International Field Trial Day Agrologica 18/7 - 2022

Introduction: Dennis Kjær Christensen

- Education: Engineer in electronics
- Profession: SW developer / Tester
- Hobby: Micro wheat breeder / Common bunt resistance gene mapping

Setup

- Anders Borgen: Field trials
- Dennis: Marker analysis
- Mapping Population: Anders Borgen, F&Z Dottenfelderhof, DE, Cultivari, DE, Genebank Material. Currently 360 lines
- Validation Population: BOKU Blizzard/ Bonneville and Bt12 mapping populations, NordGen. 635 lines total
- Nordic Seed: DNA extraction
- TraitGenetics: Genotyping
- Funding: Liveseed, Boost, Boost (FØL)

Methods and Tools

- GWAS: GAPIT, MLM (R)
- Linkage analysis, detailed recombination analysis, markers versus lines: Homemade program (Delphi)
- Excel: Marker positions: T3Wheat RefSeq 1.0 and 2.1 , Jbrowse

- (https://wheat.triticeaetoolbox.org/search/variants/results?marker_name), JBrowse (https://urgi.versailles.inra.fr/jbrowseiwgsc/gmod_jbrowse)

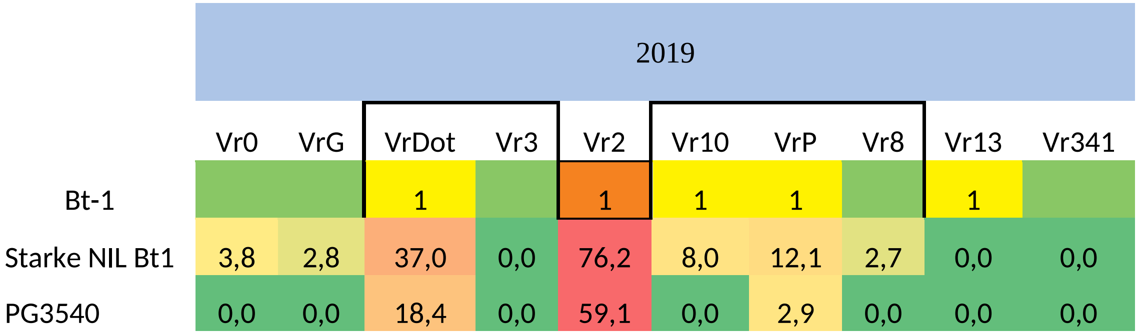
Genepostulation

- Genepostulation is based on phenotyping + pedigree information

Gene Postulation	2019/2021									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-1			1		1	1	1		1	
Bt-2		2	2	2	2	2				2
Bt-3				3						
Bt-4										
Bt-5		5								
Bt-6										6
Bt-7		7			7	7	7	7	7	
Bt-8										
Bt-9										
Bt-10						10	10	10		
Bt-11										
Bt-12										12
Bt-13									13	
Bt-Z						Z	Z			Z

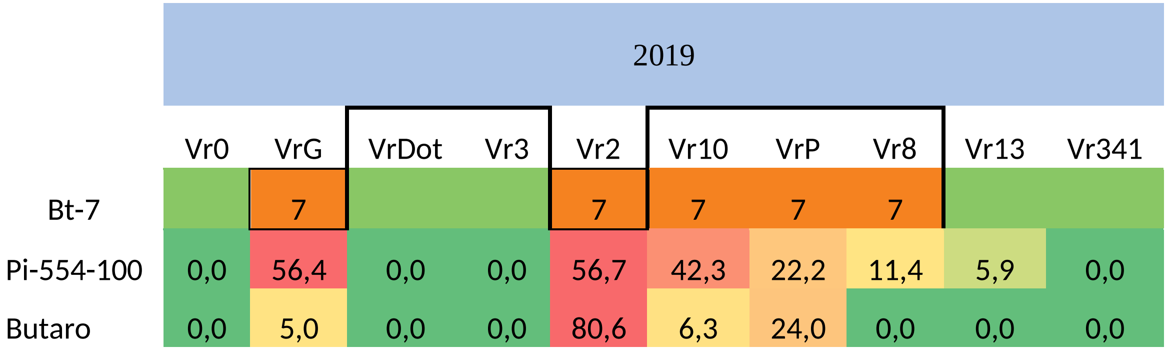
Starke NIL Bt1	Bt1		3,8	2,8	37,0	0,0	76,2	8,0	12,1	2,7	0,0	0,0
PI 554103	Bt2		0,0	47,4	71,4	53,6	37,8	20,0	3,8	2,9	0,0	3,3
Ridit	Bt3		0,0	0,0	0,0	60,6	0,0	0,0	5,9	0,0	0,0	0,0
PI-554115	Bt4	M81-152	17,6	13,0	0,0	12,0	3,2	4,3	0,0	6,3	0,0	-
Hohenheimer	Bt5		0,0	48,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Rio	Bt6		0,0	0,0	0,0	-	0,0	0,0	0,0	0,0	0,0	62,5
PI 554100	Bt7	Selection 50077	0,0	56,4	0,0	0,0	56,7	42,3	22,2	11,4	5,9	0,0
PI 554120	Bt8	M72-1250	0,0	0,0	0,0	0,0	0,0	0,0	6,7	0,0	0,0	0,0
PI 554099	Bt9	R63-6968	0,0	0,0	0,0	10,5	0,0	0,0	0,0	0,0	0,0	0,0
Weston	Bt10		0,0	6,9	0,0	0,0	0,0	52,4	39,3	31,0	0,0	0,0
Pi554098	Bt11		0,0	0,0	0,0	0,0	16,7	0,0	-	-	0,0	0,0
Pi-119333	Bt12		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	18,2
Thule III	Bt13		0,0	0,0	3,7	0,0	0,0	0,0	0,0	2,0	41,3	18,4
Graziaro	BtZ		0,0	0,0	0,0	0,0	0,0	38,5	-	-	0,0	36,7

An example



	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-1			1		1	1	1		1	
Starke NIL Bt1	3,8	2,8	37,0	0,0	76,2	8,0	12,1	2,7	0,0	0,0
PG3540	0,0	0,0	18,4	0,0	59,1	0,0	2,9	0,0	0,0	0,0

PG3540 matches the Bt1 infection pattern and probably have Bt1

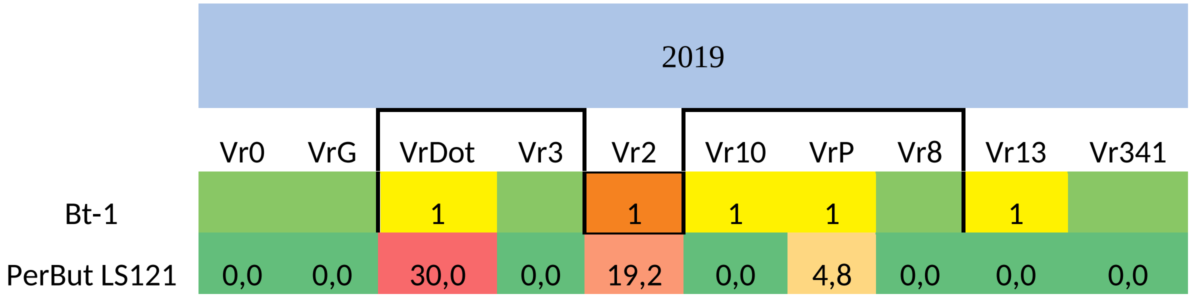


	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-7		7			7	7	7	7		
Pi-554-100	0,0	56,4	0,0	0,0	56,7	42,3	22,2	11,4	5,9	0,0
Butaro	0,0	5,0	0,0	0,0	80,6	6,3	24,0	0,0	0,0	0,0

Butaro matches (not perfectly!) the Bt7 infection pattern and probably have Bt7

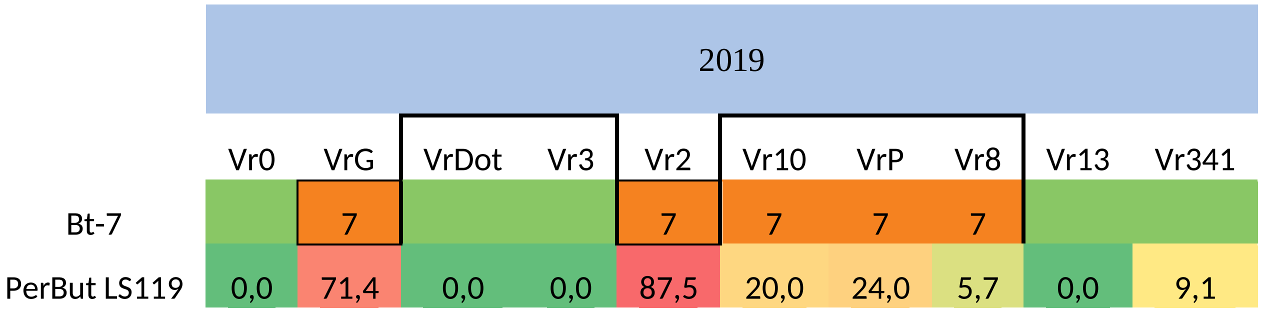
Lines from a cross between PG3540 and Butaro can have Bt1, B7 or Bt1+Bt7

