Using Maker-Assisted Selection to Improve Hard Winter Wheat FHB Resistance

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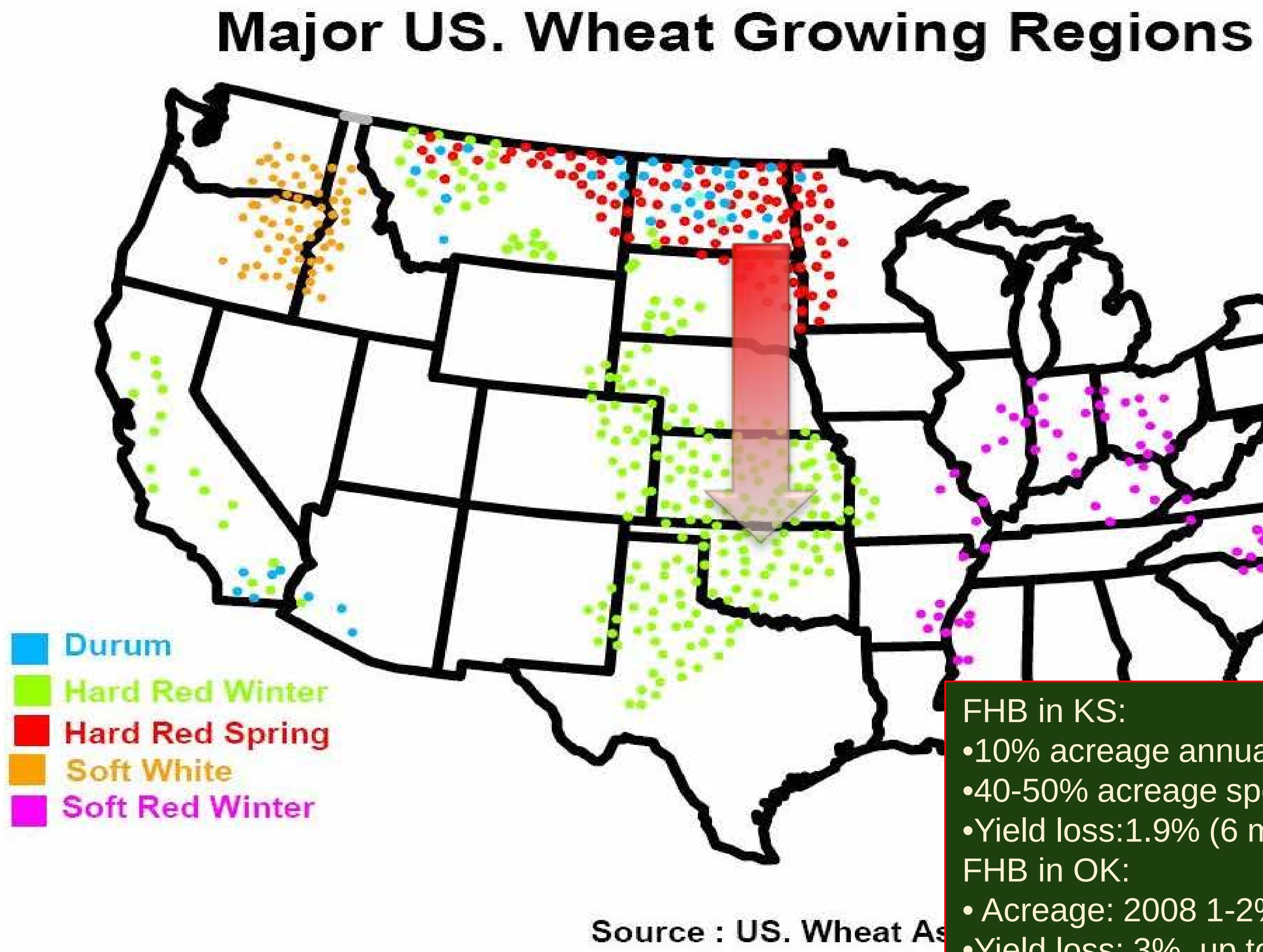
FHB moves to southern Great Plains

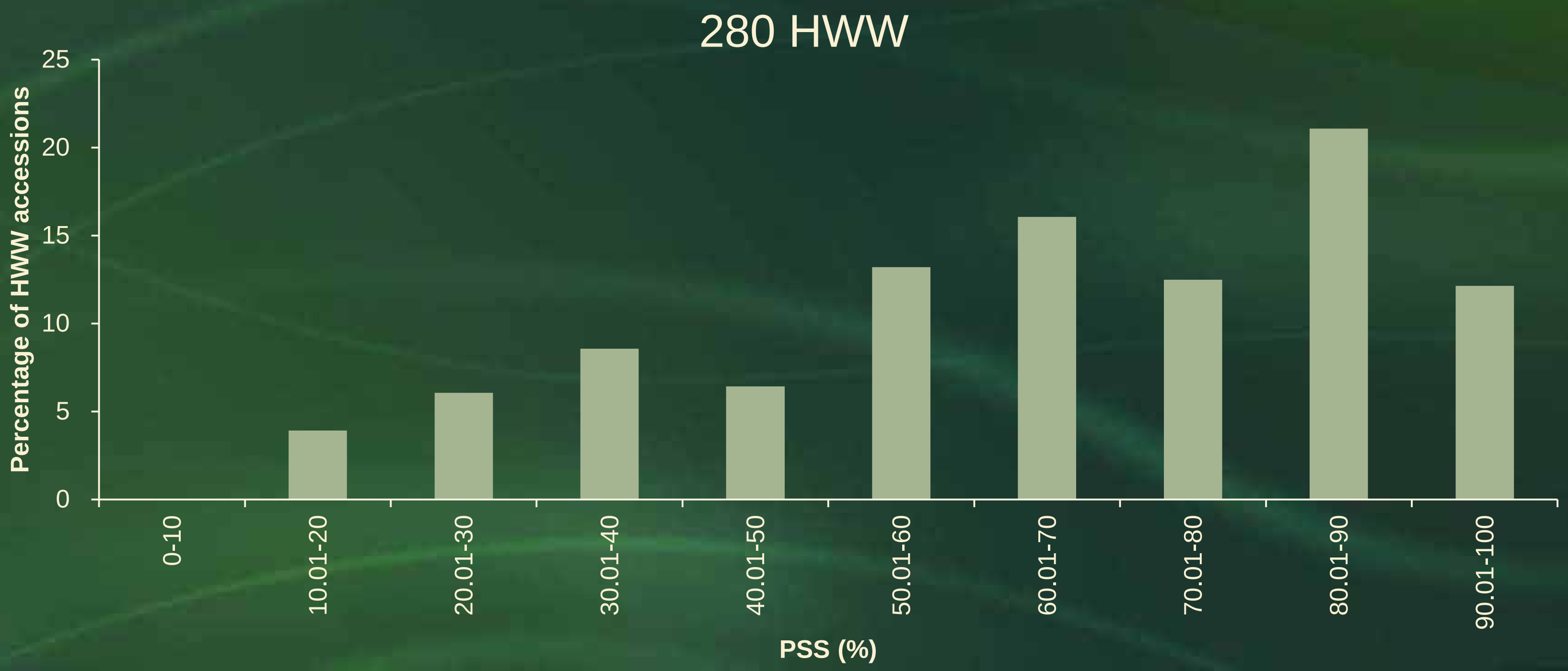


Source : US. Wheat As

FHB in KS:
- 10% acreage annually, eastern quarter
- 40-50% acreage sporadically, central KS
- Yield loss:1.9% (6 mi bushels) in 2008

FHB in OK:
- Acreage: 2008 1-2%; 2009 >20%
- Yield loss: 3%, up to 25% in eastern districts

