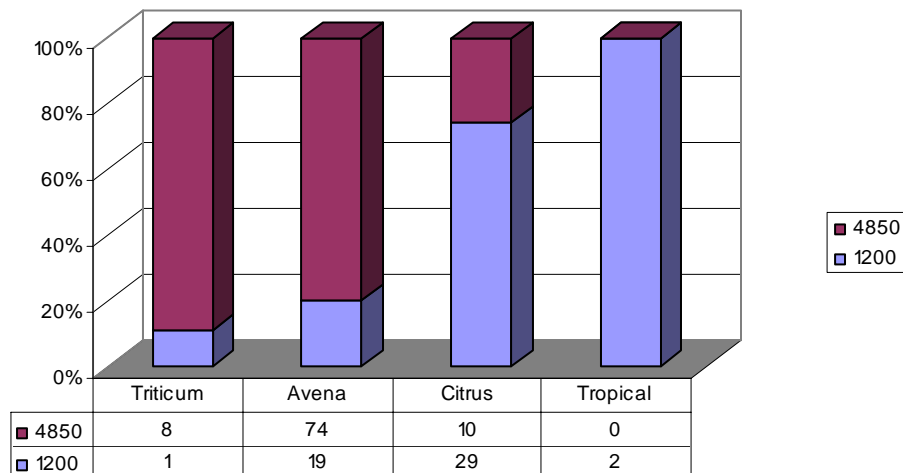
HvBM5  
intron 1

- 1200 bp
- 4850 bp

## Winter type

Chi-square 42.097

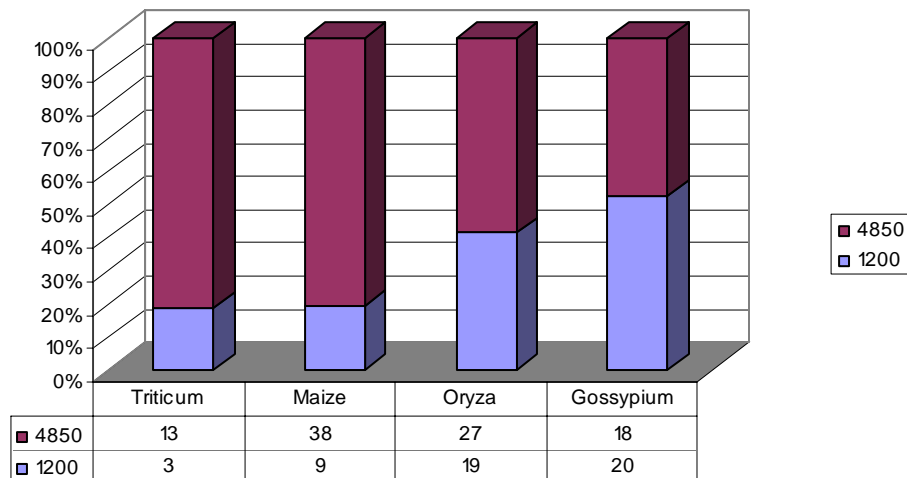
Prob <0.0001



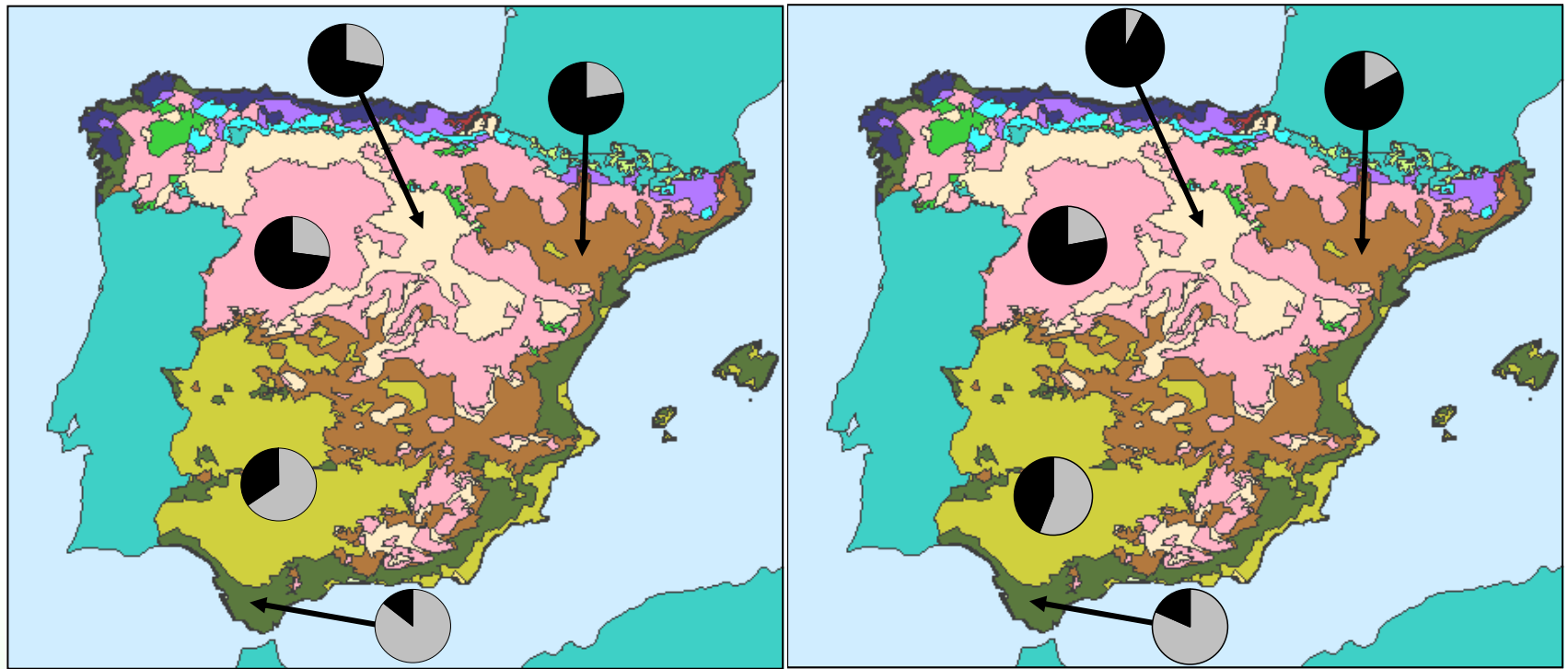
## Summer type

Chi-square 14.339

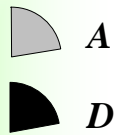
Prob 0.0063



# GERMPLASM CHARACTERIZATION AND MANAGEMENT

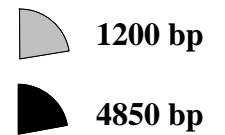


**MWG699**



MWG699	HvBM5		Total
	1200	4850	
A	42	21	63
D	8	72	80
<b>Total</b>	<b>50</b>	<b>93</b>	