An example



	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-1			1		1	1	1		1	
PerBut LS121	0,0	0,0	30,0	0,0	19,2	0,0	4,8	0,0	0,0	0,0

PerBut LS121 matches the Bt1 pattern and probably has Bt1 (Per = PG3540)

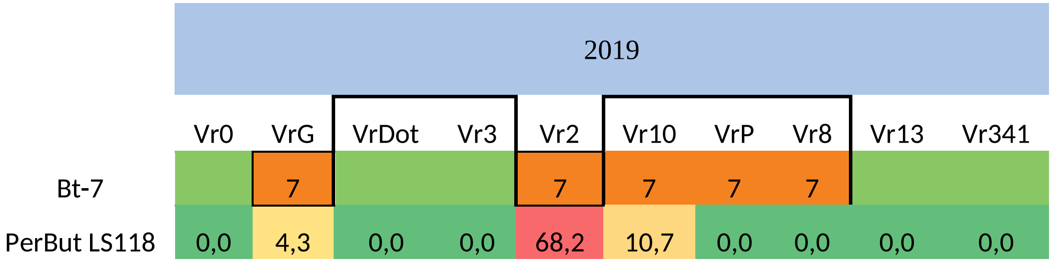


	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-7		7			7	7	7	7		
PerBut LS119	0,0	71,4	0,0	0,0	87,5	20,0	24,0	5,7	0,0	9,1

PerBut LS119 matches the Bt7 pattern and probably has Bt7

Constructing the Bt1 + Bt7 pattern from the Bt1 and Bt7 patterns

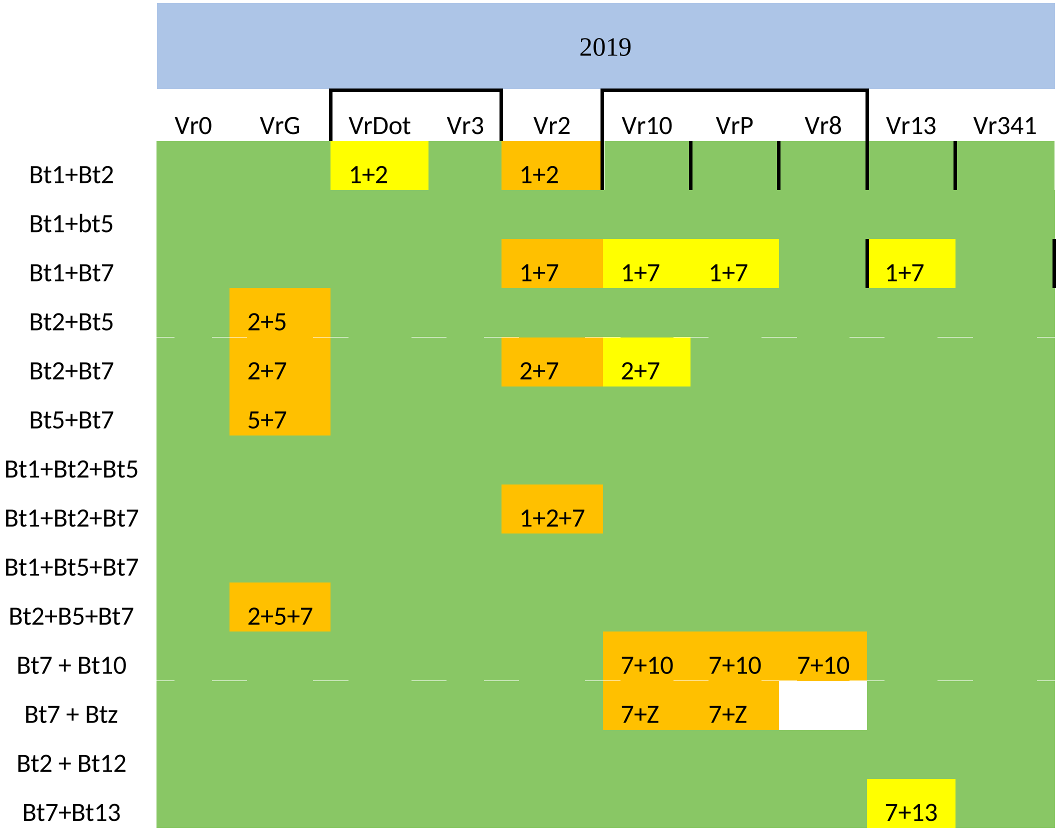
- A line having Bt1 + Bt7 would be resistant to VrG and VrDot, because VrG does not have virulence towards Bt1 and VrDot does not have virulence against Bt
- Vr2 has virulence against Bt1 and Bt7 and a line with both genes will be infected
- Vr10 cannot infect lines with Bt1 and a line with both genes will not be infected
- VrP, Vr8 and Vr13 will give a weak signal



	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt-7		7			7	7	7	7		
PerBut LS118	0,0	4,3	0,0	0,0	68,2	10,7	0,0	0,0	0,0	0,0

PerBut LS118 matches the Bt1 + Bt7 pattern ->
Gene postulate = Bt1+Bt7

A table with infection patterns for gene combinations can be made



	2019									
	Vr0	VrG	VrDot	Vr3	Vr2	Vr10	VrP	Vr8	Vr13	Vr341
Bt1+Bt2			1+2		1+2					
Bt1+bt5										
Bt1+Bt7					1+7	1+7	1+7		1+7	
Bt2+Bt5		2+5								
Bt2+Bt7		2+7			2+7	2+7				
Bt5+Bt7		5+7								
Bt1+Bt2+Bt5										
Bt1+Bt2+Bt7					1+2+7					
Bt1+Bt5+Bt7										
Bt2+B5+Bt7		2+5+7								
Bt7 + Bt10						7+10	7+10	7+10		
Bt7 + Btz						7+Z	7+Z			
Bt2 + Bt12										
Bt7+Bt13									7+13	

Not all gene combinations can be detected.

Exact infection percentages not needed. Just needs to be “good enough”

GWAS

Input for GWAS

- Genotype table with marker names, marker positions and marker SNP values for all lines
- Phenotype table with genepostulates for all lines

Rs	alleles	chrom	pos	LS10	LS100	LS101	LS102
Excalibur_c10657_796	NA	1A	1174240	G	T	T	T
wsnp_Ex_c10657_17376086	NA	1A	1176288	C	Y	T	T

Id	Bt0	Bt1	Bt2	Bt3	Bt4	Bt5	Bt6	Bt7	Bt8	Bt9	Bt10	Bt11	Bt12	Bt13	Bt14	BtQ	BtZ
LS10	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
LS100	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0

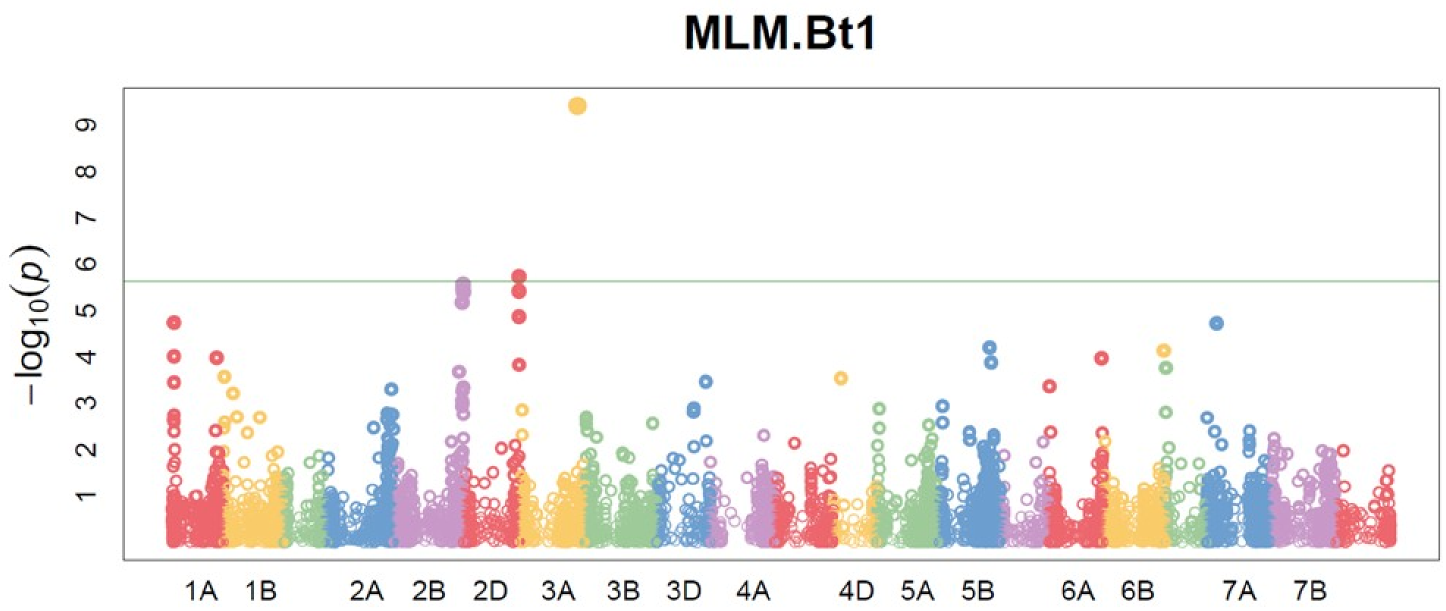
GWAS

Output for GWAS

- Manhattan plot
- Table with markers and their significance
- Q-Q plots
- ……

Bt1

- The Martin gene - Discovered by Briggs and Holton (1950).
- Located using nullisomic and monosomic lines to chromosome 13 = 2B !!