HWW FHB in greenhouse experiments



Status of FHB resistance in US HWW

- HWW cultivars are mainly MS to S
- *Fhb1* has be used in breeding for more than 10 years, to date *Fhb1* presents only in a few elite breeding lines, not in any cultivar
- Several local HWW were identified with FHB resistance, but QTL have not been determined

FHB related research in my lab

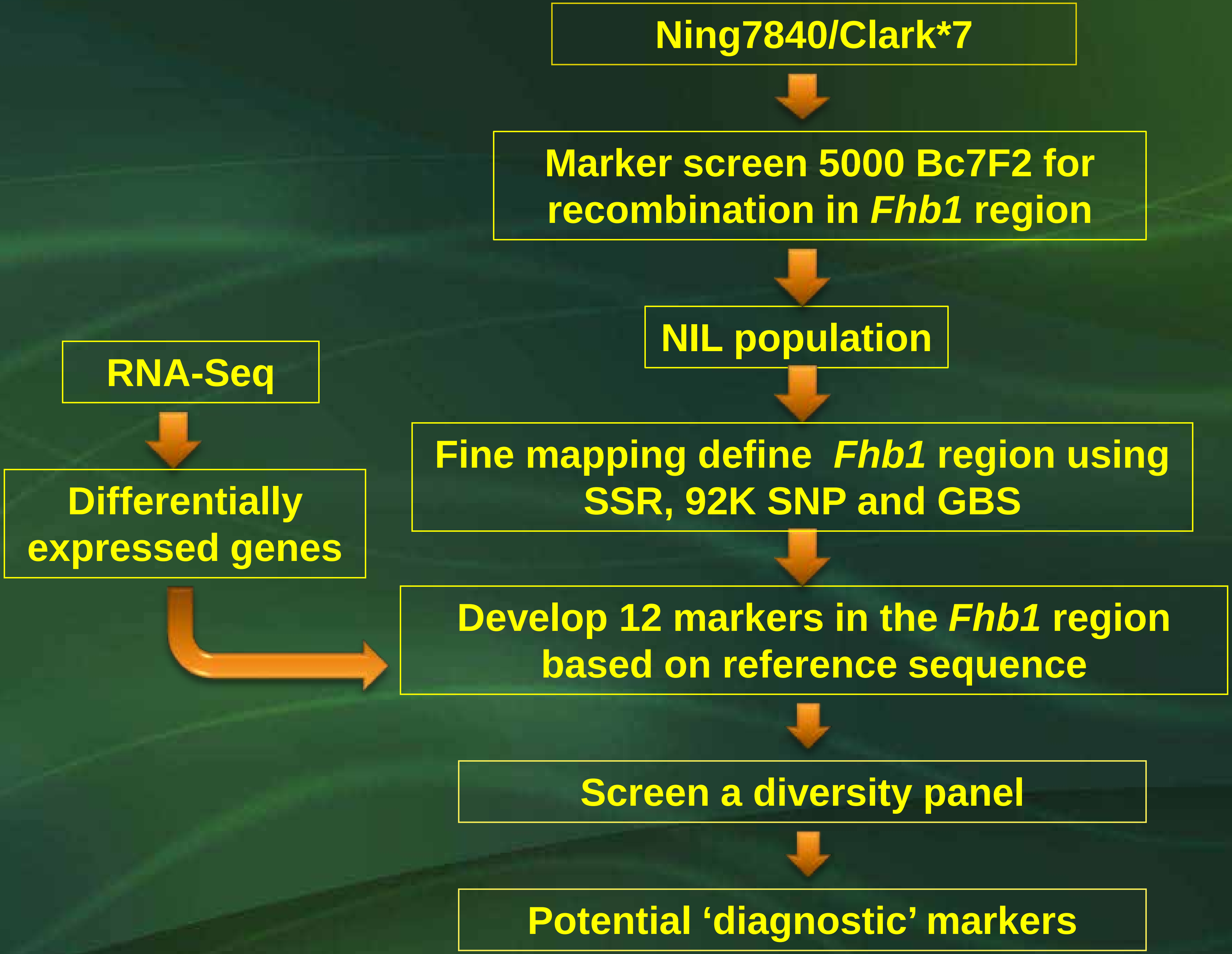
- Identify ‘diagnostic’ markers for *Fhb1*

- Develop markers for other QTL from Chinese and US resistant sources

- Develop high-throughput markers using next-generation-sequencing (GBS, GBMAS, *Genotyping by multiple amplicon sequencing*)

- Transfer *Fhb1* into US HWW backgrounds using marker-assisted backcross

Develop ‘diagnostic’ markers for *Fhb1* through fine mapping



Ning7840/Clark*7

Marker screen 5000 Bc7F2 for recombination in *Fhb1* region

NIL population

Fine mapping define *Fhb1* region using SSR, 92K SNP and GBS

RNA-Seq

Differentially expressed genes

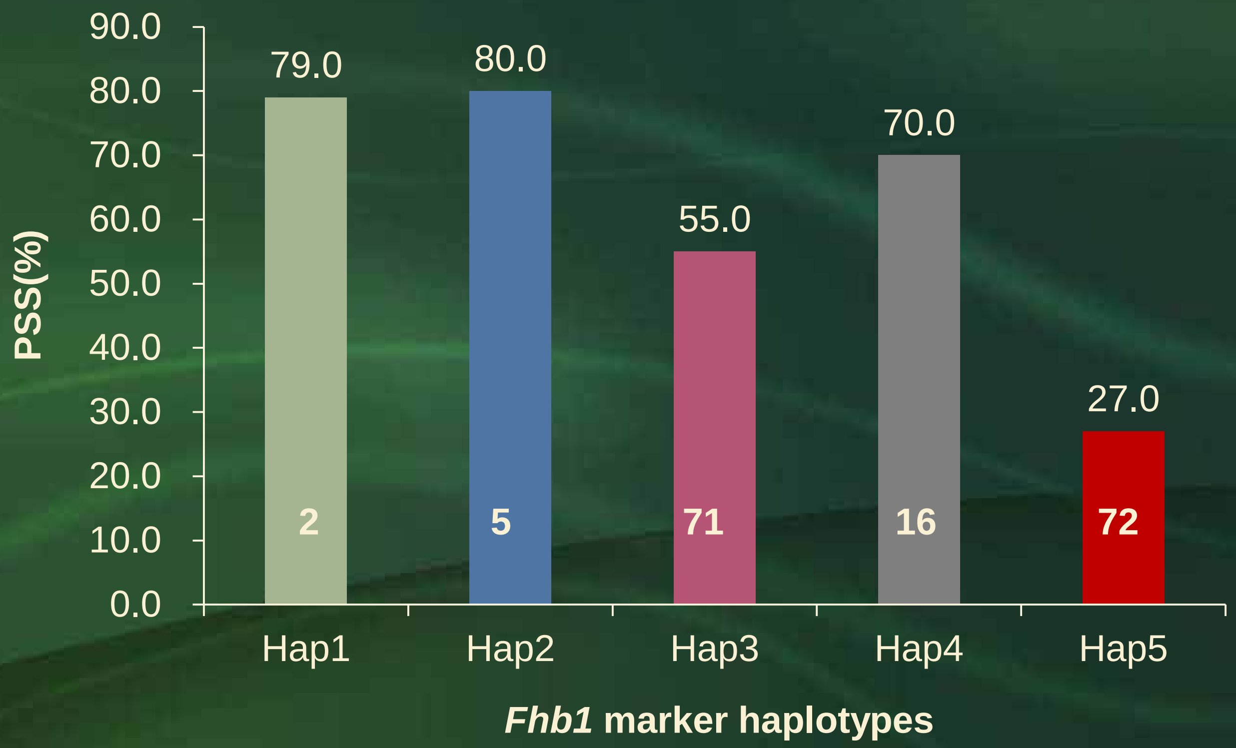
Develop 12 markers in the *Fhb1* region based on reference sequence

