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GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

FUNCTIONAL SNP MARKERS FOR THE VERNALIZATION REQUIREMENT IN BARLEY: AN OPTION 1 APPROACH

Document prepared by experts from the United Kingdom

Plant Science into practice

NIAB
National Institute of Agricultural Botany

Presenter Name Carol Norris • Date May 2010

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Functional SNP Markers for the Vernalization Requirement in Barley

Carol Norris
James Cockram
Donal O'Sullivan

Outline:

- Project background
- Vernalization and photoperiod response genes
- "Alternative" varieties
- Detection of off-types
- Conclusions

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Seasonal Growth Habit (SGH) –major phenotypic division in barley

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SGH – UPOV TG/19/10 characteristic 29

SGH states:

1 = spring

2 = alternative

3 = winter

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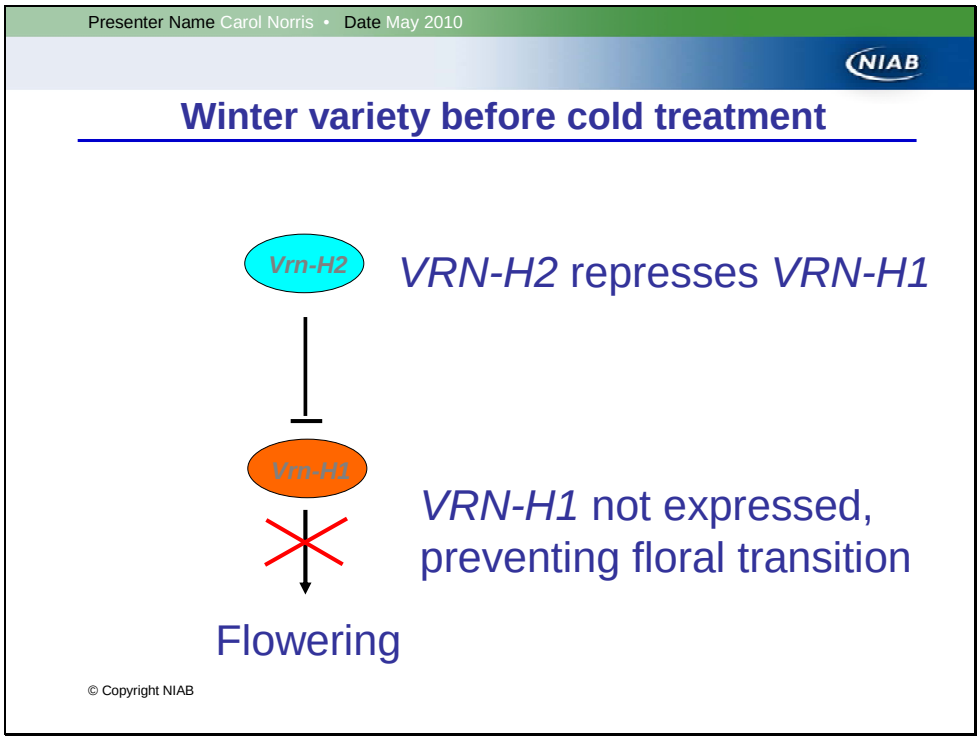
Genetic control of SGH