Signals at 2B/2D

Observe the 3A marker Kukri_c18420_705.

Unaccounted for population structure?

Kukri_c18420_705 found to be associated with spike fertility in another study.

Bt1

Significant Markers

SNP	Chromosome (TG)	Position (TG)	FDR_Adjusted_P-values
Kukri_c18420_705	3A	663180801	8,267E-06
Excalibur_c2311_1563	2D	640215489	0,014460379
Excalibur_c25043_357	2B	797328180	0,014460379
Kukri_c900_1334	2B	796807569	0,014460379
GENE-0676_649	2D	640211018	0,014460379
Excalibur_rep_c106698_235	2B	801217330	0,014460379
CAP7_c4827_53	2B	787058697	0,020599375
AX-94644192	2D	640216098	0,03641165
wsnp_Ex_c48087_53105842	1A	24943716	0,040839883
wsnp_Ex_c40247_47349166	7A	116113287	0,040839883

Bt1

Where are the markers really?

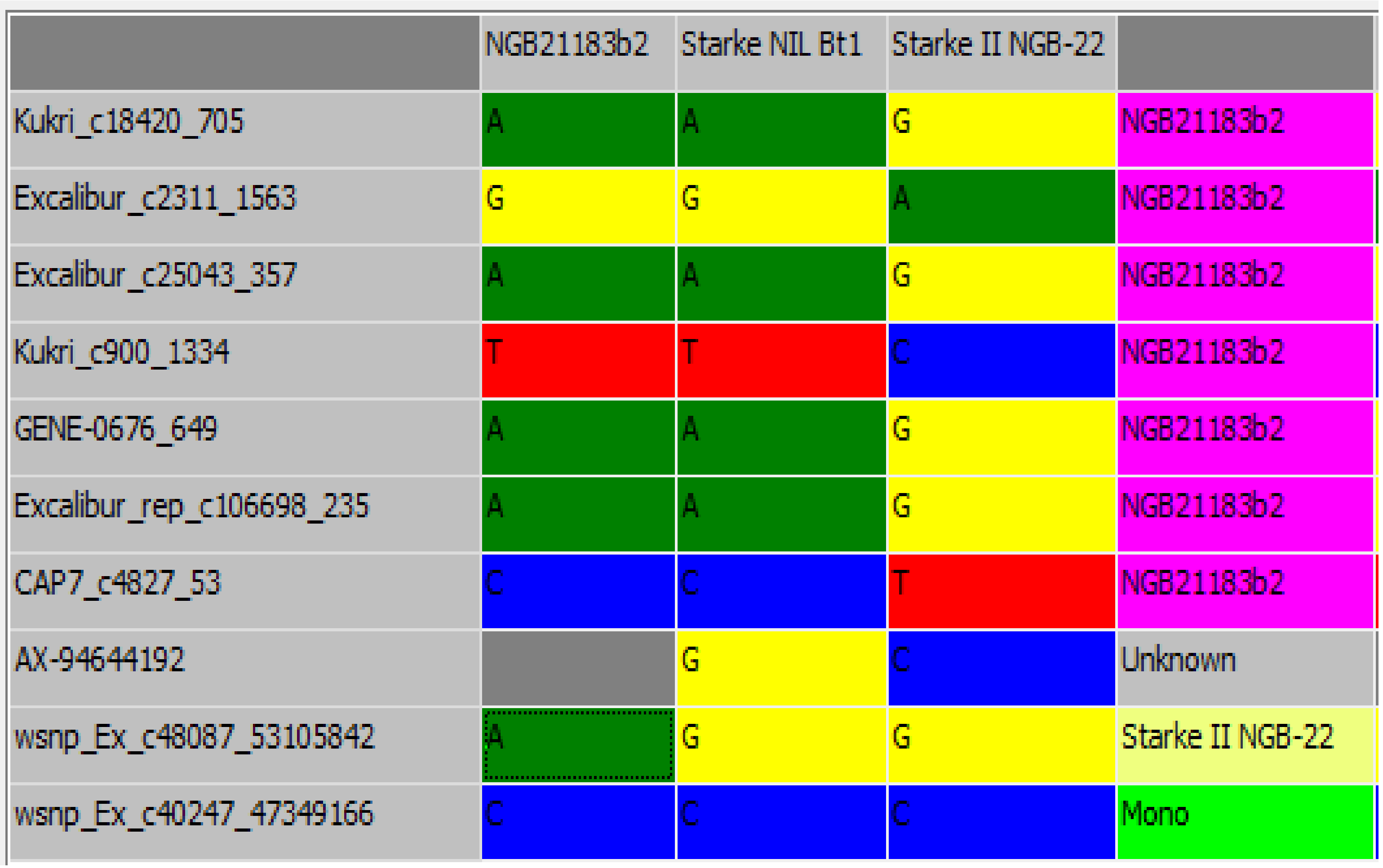
	TG		RefSeq 1.0		RefSeq 2.1		Jbrowse	
wsnp_Ex_c48087_53105842	1A	24943716	1A	24943674	-	-	1A	24943574..24943768
Excalibur_c25043_357	2B	797328180	2B	797328204	2D	652726972	2A/2B/2D	-
Kukri_c900_1334	2B	796807569	2B	796807569	Un		-	-
Excalibur_rep_c106698_235	2B	801217330	2B	801217354	2A	781357606	2A/2B/2D	
CAP7_c4827_53	2B	787058697	Un		Un		-	-
Excalibur_c2311_1563	2D	640215489	2D	640215513	-	-	2B/2D	-
GENE-0676_649	2D	640211018	2D	640211042	2B	806014894	2A/2B/2D	
AX-94644192	2D	640216098	-	-	-	-	-	-
Kukri_c18420_705	3A	663180801	3A	663180825	Un	-	-	-
wsnp_Ex_c40247_47349166	7A	116113287	7A	116113239	-	-	7A/7B	-

Markers placed at the same chromosome at approximately the same postion + only mapped to one position in JBrowse -> “High quality” markers.

“High quality” markers are used for detailed analysis

Bt1

Markers from GWAS versus Starke II NIL



	NGB21183b2	Starke NIL Bt1	Starke II NGB-22	
Kukri_c18420_705	A	A	G	NGB21183b2
Excalibur_c2311_1563	G	G	A	NGB21183b2
Excalibur_c25043_357	A	A	G	NGB21183b2
Kukri_c900_1334	T	T	C	NGB21183b2
GENE-0676_649	A	A	G	NGB21183b2
Excalibur_rep_c106698_235	A	A	G	NGB21183b2
CAP7_c4827_53	C	C	T	NGB21183b2
AX-94644192		G	C	Unknown
wsnp_Ex_c48087_53105842	A	G	G	Starke II NGB-22
wsnp_Ex_c40247_47349166	C	C	C	Mono

2A/2B signal confirmed

Kukri_c18420_705 “confirmed”

1A discarded

7A unknown

Is the signal at 2A or 2B?