**HvBM5  
intron 1**

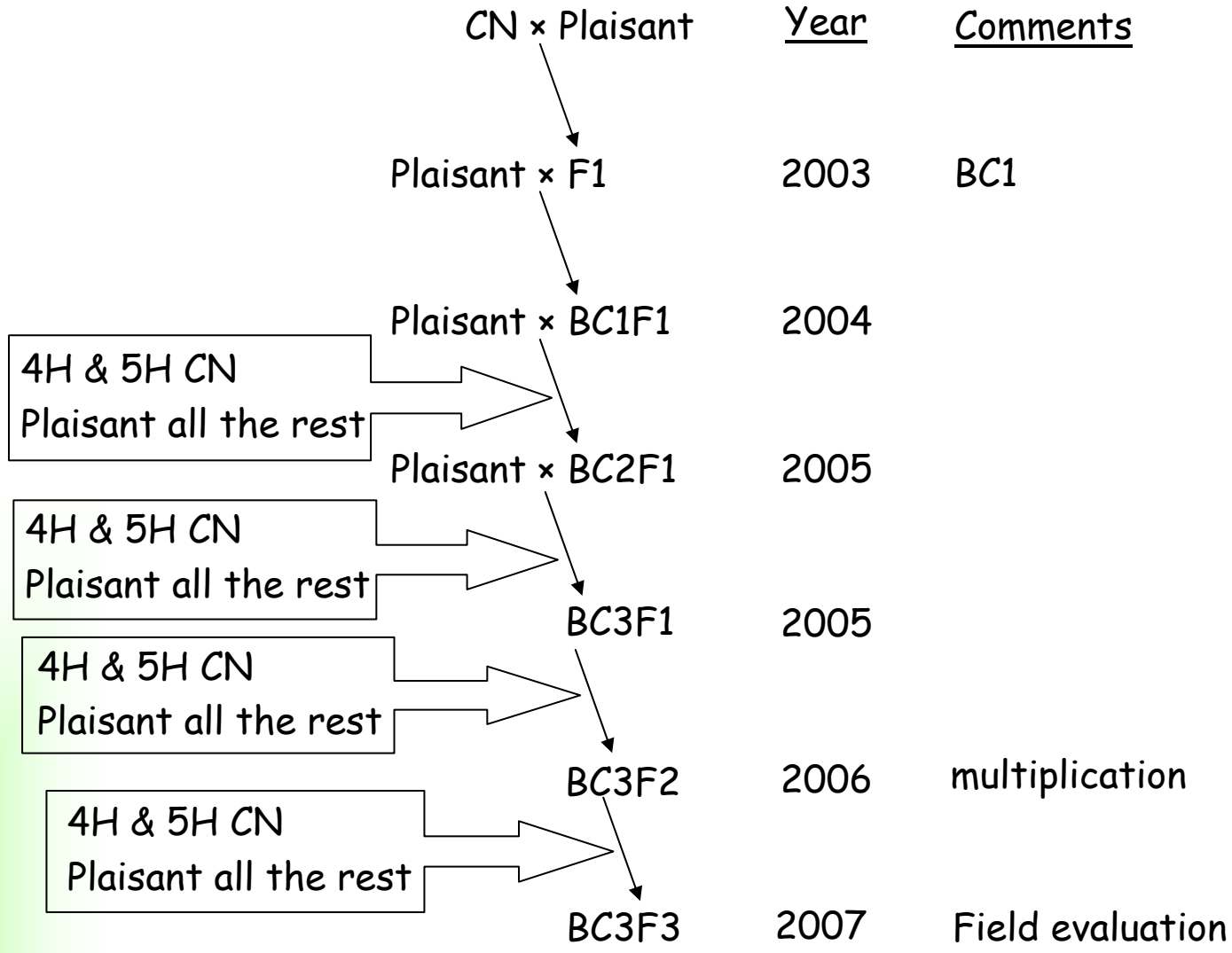


# Use of exotic germplasm in breeding

MAS Backcrossing for introgression of specific regions



# MAS-BC to introgress *VrnH1* and *VrnH2*



## *MAS-BC to introgress VrnH1 and VrnH2*

The goal is to test the effect of four allelic combinations:

1. Recurrent on 4H & 5H
2. Spanish (CN) on 4H, recurrent on 5H
3. Recurrent on 4H, CN on 5H
4. CN on 4H & 5 H

## *MAS-BC to introgress VrnH1 and VrnH2*

- BC1 - 36 plants, 7 markers
  - Selection for CN (2 on 4H, 3 on 5H)
- BC2 - 112 plants, 15 markers
  - Selection for CN (2 on 4H, 3 on 5H)
  - Pleasant the rest (2H, 4H and 5H)
- BC3F1 - 110 plants, 23 markers
  - Selection for CN (2 on 4H, 3 on 5H)
  - Pleasant the rest (1H, 2H, 4H, 5H and 7H)
- BC3F2 - 185 plants, 44 markers
  - Selection for CN (2 on 4H, 3 on 5H)
  - Pleasant the rest (mainly 3H and 6H)

## *MAS-BC to introgress VrnH1 and VrnH2*

11 families BC3F3 for field testing:

G1 - Plaisant (4H & 5H), 3 families, 95.4-100 % Plaisant

G2 - Spanish (4H), 3 families, 90.9-93.2 % Plaisant

G3 - Spanish (5H), 3 families, 77.3-88.6 % Plaisant

G4 - Spanish (4H & 5H), 2 families, 81.8-84.1% Plaisant

## Barley breeding (Zaragoza)

Dr. Ana M. Casas

Dr. M. Pilar Gracia

Dr. Ernesto Igartua

Dr. José M. Lasa

Alfonso Cuesta

Dr. Samia Yahiaoui

## Spanish barley breeding programme

UdL-IRTA, Lérida

(Dr. Jose L. Molina-Cano)

ITA-JCyL, Valladolid

(Dr. Francisco Ciudad)

## External collaborators

Prof. Patrick M. Hayes, OSU

Dr. Ildikó Karsai HAS-Martonvásár

Dr. Peter Szűczs