- Two major loci in European barley (*VRN-H1* and *VRN-H2*)
- *VRN-H1* responsible for flowering mechanism
- *VRN-H2* represses *VRN-H1*
- During cold treatment repression is removed
- Mutation in either gene = spring type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Winter variety before cold treatment
Vrn-H2
VRN-H2 represses VRN-H1
Vrn-H1
VRN-H1 not expressed,
preventing floral transition
Flowering
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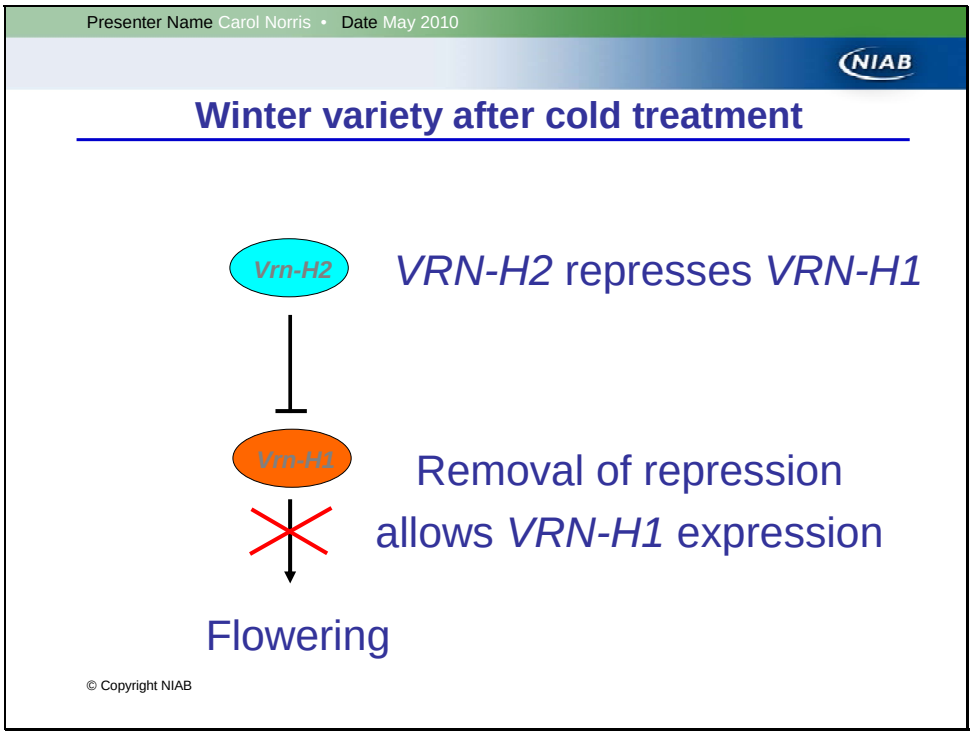
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Winter variety after cold treatment
Vrn-H2
VRN-H2 represses VRN-H1
Vrn-H1
Removal of repression
allows VRN-H1 expression
Flowering
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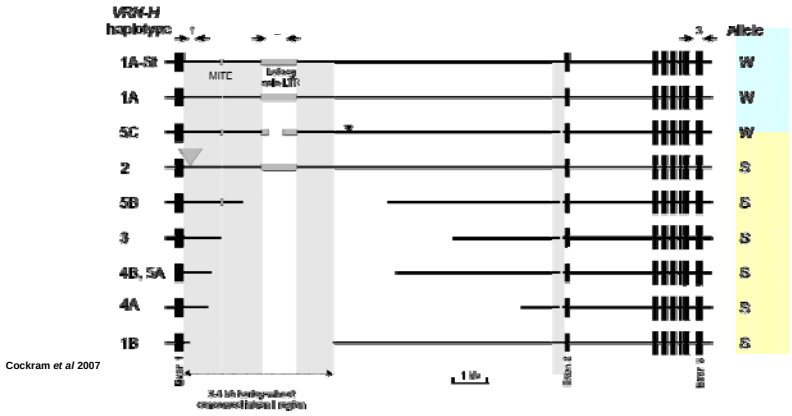
VRN-H1: 6 spring, 2 winter alleles



Cockram *et al* 2007

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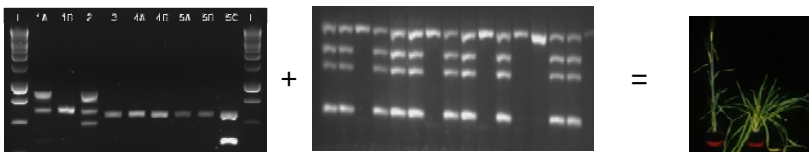
VRN-H1 assay + *VRN-H2* assay

Combined haplotypes predict SGH in 396 UK varieties

VRN-H1 assay + *VRN-H2* assay = predicted SGH



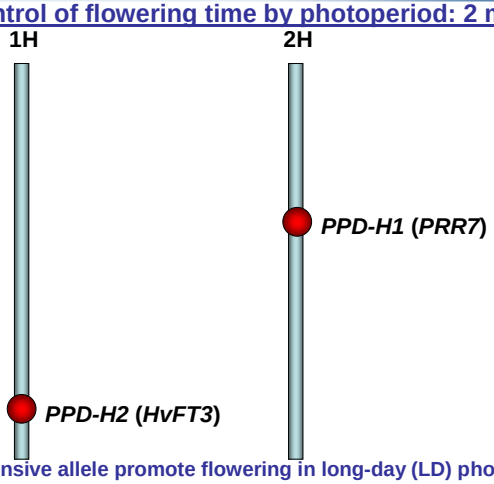
(Cockram *et al* 2008) (Karsai *et al* 2005)

Generated a reference database of *VRN-H1* and *VRN-H2* alleles

Since September 2008…

- Tested and sequenced "alternative" varieties
- Investigated the role of photoperiod (PPD) genes in SGH
- Developed PCR marker to detect and quantify off-types (uniformity)