Bt1

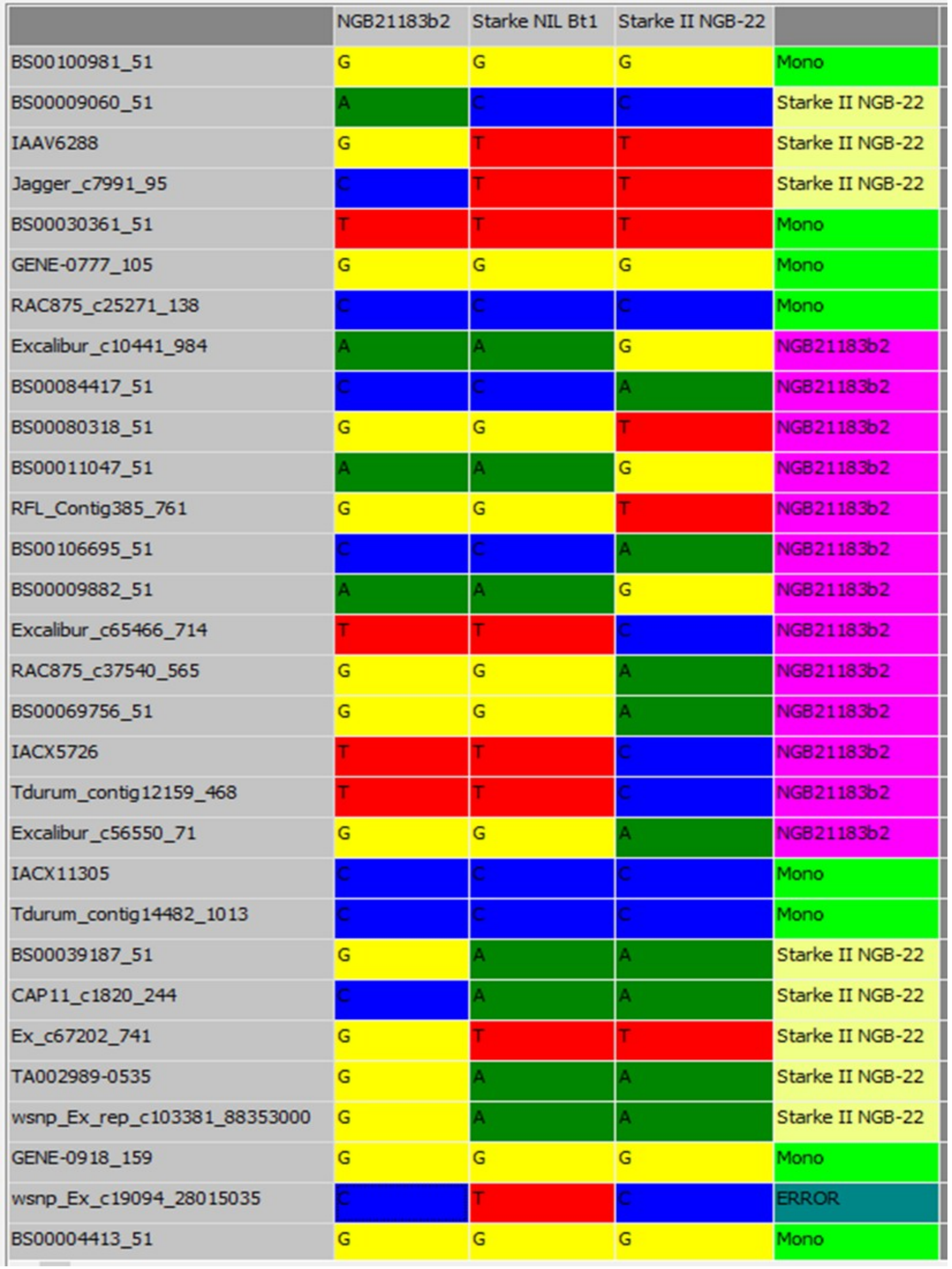
2B analysis
Using "High quality" 2B markers

Interval: Jagger_c7991_95 - BS00039187_51 = 786,230,010 – 790,753,463 bp (TraitGenetics = RefSeq 1.0 positions)

Interval Size: 4,523,453 bp

Recombination events in selected lines -> refines interval

More lines and work needed!!



	NGB21183b2	Starke NIL Bt1	Starke II NGB-22	
BS00100981_51	G	G	G	Mono
BS00009060_51	A	C	C	Starke II NGB-22
IAAV6288	G	T	T	Starke II NGB-22
Jagger_c7991_95	C	T	T	Starke II NGB-22
BS00030361_51	T	T	T	Mono
GENE-0777_105	G	G	G	Mono
RAC875_c25271_138	C	C	C	Mono
Excalibur_c10441_984	A	A	G	NGB21183b2
BS00084417_51	C	C	A	NGB21183b2
BS00080318_51	G	G	T	NGB21183b2
BS00011047_51	A	A	G	NGB21183b2
RFL_Contig385_761	G	G	T	NGB21183b2
BS00106695_51	C	C	A	NGB21183b2
BS00009882_51	A	A	G	NGB21183b2
Excalibur_c65466_714	T	T	C	NGB21183b2
RAC875_c37540_565	G	G	A	NGB21183b2
BS00069756_51	G	G	A	NGB21183b2
IACX5726	T	T	C	NGB21183b2
Tdurum_contig12159_468	T	T	C	NGB21183b2
Excalibur_c56550_71	G	G	A	NGB21183b2
IACX11305	C	C	C	Mono
Tdurum_contig14482_1013	C	C	C	Mono
BS00039187_51	G	A	A	Starke II NGB-22
CAP11_c1820_244	C	A	A	Starke II NGB-22
Ex_c67202_741	G	T	T	Starke II NGB-22
TA002989-0535	G	A	A	Starke II NGB-22
wsnp_Ex_rep_c103381_88353000	G	A	A	Starke II NGB-22
GENE-0918_159	G	G	G	Mono
wsnp_Ex_c19094_28015035	C	T	C	ERROR
BS00004413_51	G	G	G	Mono

References

IDENTIFICATION OF THE CHROMOSOME CARRYING THE MARTIN GENE FOR RESISTANCE OF WHEAT TO BUNT. SEARS, E. R., SCHALLEARN, C., & BRIGG, D. F. (1960). Briggs, F., & ton., C. H. (1950).

Reaction of wheat varieties with known genes for resistance to race of bunt, Tilletia caries and I. foetida. *Agron. Jour. 32*, 483-486.

Mapping QTL for spike fertility and related traits in two doubled haploid wheat (Triticum aestivum L.) populations. Nicole Pretini, Leonardo S. Vanzetti, […], and Fernanda G. González

Bt2

- Originally called the Hussar factor.
- Hussar has Bt1 + Bt2.
- Differential lines are Selection 1102 / PI 554097 and Selection 2075 / PI 554103
- *No results yet.*

References

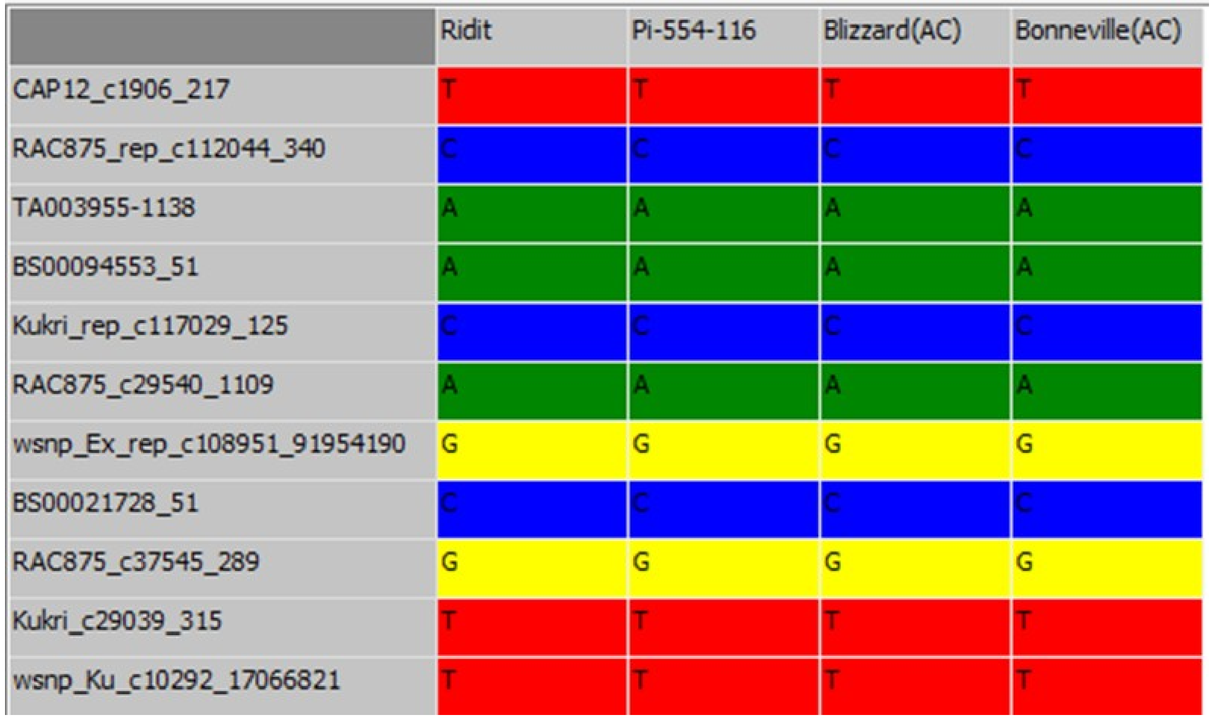
INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, TILLETIA TRITICI, IN HUSSAR WHEAT By F. N. BRIGGS