Screen a diversity panel

Potential ‘diagnostic’ markers

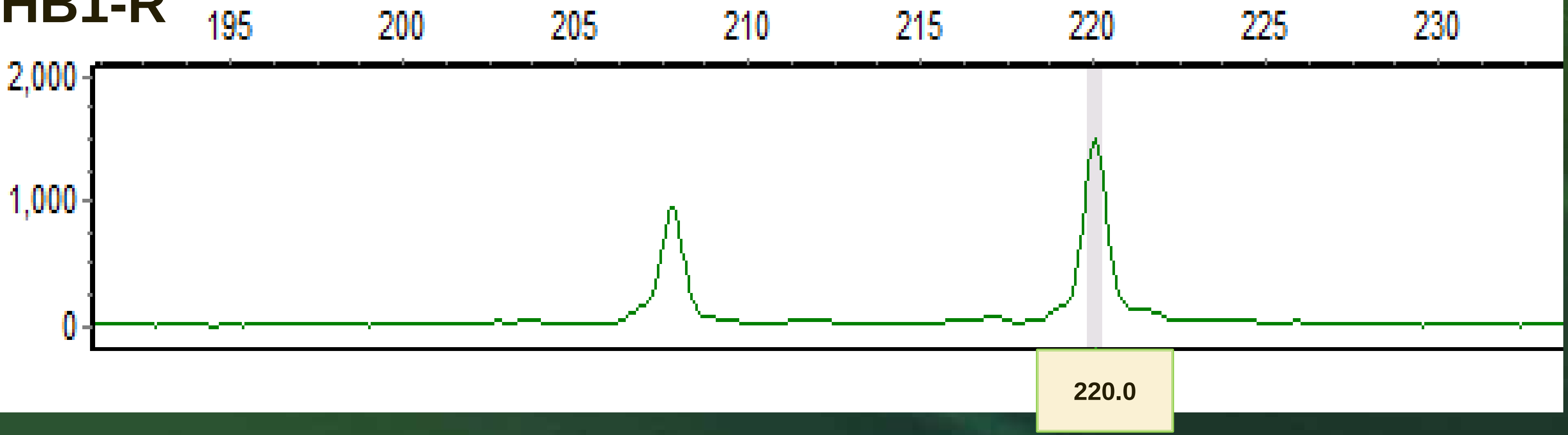
Fhb1 region marker haplotypes assayed in an diversity panel (166 acc.)

Haplotype	cfb6067	SNP319	umn10	cfb6059	FHB1-STS1	FHB1-STS2	FHB1-STS4	cfb6057	cfb6056	cfb6055	STS256	cfb6078	Represent Var.
Hap1	293	G	247	240	other	237	332	273	317	225	243	208	Chinese Spring
Hap2	293	G	255	238	other	237	332	273	317	224	244	null	Dahuangpi
Hap3	290	G	255	244	other	237	332	279	317	225	243	208	Clark
Hap4	293	A	258	null	other	246	302	null	null	224	250	205	ND2419
Hap5	null	A	258	null	220	246	302	null	null	225	250	208	Sumai 3