Control of flowering time by photoperiod: 2 major loci



***PPD-H1*: responsive allele promote flowering in long-day (LD) photoperiods. non-responsive allele has no promotional effect in LDs**
***PPD-H2*: responsive allele represses flowering in short-day (SD) photoperiods. non-responsive allele (presence of the gene) has no effect in SDs.**

Name	AFP	SGH	VRNH1_Mpx	VRN-H2 Mpx	HnFT3_Mpx	PPDH1 T-308T/G
JEWEL	1320	winter	1A	1	0	G
HONEY	1324	winter	1A	1	0	G
LARK	1325	winter	1A	1	0	G
BARTON	1326	winter	1A	1	0	G
TOFFEE	1327	winter	1A	1	0	G
SEVILLA	1396	winter	1A	1	0	G
ETHNO	1398	winter	1A	1	0	G
HELIGAN	1402	winter	1A	1	0	G
MASQUERADE	1403	winter	1A	1	0	G
TABETHA	1404	winter	1A	1	0	G
MOLLY	1405	winter	1A	1	0	G
OPAL	1435	winter	1A	1	0	T
MARINER	1436	winter	1A	1	0	T
TURINE	1330	winter	1A	1	0	G
BISTRO	1392	winter	1A	1	0	G
MUSETTE	1394	winter	1A	1	0	G
1		alternative	5C	1	0	G
2		alternative	1A	1	0	G
3		alternative	5A	1	0	T
4		alternative	1A*	0	0	G
5		alternative	1A*	1	0	T
6		alternative	5A	1	0	T
7		alternative	5C	0	0	G
8		alternative	1A*	0	0	T
DALLAS	1022	spring	S	0	1	T
GRAPHIC	1029	spring	S	0	1	T
CHARIOT	1031	spring	S	0	1	T
FELICE	1091	spring	S	0	1	T
HERON	1107	spring	S	0	1	T
DELIBES	1133	spring	S	0	1	T
COOPER	1146	spring	S	0	1	T
BREWSTER	1147	spring	S	0	1	T
CORK	1107	spring	S	0	1	T
OPTIC	1188	winter	S	0	1	T
BRAHMS	1191	spring	S	0	1	T
CANASTA	1194	spring	S	0	1	T
REGGAE	1196	spring	S	1	1	T
JIVE	1201	spring	S	1	1	T
PRIMERA	1245	spring	S	0	1	T
CLARITY	1246	spring	S	0	1	T

Winter
***VRN-H1* = W**
***VRN-H2* = W**
***PPD-H2* = W (responsive/delayed flowering under SD)**
***PPD-H1* = variable**

Alternative
***VRN-H1* = W or S**
***VRN-H2* = W or S**
***PPD-H2* = W**
***PPD-H1* = variable**

Spring
***VRN-H1* = S**
***VRN-H2* = S/W**
***PPD-H2* = S (non responsive/ no delayed flowering under SD)**
***PPD-H1* = non-responsive to LD**

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Genotyping ''alternative'' varieties

Genotyped for SNPs and SSRs

	VRN-H1 Mpx	SNP1	SNP2	SNP3	SSR	Del	SNP4	SNP5	SNP6	VRN-H2	haplotype	predicted pheno	PPD-H1	PPD-H2
Strider	1A	T	A	C	4	0	T	G	C	+Z	1A+Z	Winter		
2	1A	T	A	C	4	0	T	G	C	+Z	1A+Z	Winter	G	0
5	1A	T	A	C	4	0	T	G	C	+Z	1A+Z	Winter	T	0
8	1A	T	A	C	4	0	T	G	C	-Z	1A-Z	weak spring	T	0
4	1A	T	A	C	4	0	T	G	C	-Z	1A-Z	weak spring	G	0
Express	5C	T	G	C	5	5C	C	A	G	+Z	5C+Z	Winter		
1	5C	T	G	C	5	5C	C	A	G	+Z	5C+Z	Winter	G	0
7	5C	T	G	C	5	5C	C	A	G	-Z	5C-Z	weak spring	G	0
3	S	T	G	C	5	5A	C	A	G	+Z	5A+Z	Spring	T	0
6	S	T	G	C	5	5A	C	A	G	+Z	5A+Z	Spring	T	0

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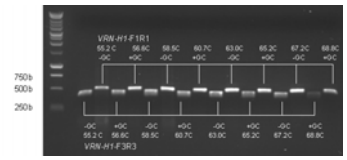
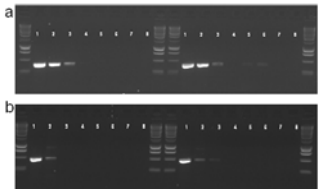
Detection of off-types