Bt3

- Initially known as the Florence factor.
- Ridit is a selection from a Florence x Turkey cross and is used as the Bt3 differential line.
- Mapped by BOKU in Blizzard/Bonneville.
- Chromosome 1A - 492.81 - 515.55 Mbp

Bt3

Comparing haplotypes in Blizzard, Bonneville, Ridit, Red Bobs Bt3 NIL (M83-1551 / PI 554116)



	Ridit	Pi-554-116	Blizzard(AC)	Bonneville(AC)
CAP12_c1906_217	T	T	T	T
RAC875_rep_c112044_340	C	C	C	C
TA003955-1138	A	A	A	A
BS00094553_51	A	A	A	A
Kukri_rep_c117029_125	C	C	C	C
RAC875_c29540_1109	A	A	A	A
wsnp_Ex_rep_c108951_91954190	G	G	G	G
BS00021728_51	C	C	C	C
RAC875_c37545_289	G	G	G	G
Kukri_c29039_315	T	T	T	T
wsnp_Ku_c10292_17066821	T	T	T	T

wsnp_Ku_c21316_31053745	T
CAP12_c1906_217	T
RAC875_rep_c112044_340	C
TA003955-1138	A
BS00094553_51	A
Kukri_rep_c117029_125	C
RAC875_c29540_1109	A
wsnp_Ex_rep_c108951_91954190	G
BS00021728_51	C
RAC875_c37545_289	G
Kukri_c29039_315	T
wsnp_Ku_c10292_17066821	T
wsnp_Ku_c1642_3232242	T

Interval: 498,451,021 - 506,854,738 bp

Interval Size: 8,403,717 bp

Markers in green are usefull for MAS.

Markers in brown define the interval.

References

Inheritance of Resistance to Three Physiologic Forms of Bunt in Ridit x Utah-Kanred Cross. Muhammad Ibrahim Shah 1935

Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. Muellner, Almuth et. Al. 2021

Bt4

- Known as the Turkey Gene.
- Mapped to 1B in 1969 by Monosomic analysis.
- Linked to Bt5 and Bt6. Most closely to Bt6 = 1BS.
- Having a chromosome only some triplets of lines – parents1, parent2 and sibling is needed to find candidate interval(s)
- Such lines are in the phenotyping/ genotyping pipelines

References

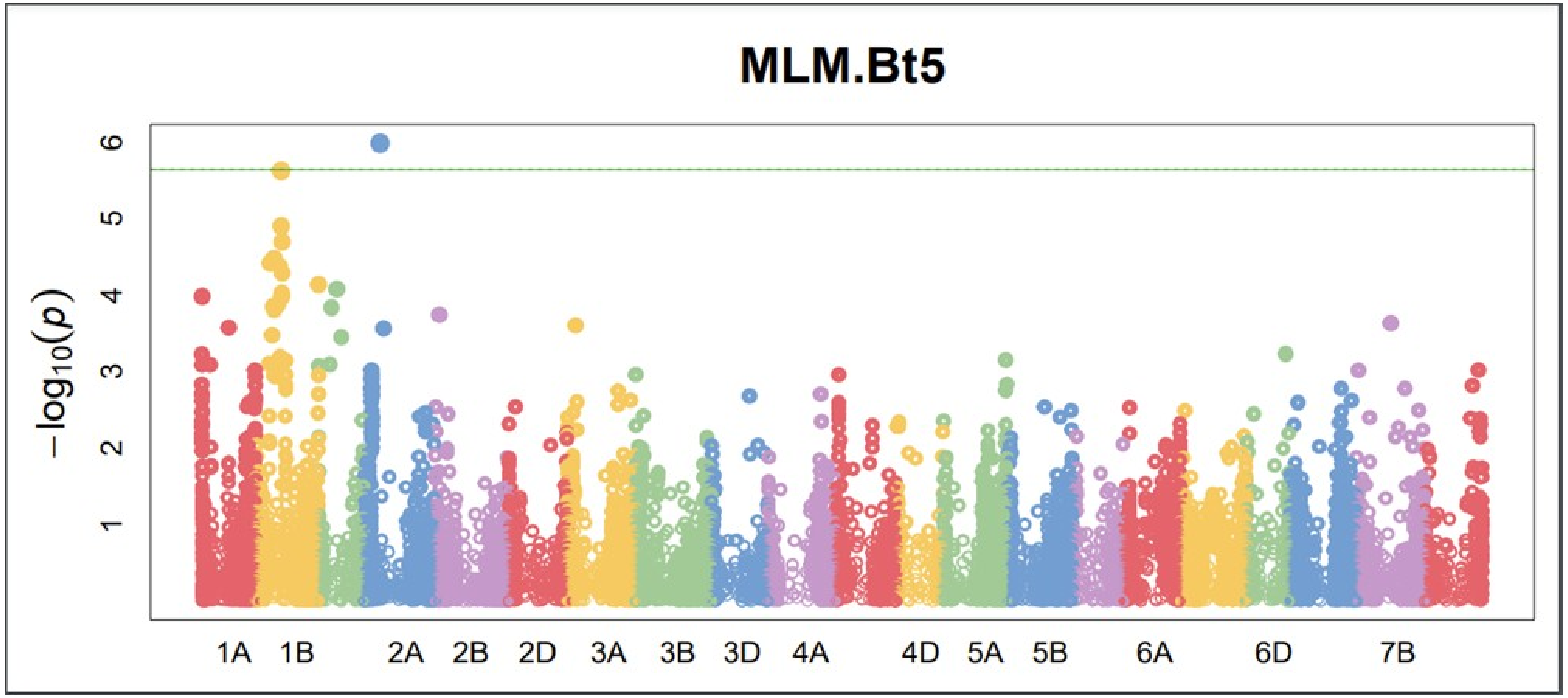
Monosomic Analysis for Bunt Resistance in Derivatives of Turkey and Oro Wheats. J. W. Schmidt, R. Morris, and V. A. Johnson 1969

Linkage Relationships of the Martin, Hussar, Turkey, and Rio Genes For Burnt Resistance in Wheat C. W. Schaller and F. N. Briggs 1955

Bt5

Discovered in the cultivar Hohenheimer which is still used as differential line (Does it have Bt7 also?)

At 1B linked to Bt4 and Bt6



No of principal components = 1 !!!

Bt5

SNP	Chromosome	Position	FDR_Adjusted_P-values
AX-110385663	1B	277458400	0,01791401
AX-158609274	2A	177991455	0,01791401
AX-158545071	1B	277958158	0,034776635
AX-110936551	1B	288649553	0,037952615
wsnp_BE637864B_Ta_1_1	1B	199575153	0,037952615
AX-109342634	1B	259025474	0,037952615
BS00023130_51	1A	5024210	0,037952615
AX-158540319	1B	156598386	0,03962259
AX-110366212	1B	288649630	0,046669231

Interval 156,598,386 – 288,649,630 bp.

Manual inspection of recombination events reduced interval

Interval: 118,731,133 – 284,939,025 bp.

Interval Size: 166,207,892 bp

Recombination events are rare

Bt5

Markers in green are usefull for MAS.

Markers in brown define the interval.

BS00022473_51	C
Ra_c455_283	T
RAC875_c60360_389	C
BS00093740_51	G
BS00010992_51	T
BS00073034_51	C
BS00063574_51	T
BS00068512_51	T
BS00068246_51	G
BS00022218_51	G
BS00066165_51	G
wsnp_BE637864B_Ta_1_1	G
BS00022317_51	A
AX-109342634	A
AX-110385663	C
Excalibur_c6497_535	G

Markers in this interval correctly identifies 59 (61) out of 64 lines postulated to have Bt5, yielding a 92 (95) % identification rate.

15 out of 165 lines not postulated to have Bt5 were incorrectly identified as having Bt5, giving a false positive rate of 9 %.