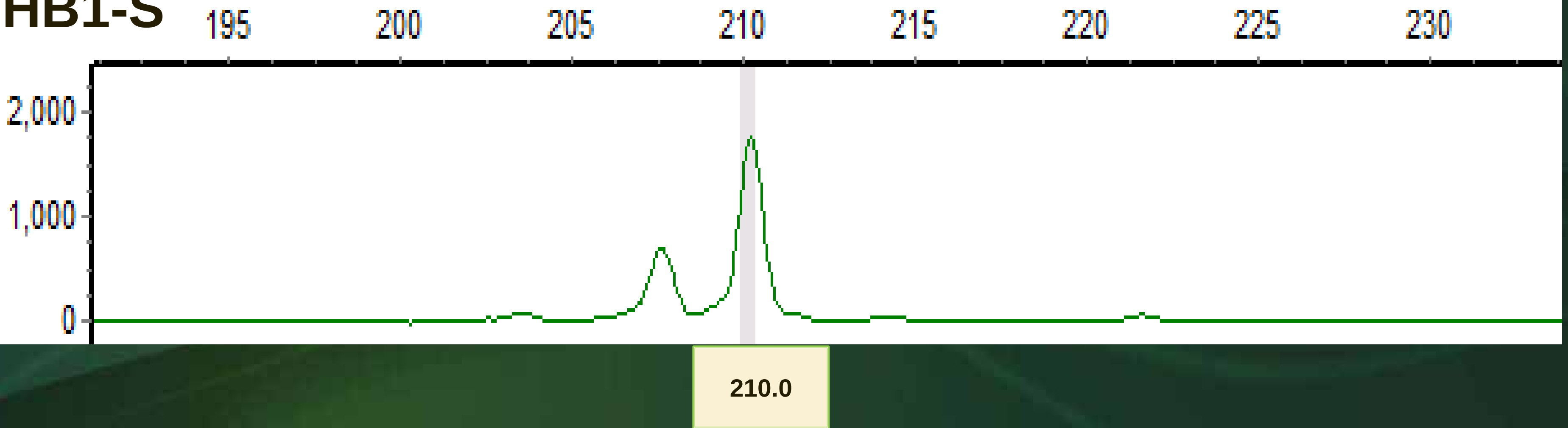


FHB1-STS1 for *Fhb1*

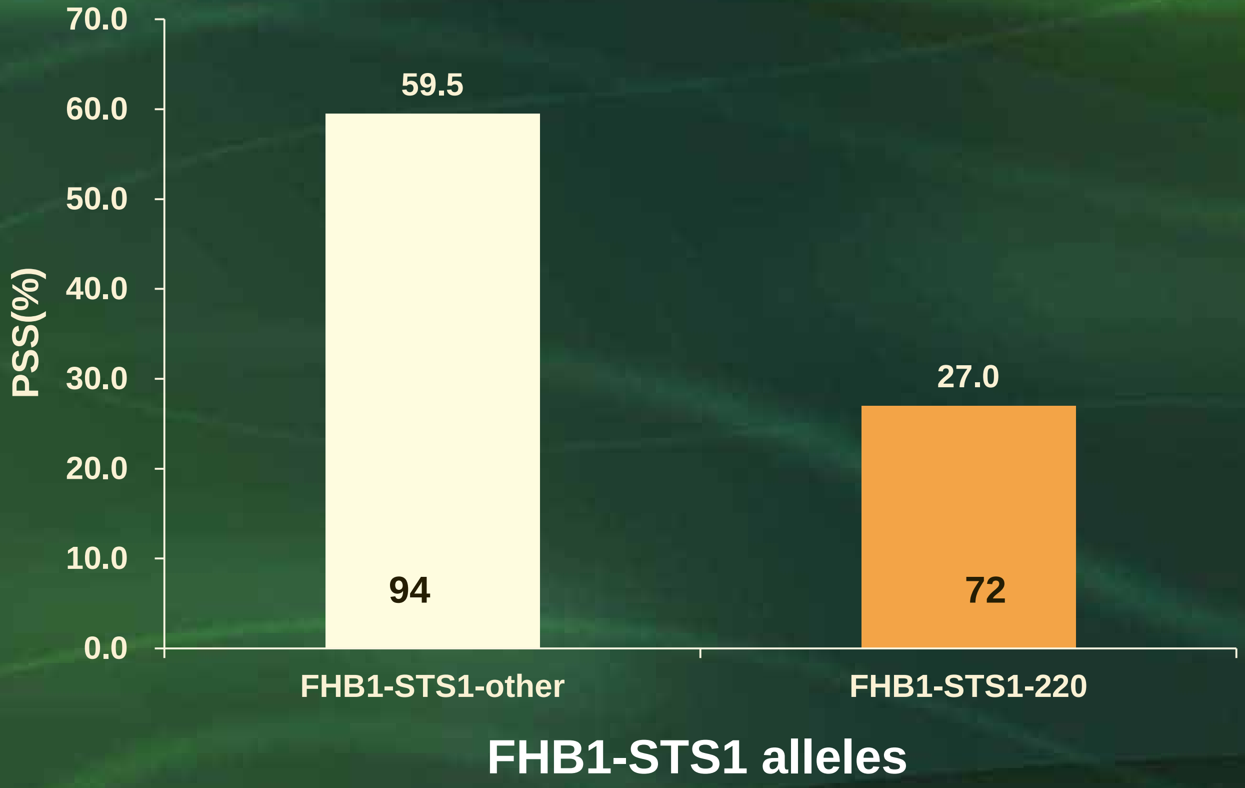
FHB1-R



FHB1-S



Mean PSS for two groups in the diversity panel contrasting in FHB1-STS1 alleles



Meta-analysis of FHB resistance QTL in Chinese wheat landraces

FHB resistant Chinese landraces

Cultivar	PSS (%, greenhouse)	PSS (%, field)
Ning7840	9	11
Wangshuibai	10	11
Huangcandou (HCD)	14	10
Haiyanzhong(HYZ)	9	15
Huangfangzhu(HFZ)	13	12
Baisanyuehuang(BSYH)	7	11

5 Chinese landraces/Wheaton or Jagger

F_{6-8} RILs

FHB phenotyping

GBS/SSR

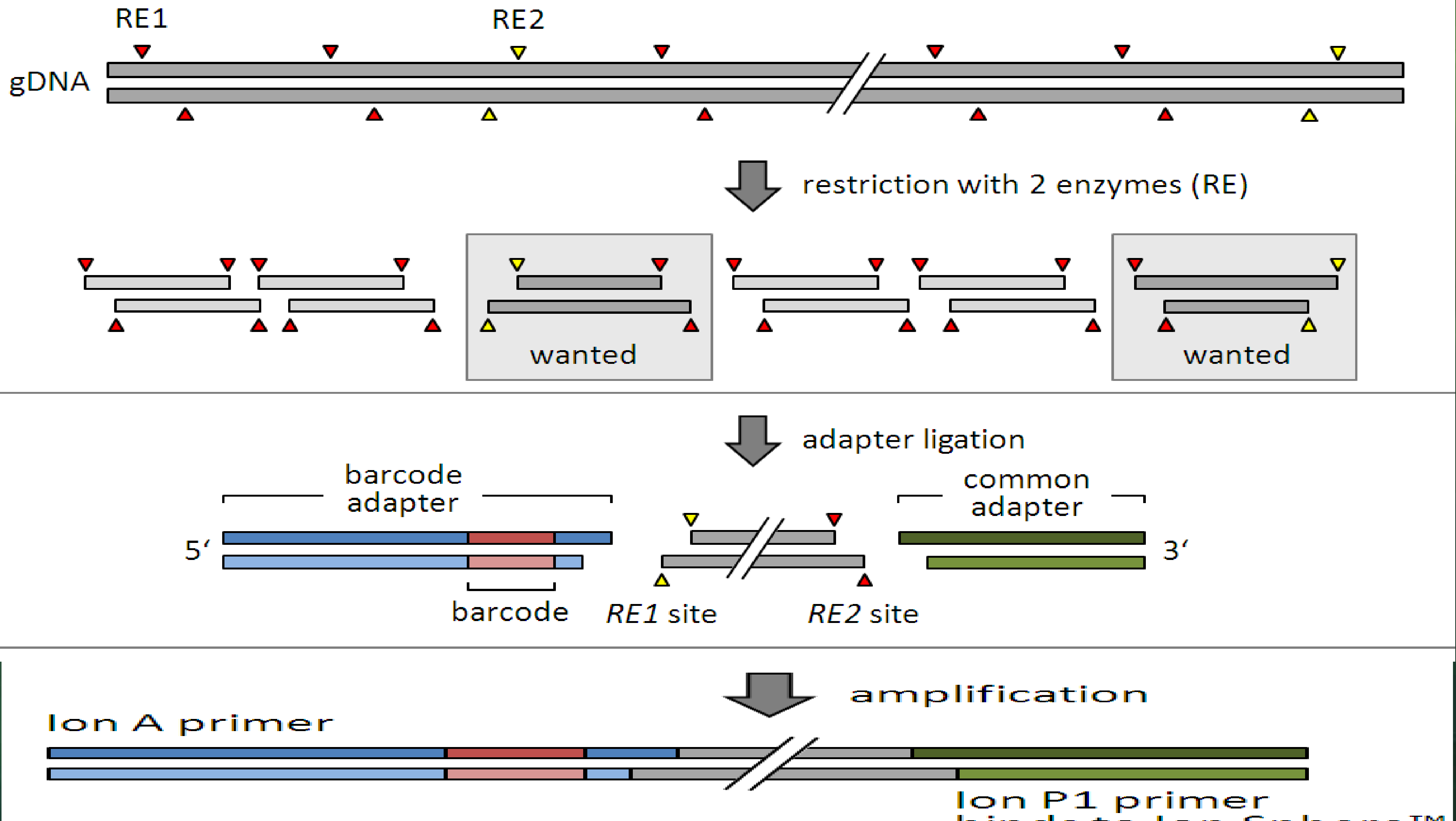
QTL mapping for each pop

Consensus map

Meta QTL analysis

Convert GBS to KASP-SNP and GBMAS for MAS

Genotyping-by-Sequencing



RE1

RE2

gDNA

restriction with 2 enzymes (RE)

wanted

wanted

adapter ligation

barcode adapter

common adapter

5'

3'

barcode

RE1 site

RE2 site

amplification

Ion A primer

Ion P1 primer binds to Ion Sphere™

emulsion PCR (pooled)