(1)Optimisation of primers



(2) Determine primer sensitivity



DNA concentations of 0.15ng/µl detected
Minimum sensitivity equates to 1 off-type in 400

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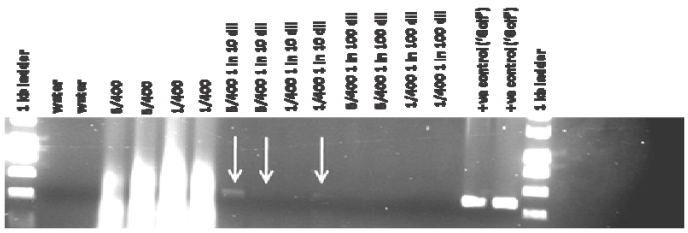
Detection of *VRN-H1* spring off-types by PCR

➢**Detection of 5 in 400 and 1 in 400 off-type seed possible (but need to dilute the DNA extraction 1/10). Detection of off-types even after dilution means that the approach should be robust.**

➢**Need to optimise DNA extraction from 400 seed, as high carry over of PCR inhibitors (DNA extraction very viscous).**



Conclusions

- A single-well multiplex PCR assay diagnostic of all known spring and winter alleles at the major flowering-time locus *VRN-H1* has been developed.
- Genotyping of the flowering-time genes *VRN-H1, VRN-H2, PPD-H1* and *PPD-H2* in a panel of 396 UK barley varieties has been completed.
- *VRN-H1, VRN-H2, PPD-H1* and *PPD-H2* have been genotyped in the eight "alternative" varieties identified.
- Although no set of polymorphisms perfectly diagnostic for "alternative" seasonal growth habit has been identified, six of the eight "alternative" varieties can be predicted to lack a full vernalization requirement, and would suggest that these be targeted for field testing in a spring vernalization trial.
- Detection of off-types to the standards in the UPOV Test Guidelines has been demonstrated using DNA extracted from ground seed.

Thanks to

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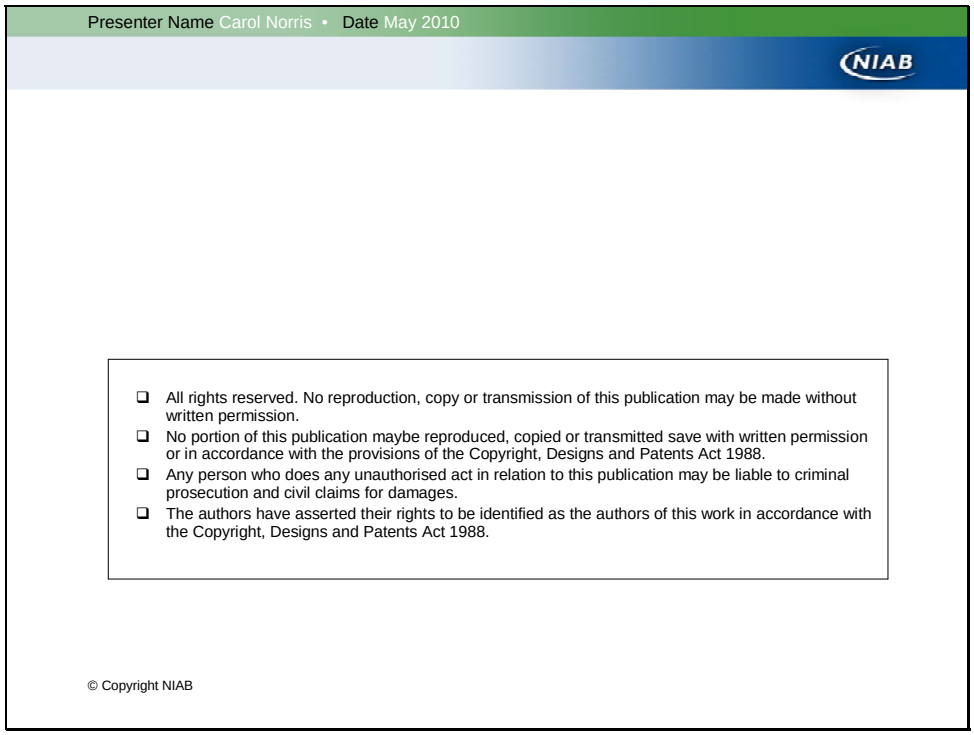
James Cockram
Donal O'Sullivan
Eunhee Soh who carried out much of the sequencing



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