10 + crosses done in 2022 between Bt5 donors and lines with good marker contrast in the interval

References

Monosomic Analysis for Bunt Resistance in Derivatives of Turkey and Oro Wheats. J. W. Schmidt, R. Morris, and V. A. Johnson 1969

Linkage Relationships of the Martin, Hussar, Turkey, and Rio Genes For Burnt Resistance in Wheat C. W. Schaller and F. N. Briggs 1955

Genetic Mapping of Common Bunt Resistance Gene Bt5. Dennis Kjær Christensen, Anders Borgen, Gunter Backes, Carl Vollenweider and Karl-Josef Müller. 2022

Bt6

- Known as the Rio gene
- Linked to Bt4 and Bt5 at 1B
- BOKU mapped something at 1B in Blizzard/Bonneville.
- Interval BOKU: 8 - 22 Mbp
- We located a haploblock from Rio in that interval
- One marker - BS00074962_51 – fails in all lines having Bt6
- Positioned in gene TraesCS1B02G032200
- Detection of recombination events in the Blizzard/Bonneville x Rainer populations done
- The presence of three genes – Bt3, Bt6 and something at 7A – and phenotyping with only one virulence races makes it hard to refine intervals

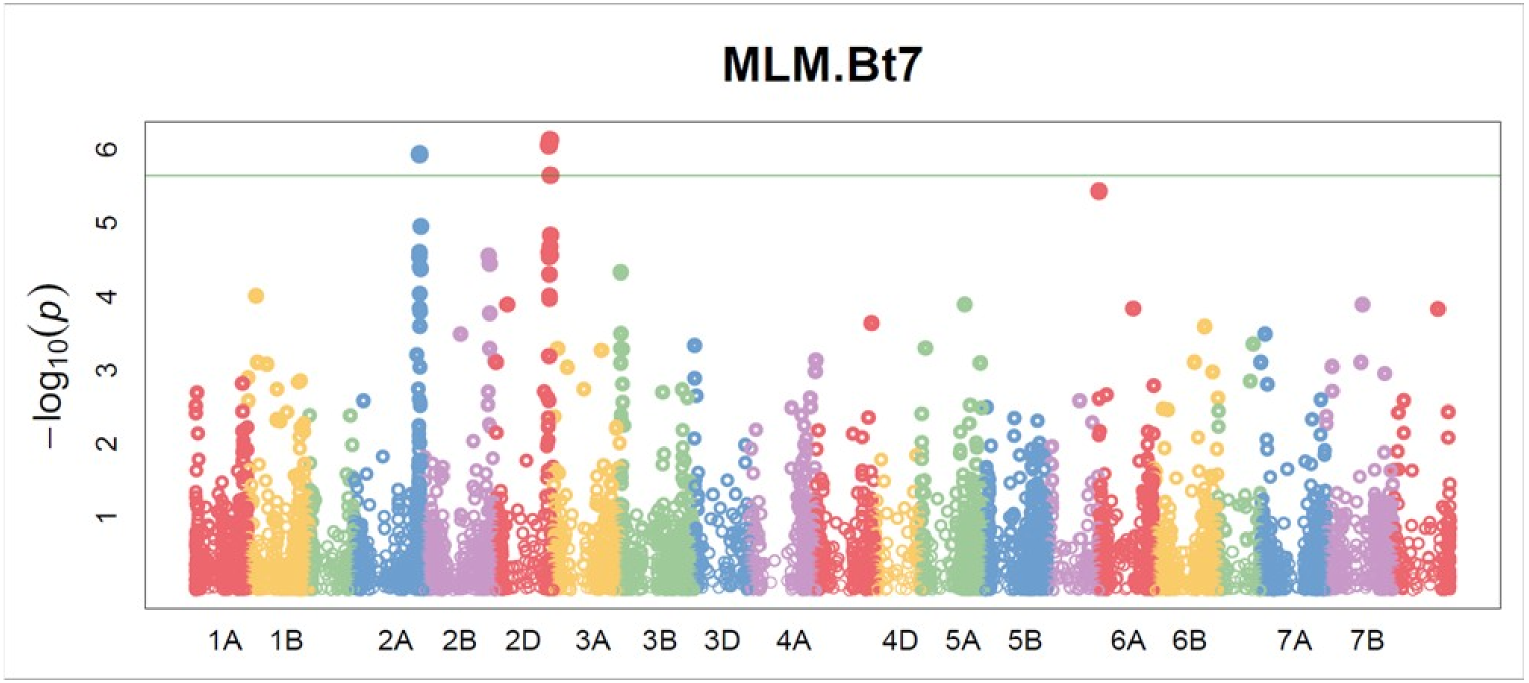
References

Linkage Relationships of the Martin, Hussar, Turkey, and Rio Genes For Burnt Resistance in Wheat C. W. Schaller and F. N. Briggs 1955

Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. Muellner, Almuth et. Al. 2021

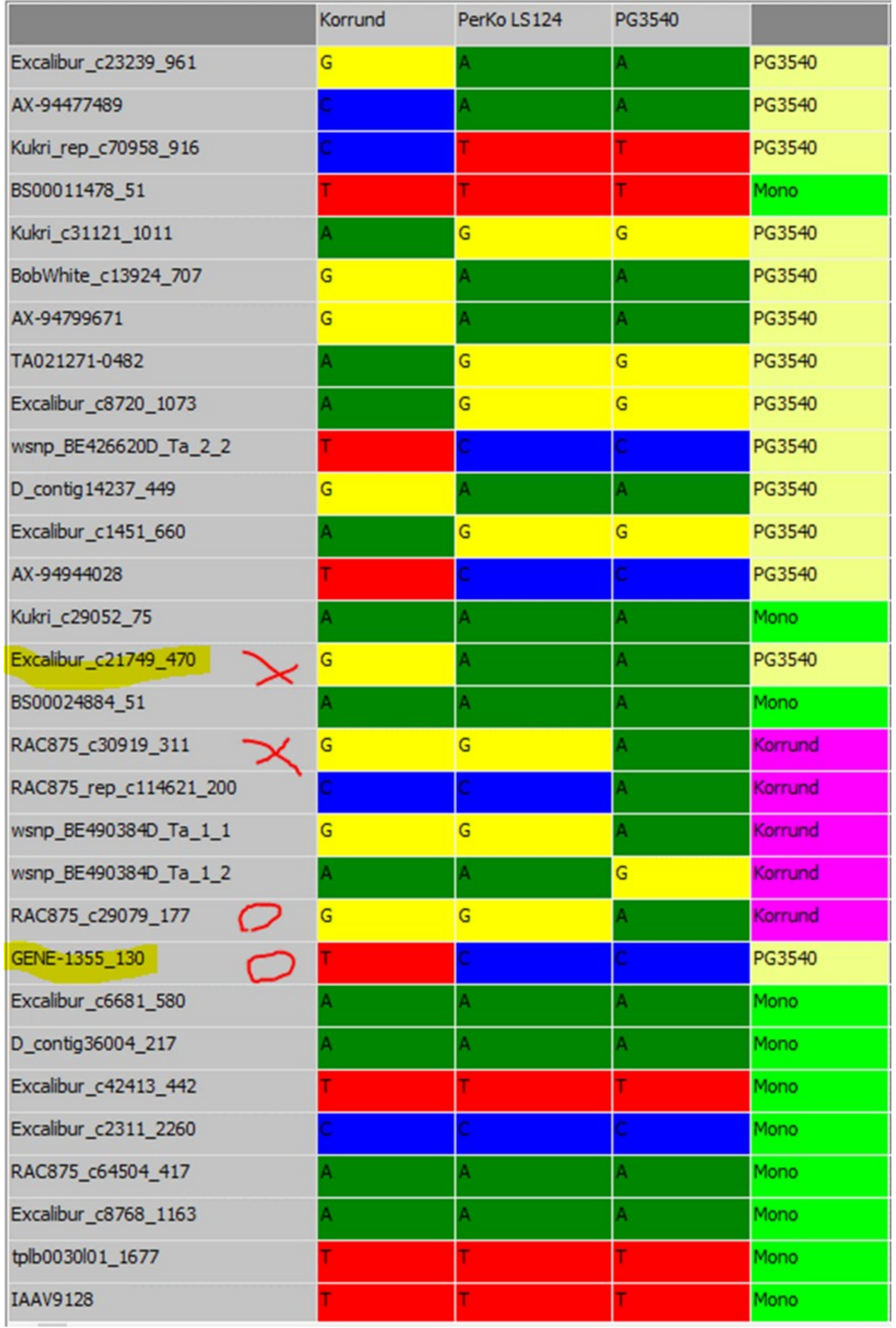
Bt7

- Initially known as the Martin gene number 2.
- Selection 50077 / PI 554100 uses as differential.
- Mapped by monosomic analysis to chromosome 2D.
- The GWAS gave 45 significant markers on 2A, 2B and 2D.



Bt7

- Marker positions are from TraitGenetics and are wrong for our population.
- Linkage analysis and detailed analysis of recombination events confirmed one signal at 2D.
- Detailed analysis of recombination events in lines where the existence of not of Bt7 is certain and both parents have been genotyped confirmed the 2D signal.
- One line - PerKo LS124 - was very interesting. It has Bt7 and have two recombination events in the mapped interval



		Korrund	PerKo LS124	PG3540	
Excalibur_c23239_961		G	A	A	PG3540
AX-94477489		C	A	A	PG3540
Kukri_rep_c70958_916		C	T	T	PG3540
BS00011478_51		T	T	T	Mono
Kukri_c31121_1011		A	G	G	PG3540
BobWhite_c13924_707		G	A	A	PG3540
AX-94799671		G	A	A	PG3540
TA021271-0482		A	G	G	PG3540
Excalibur_c8720_1073		A	G	G	PG3540
wsnp_BE426620D_Ta_2_2		T	C	C	PG3540
D_contig14237_449		G	A	A	PG3540
Excalibur_c1451_660		A	G	G	PG3540
AX-94944028		T	C	C	PG3540
Kukri_c29052_75		A	A	A	Mono
Excalibur_c21749_470	X	G	A	A	PG3540
BS00024884_51		A	A	A	Mono
RAC875_c30919_311	X	G	G	A	Korrund
RAC875_rep_c114621_200		C	C	A	Korrund
wsnp_BE490384D_Ta_1_1		G	G	A	Korrund
wsnp_BE490384D_Ta_1_2		A	A	G	Korrund
RAC875_c29079_177	O	G	G	A	Korrund
GENE-1355_130	O	T	C	C	PG3540
Excalibur_c6681_580		A	A	A	Mono
D_contig36004_217		A	A	A	Mono
Excalibur_c42413_442		T	T	T	Mono
Excalibur_c2311_2260		C	C	C	Mono
RAC875_c64504_417		A	A	A	Mono
Excalibur_c8768_1163		A	A	A	Mono
tplb0030l01_1677		T	T	T	Mono
IAAV9128		T	T	T	Mono

- One recombination event between the two markers highlighted with a X and one between markers with O.
- Bt 7 in the interval]Excalibur_c21749_470; GENE-1355_130[=]613,322,375; 621,845,161[bp

Bt7

Markers in green are usefull for MAS.