PGM™ Sequencing

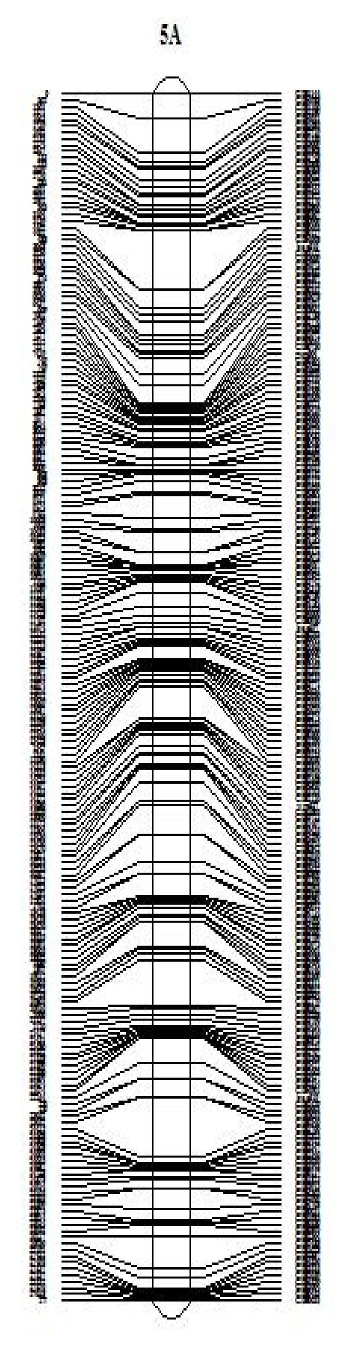
alignment of reads, SNP calling

GBS-SNP map for HYZ/Wheat

6232 SNPs were called with 20% missing data

~5000 SNPs were mapped to 36 linkage groups

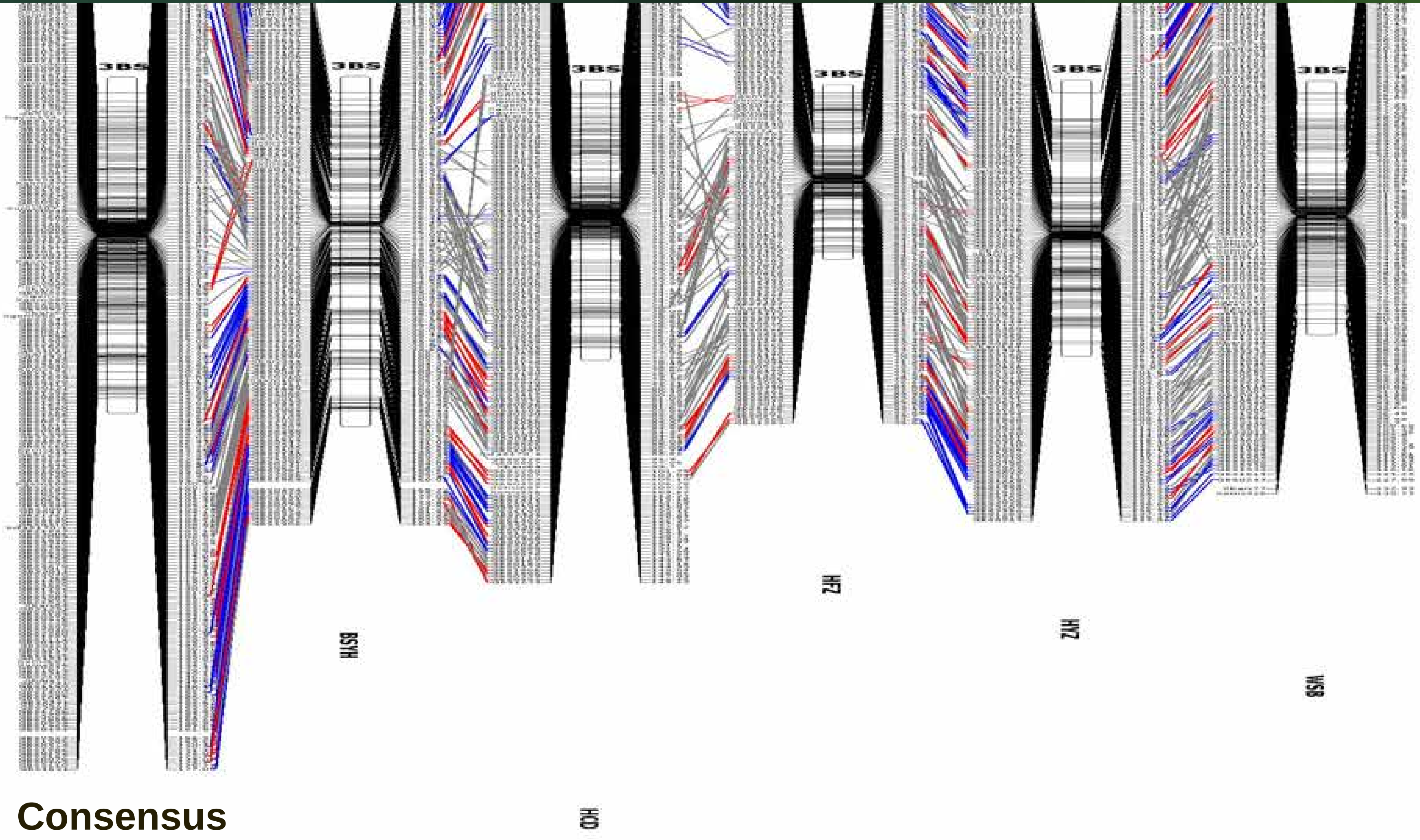
Average marker density was 0.755cM per marker



QTL in HYZ using a GBS map

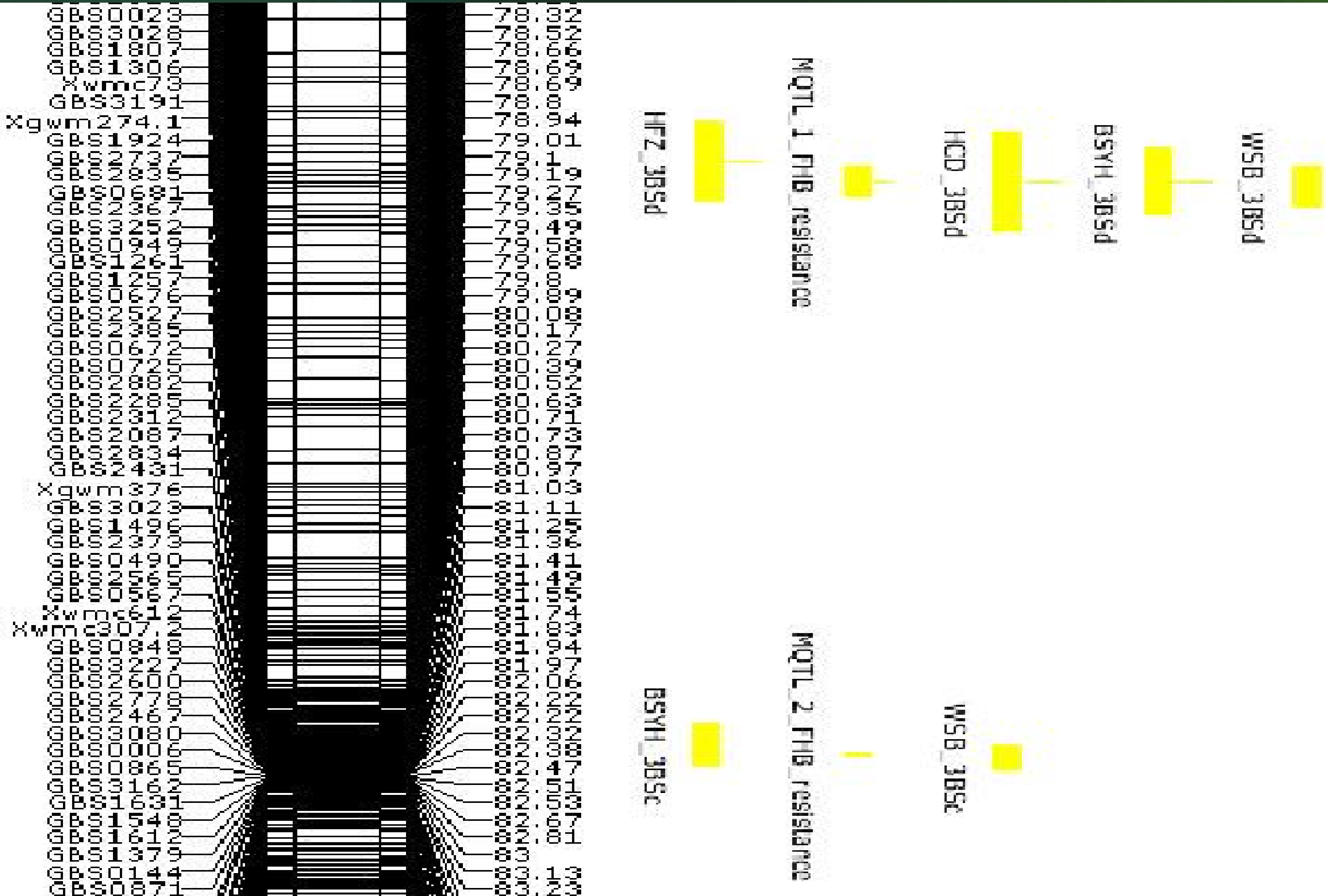
Locus	Flanking markers	Combined mean	
		LOD	R^2 %
5A	GBS3127~Xbarc316	6.0	9.4
2B	GBS1711~GBS5561	5.0	7.6
6B	GBS0576~GBS0208	5.3	8.5
7D	GBS0983~Xwmc121	2.9	4.8
2D	GBS5276~Xcfd51	3.9	5.8
4D	Xwmc52~GBS4813	4.1	6.2
6D	GBS0984~GBS5458	3.4	5.0

Consensus map for chromosome 3BS

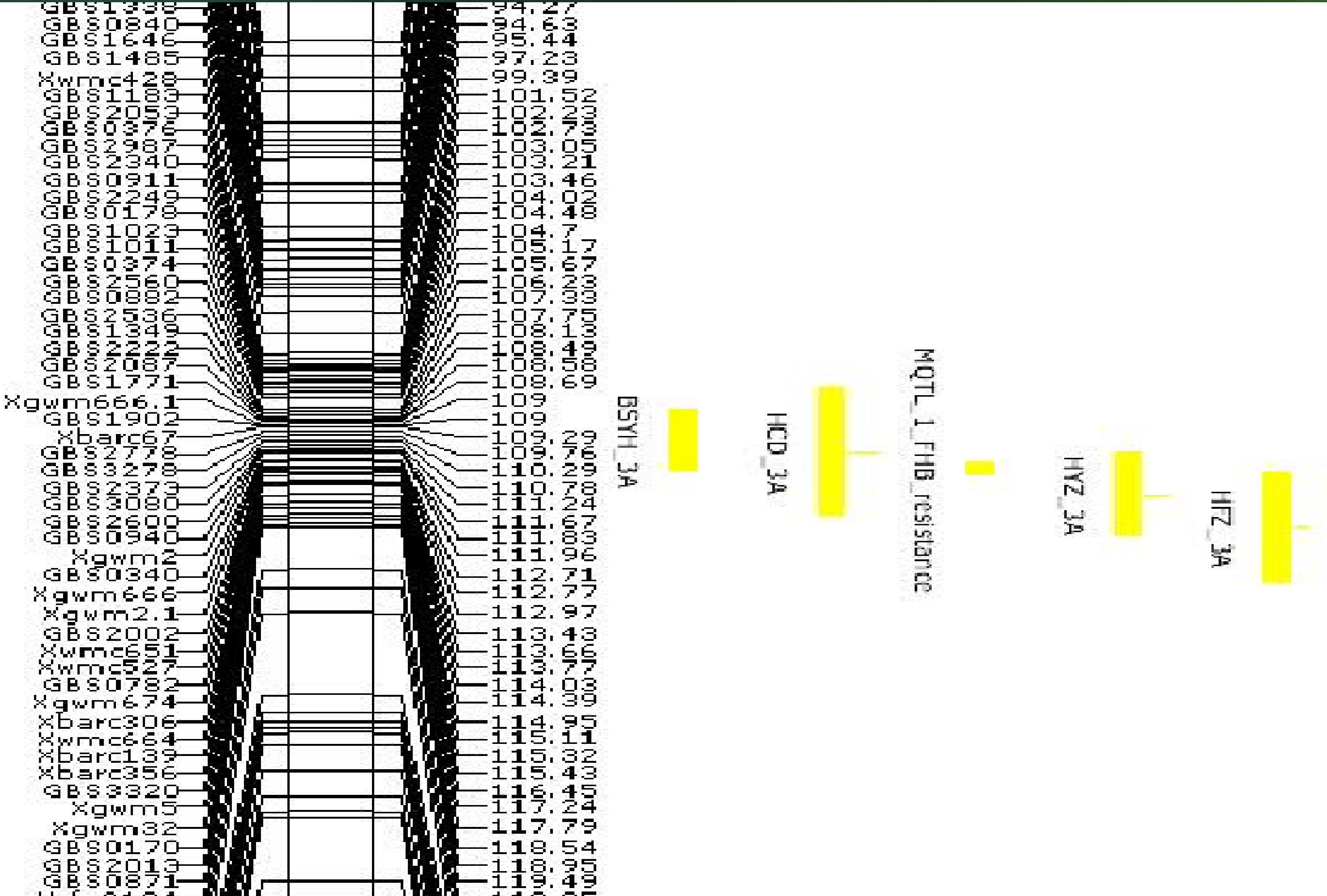


Consensus map

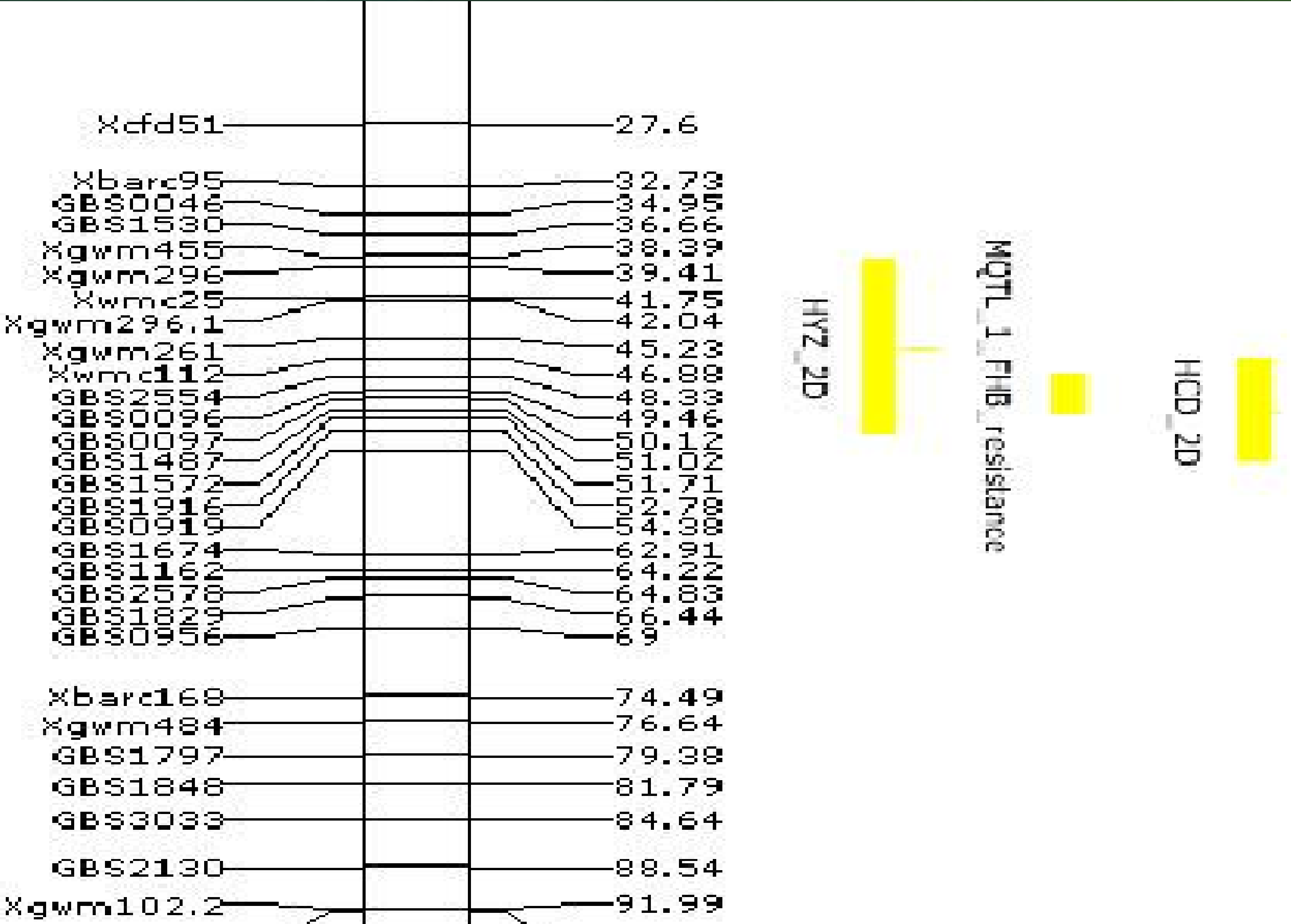
Meta-QTLs on 3BS



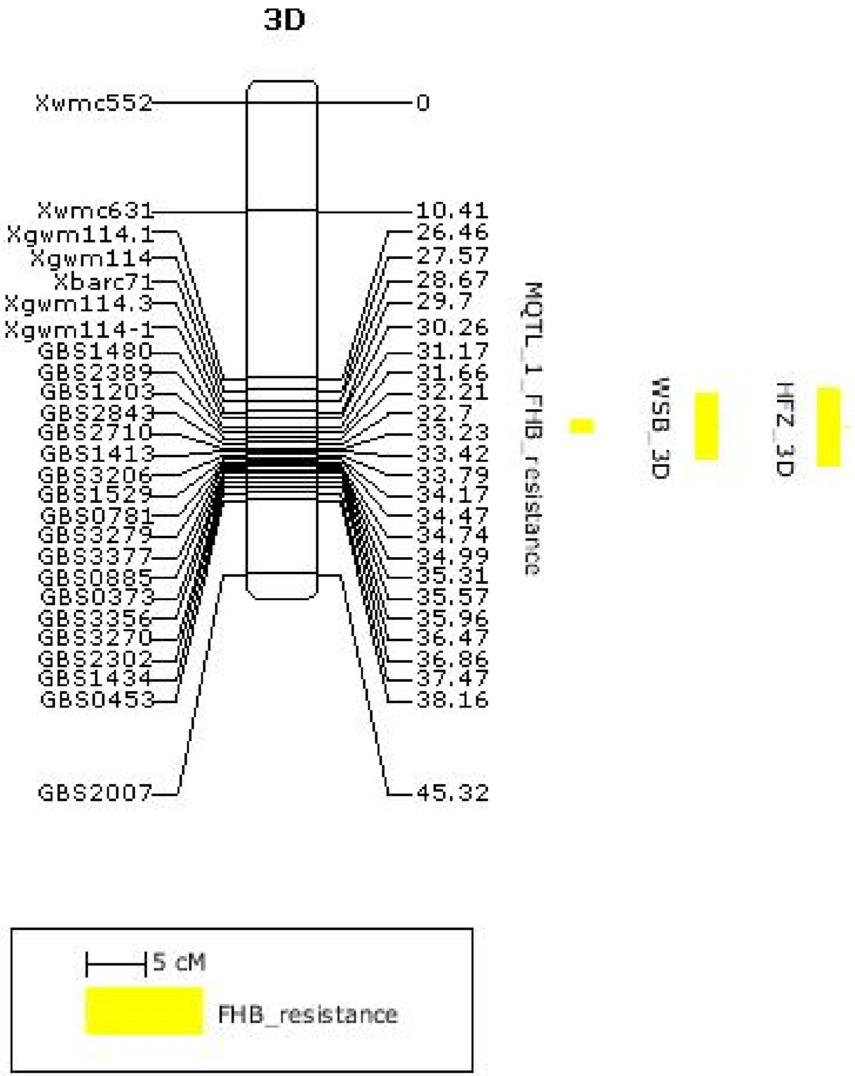
Meta-QTL on 3AS

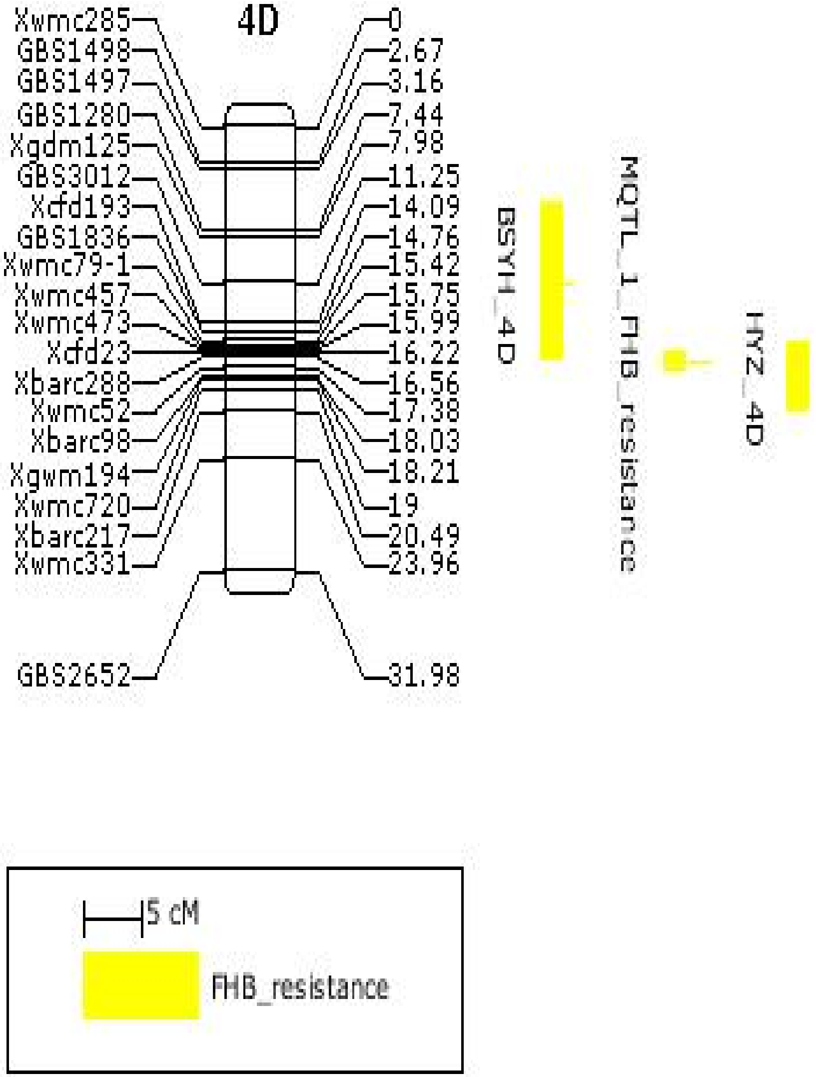


Meta-QTLs on 2D



Meta-QTLs on 3D and 4D

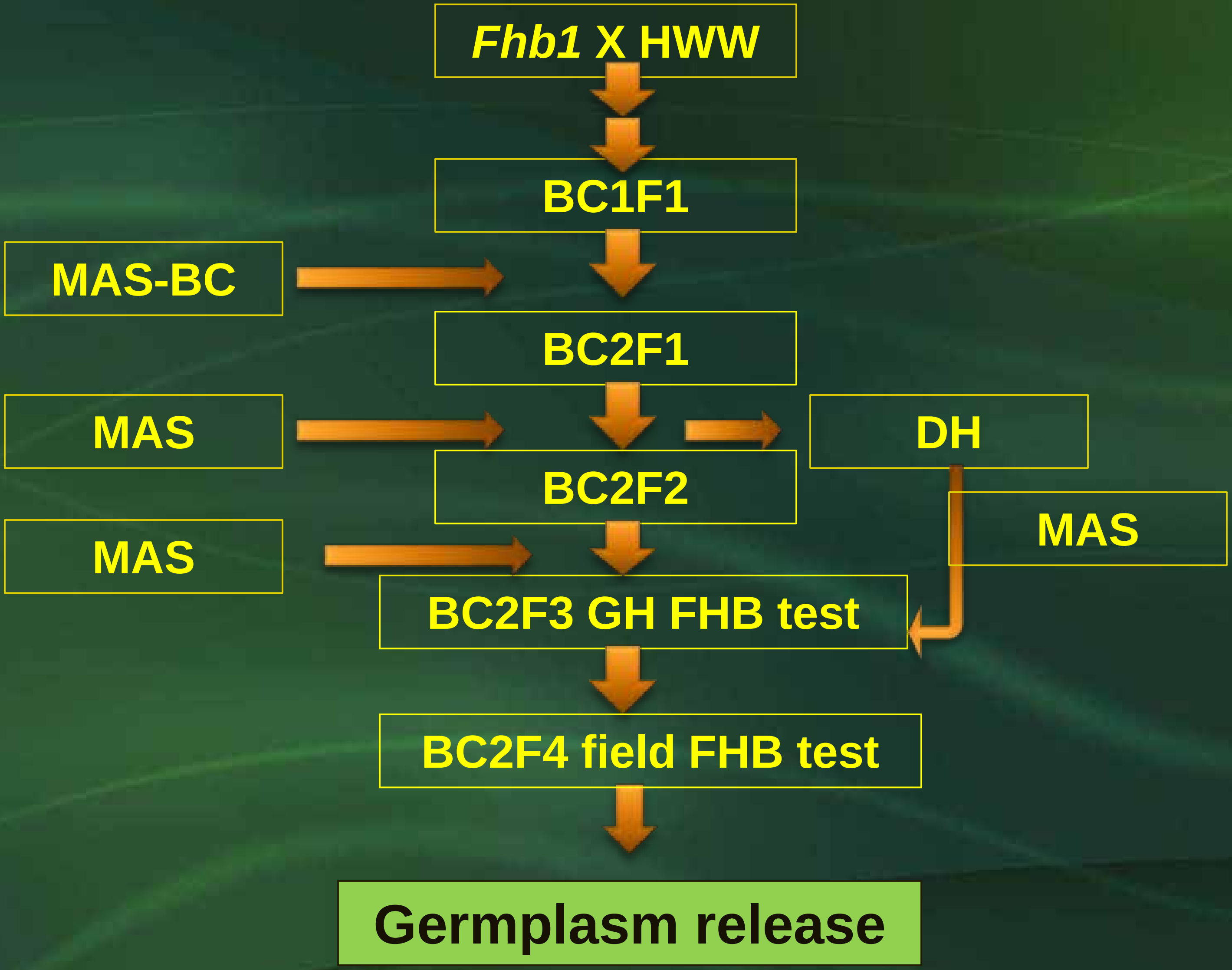




Summary

- Many important QTL reported to date can be identified in Chinese landraces
- *Fhb1* is the QTL with the largest effect in most Chinese resistance landraces, but not in HYZ
- HYZ lacks *Fhb1* and its resistance is conditioned by seven minor QTL, suggesting accumulation of multiple minor QTL also can achieve a high level of resistance
- QTL on 3AS, 3BS(2), 3D, 2D and 4D detected in multiple populations are more stable QTL for breeding
- GBS-SNP were found for most of these QTL and conversion of them to KASP and high-throughput GBMAS markers is in progress

Marker-assisted backcross to transfer *Fhb1* into HWW



Fhb1 X HWW

BC1F1

MAS-BC

BC2F1

MAS

DH

BC2F2

MAS

MAS

BC2F3 GH FHB test

BC2F4 field FHB test

Germplasm release

Transfer *Fhb1* to Wesley, Trego and Harding

- *Fhb1* donors: Sumai 3 and its soft wheat derivatives
- Recurrent parents: Wesley, Trego, Harding
- ~ 100 Bc2F4-5 lines per cross tested in Tri-state breeding nurseries

WesleyFhb1 lines and Wesley in field (2012)

WesleyFhb1 lines and Wesley in field (2013)

WesleyFhb1 and Wesley in greenhouse