Markers in brown define the interval.

Excalibur_c21749_470	G
BS00024884_51	A
BS00100106_51	C
RAC875_c30919_311	G
RAC875_rep_c114621_200	C
wsnp_Ex_c42970_49408712	A
wsnp_BE490384D_Ta_1_1	G
wsnp_BE490384D_Ta_1_2	A
RAC875_c29079_177	G
GENE-1355_130	T

Markers are working very well. “Perfect” detection rate. Low false positive rate.

Bt7
Example of validation results

No of varieties: 389
No of blocks: 55
No Markers Found: 888

Not all Markers Found: 999

Heterozygos Markers Found: 777
Heterozygos and Failing Markers Found: 666

Name: Cbt_Bt7_2D_MAS
Chromosome: 2D
NoOfMarkers: 8
PhysPosMin: 617089362
PhysPosMax: 619576292
Size: 2486930

Starke II NGB-22	Bt0	13
PG3540	Bt1	25
Starke NIL Bt1	Bt1	13
Courier	Bt1	100
ArPG LS116	Bt1	25
PerBut LS21	Bt1	777
PerKo LS123	Bt1	25
PerXe LS125	Bt1	777
CorPG LS132	Bt1	13
CorPG LS133	Bt1	25
CorPG LS134	Bt1	13
FoPG LS137	Bt1	25
CCP 16	Bt1	777
PerHyp7	Bt1	100
Aristaro	Bt1	13
WIF109Bt.01	Bt1	100
Pi-554-097	Bt2	777
Pi-554-103	Bt2	13
Bussard	Bt2	13

Bt7

Pi-554-100	Bt7	100
Tambor	Bt7	777
Butaro	Bt7	100
Korrund	Bt7	100
Xenos	Bt7	100
Segor	Bt7	100
Ring 59: HSI-672-10	Bt7	100
Ring 60: HSI-367-10	Bt7	100
Ring 61: HSI-664-11	Bt7	100
Jacoby	Bt7	25
SegQue LS81	Bt7	777
ButQue LS88	Bt7	100

Bt7

Bt0	0
Bt1	21
Bt2	4
Bt3	0
Bt4	0
Bt5	16
Bt6	0
Bt7	95
Bt8	0
Bt9	0
Bt10	20
Bt11	0
Bt12	0
Bt13	50
Bt14	0
Bt15	0
BtZ	17
BtQ	40
Bt5+Bt13	0
Bt13+BtZ	0
Bt7+BtZ	50

References

E. R. Sears, C. W. Schaller, and F. N. Briggs. IDENTIFICATION OF THE CHROMOSOME CARRYING THE MARTIN GENE FOR RESISTANCE OF WHEAT TO BUNT. *Canadian Journal of Genetics and Cytology*. **2**(3): 262-267. https://doi.org/10.1139/g60-026

Mapping Common Bunt Resistance Gene Bt7. Dennis Kjær Christensen, Anders Borgen, Gunter Backes, Hermann Gregor Dallinger, Carl Vollenweider, Karl-Josef Müller and Pernille Merete Sarup. 2022

Bt8

- Discovered in Yayla 305 / PI 178210
- Differential line is M72-1250 / PI 554120
- Hypothesized to be at 6DS.

References

Inheritance of a New Factor (Bt8) for Resistance to Common Bunt in Wheat. J. L. Wad and R. J. Metzger 1970

Is Bt8 located at Chromosome 6D and closely linked to Bt10? Dennis Kjær Christensen | Anders Borgen Agrologica, Houvej 55, DK-9550 Mariager, Denmark. Virtual Bunt and Smut Workshop May 5 – 6, 2021

Bt9

- Discovered in the line CI 7090/PI 57143 in 1979
- Mapped by Steffan et. Al. to 6D
- And later to 469,830,275 – 471,017,889 bp (IWGSC RefSeq v1.1 positions) by Wang et al.
- The Starke II Bt9 NIL had inherited 10 out of 405 markers on chromosome 6D from NGB 21193. These 10 markers covered the 1,760,727 bp interval 468,879,174 – 470,639,901 bp (TG positions).

Bt9

- Interval: 468,879,174 – 470,639,901
- Interval Size: 1,760,727
- Haplogroup analysis against PI 178383
- Interval: 490,337,515 – 491,431,473 bp (RefSeq 2.1 positions)
- Interval Size: 1,093,958 bp

Markers in green are usefull for MAS.

Markers in brown define the interval.

High false positive rate in our material

Excalibur_c16566_485	G
wsnp_JG_c5646_2148296	C
wsnp_JG_c5646_2148382	C
wsnp_CAP7_c1735_859875	G
wsnp_CAP7_c1735_859744	T
AX-94589700	A
Excalibur_c2991_320	C

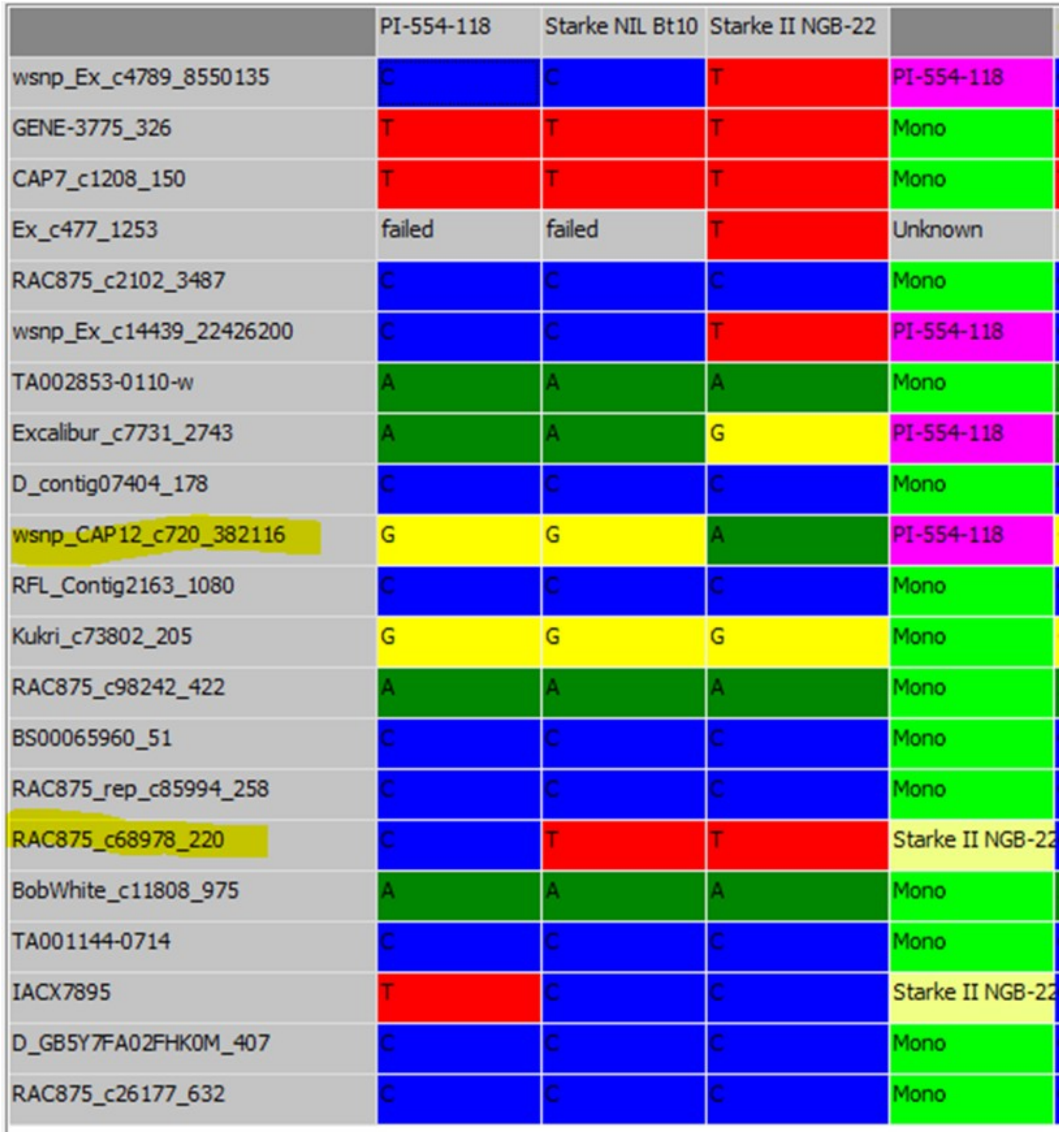
References

Inheritance of Resistance to Common Bunt in Wheat, C.I. 70901 R. J. Metzger, C. W. Schaller, and C. R. Rohde 1979
Mapping of common bunt resistance gene Bt9 in wheat. Steffan, P.; A.M. Torp; A.Borgen; G.Backes; S.K. Rasmussen (2017).
Identifcation and assessment of two major QTLs for dwarf bunt resistance in winter wheat line ‘IDO835’
Rui Wang · Tyler Gordon · David Hole · Weidong Zhao · Kyle Isham · J. Michael Bonman · Blair Goates · Jianli Chen