PSS for *Fhb1* in three HWW

Line	GH PSS	Mean
262(ND2928/Wesley*2)F3/WesleyF4	0.079	
267(ND2928/Wesley*2)F3/WesleyF4	0.090	
568(ND2928/Wesley*2)F3/WesleyF4	0.192	0.12
Wesley	**0.584**	**79.5%**
277(ND2710/Trego*2F3//TregoF4	0.324	
219(ND2710/Trego*2F3//TregoF4	0.253	
27(ND2710/Trego*2F3//TregoF4	0.203	0.26
Trego	**0.722**	**63.7%**
167(Harding*2/Sumai3)F3Harding/F4	0.534	
61(Harding*2/Sumai3)F3Harding/F4	0.580	
31(Harding*2/Sumai3)F3Harding/F4	0.350	0.49
Harding	**0.507**	**0.02**

Transfer *Fhb1* from Ning7840 to Overland, Overley and Jagger

FHB severity and DON content between parents and their Fhb1 lines in Overland, Overley and Jagger backgrounds

	GH PSS (%) (3 seasons)	Field PSS (%) (2 years)	FDK (%) (2 years)	DON (ppm) (2014)
Overland-*Fhb*1 lines (49)	21.26	33.08	20.61	14.44
Overland	42.00	46.00	41.00	32.20
FHB reduction due to *Fhb*1 (%)	49.37	28.09	49.74	55.16
Overley-*Fhb*1 lines (20)	46.31	28.94	17.20	10.94
Overley	95.00	77.00	68.00	27.60
FHB reduction due to *Fhb*1 (%)	51.25	62.42	74.70	60.36
Jagger-*Fhb*1 lines (59)	40.39	19.44	11.93	10.48
Jagger	83.00	58.00	31.00	15.10
FHB reduction due to *Fhb*1 (%)	51.33	66.49	61.50	30.57


Overland
OverlandFhb1

Transfer *Fhb1* from WesleyFhb1 to 11 HWW from 5 States

Ø11 locally adapted recurrent parents:
ND: Decade, Jerry,
NE: NE01481, NE6607,
KS: Everest, KS061406LN-47,
SD: SD08080, Lyman,
OK: Garrison, Ruby Lee, OK06617-Rht

ØMarkers: Xumn10 and Xsnp8

Progress

- Completed projects:
    - *Fhb1* carrying NILs are available for Wesley, Harding, Trego, Overland, Overley and Jagger

- 5 state MAB project in progress:
    - DH: 1st set sent to AZ for increase and 2nd set DH production in progressing
    - 11 Bc_2F_4 pop. were planted for field FHB evaluation this fall and greenhouse FHB evaluation is in progress

Summary

- *Fhb1* significantly improves resistance in most HWW backgrounds but FHB resistance levels varied with resistance levels of recurrent parents, thus avoiding use of highly susceptible cultivars as recurrent parents have a higher chance to select highly resistant lines
- Although many backcross progenies show slightly lower yield than recurrent parents, some *Fhb1* lines have similar yield as recurrent parents
- Marker selected *Fhb1* lines still show different levels of resistance in each population, phenotypic selection will be useful to improve the level of resistance in selected lines.
- Wesley*Fhb1* can be useful *Fhb1* donor for HWW

Summary

- *GBS is a cost effective system for QTL mapping and SNP discovery*
- *Single SNP marker may not be diagnostic for a QTL when a large set of germplasm are screened. Thus several SNP (haplotype) may be needed to tag a QTL*
- *GBMAS using Ion Proton can be a good system for high throughput screening of multiplex SNP for MAS in breeding*

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Thank You for Your Attention!