Bt10

- Bt10 was discovered in the lines Greece 18 / PI 116301 and Mocho / PI 116306 in 1971 by Metzger
- Has been mapped to 6DS.
- The differential line is R63-6982 / PI 554118 (Elgin x PI 178383)
- MacKay has made a Starke II NIL – Bt10 source is unknown
- Using R63-6982 / PI 554118 as donor a recombination event is found

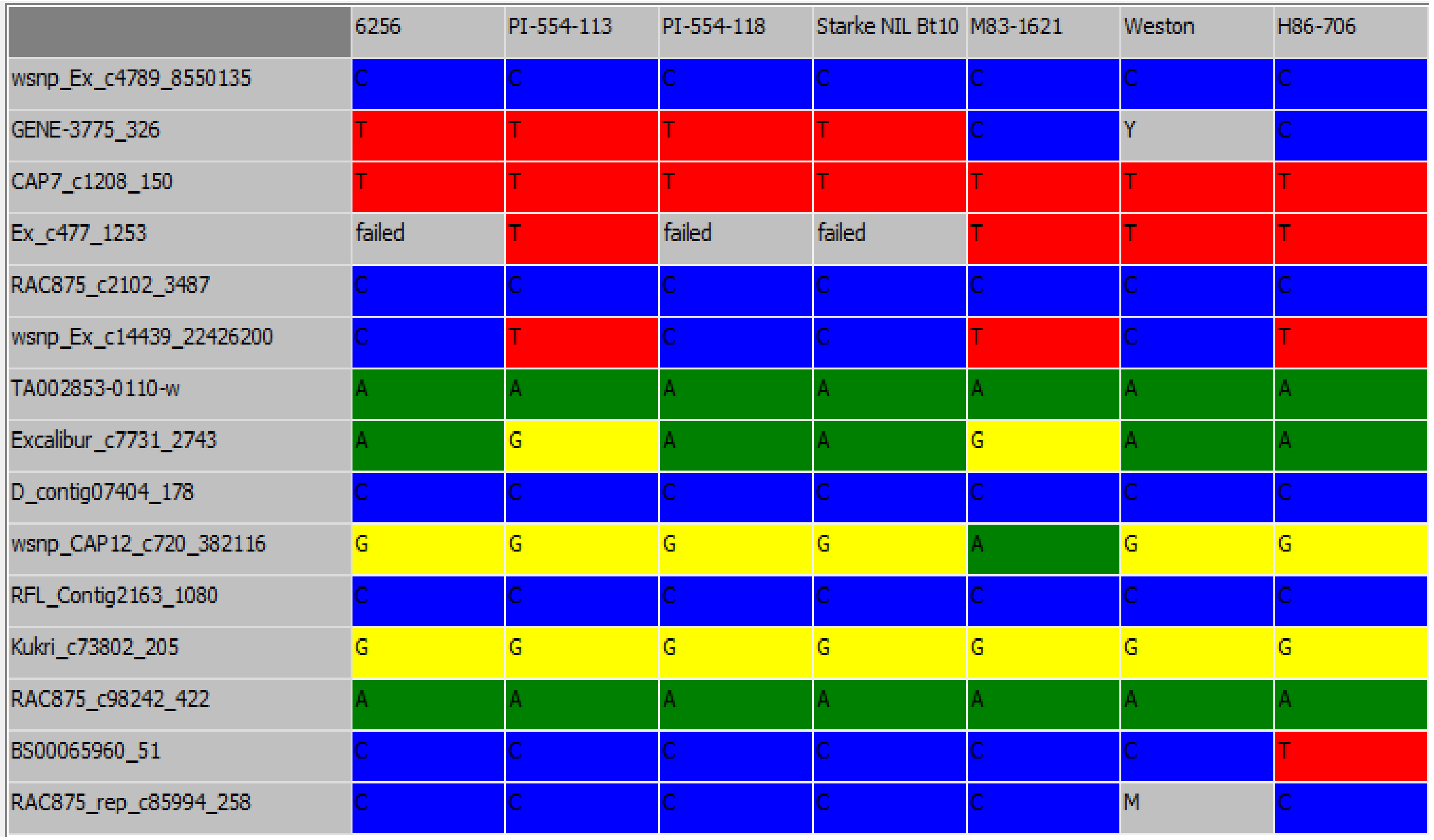
Bt10



	PI-554-118	Starke NIL Bt10	Starke II NGB-22	
wsnp_Ex_c4789_8550135	C	C	T	PI-554-118
GENE-3775_326	T	T	T	Mono
CAP7_c1208_150	T	T	T	Mono
Ex_c477_1253	failed	failed	T	Unknown
RAC875_c2102_3487	C	C	C	Mono
wsnp_Ex_c14439_22426200	C	C	T	PI-554-118
TA002853-0110-w	A	A	A	Mono
Excalibur_c7731_2743	A	A	G	PI-554-118
D_contig07404_178	C	C	C	Mono
wsnp_CAP12_c720_382116	G	G	A	PI-554-118
RFL_Contig2163_1080	C	C	C	Mono
Kukri_c73802_205	G	G	G	Mono
RAC875_c98242_422	A	A	A	Mono
BS00065960_51	C	C	C	Mono
RAC875_rep_c85994_258	C	C	C	Mono
RAC875_c68978_220	C	T	T	Starke II NGB-22
BobWhite_c11808_975	A	A	A	Mono
TA001144-0714	C	C	C	Mono
IACX7895	T	C	C	Starke II NGB-22
D_GB5Y7FA02FHK0M_407	C	C	C	Mono
RAC875_c26177_632	C	C	C	Mono

- The investigated interval is 0 – 26,245,078 bp
- It looks like Bt10 is in the interval below RAC875_c68978_220.
- Interval [0; 6,470,777[bp in RefSeq 2.1 positions
- Only PI-554-118, Starke NIL Bt10, 6256, PI-554-113, M83-1621, Weston, H86-706 available

Bt10



	6256	PI-554-113	PI-554-118	Starke NIL Bt10	M83-1621	Weston	H86-706
wsnp_Ex_c4789_8550135	C	C	C	C	C	C	C
GENE-3775_326	T	T	T	T	C	Y	C
CAP7_c1208_150	T	T	T	T	T	T	T
Ex_c477_1253	failed	T	failed	failed	T	T	T
RAC875_c2102_3487	C	C	C	C	C	C	C
wsnp_Ex_c14439_22426200	C	T	C	C	T	C	T
TA002853-0110-w	A	A	A	A	A	A	A
Excalibur_c7731_2743	A	G	A	A	G	A	A
D_contig07404_178	C	C	C	C	C	C	C
wsnp_CAP12_c720_382116	G	G	G	G	A	G	G
RFL_Contig2163_1080	C	C	C	C	C	C	C
Kukri_c73802_205	G	G	G	G	G	G	G
RAC875_c98242_422	A	A	A	A	A	A	A
BS00065960_51	C	C	C	C	C	C	T
RAC875_rep_c85994_258	C	C	C	C	C	M	C

- It is seen that markers cannot be used for MAS
- Xenos x Weston crosses in the pipeline. Crossing fingers for recombination events in this interval.

References

A New Factor for Resistance to Common Bunt in Hexaploid Wheats. R. J. Metzger and B. A. Silbaugh 1971

J. G. Menzies, R. E. Knox, Z. Popovic, and J. D. Procunier. Common bunt resistance gene Bt10 located on wheat chromosome 6D. Canadian Journal of Plant Science. 86(Special Issue): 1409-1412. https://doi.org/10.4141/P06-106

Bt11

- Discovered in Dimenit / PI 166910 in 1984. Dimenit should also have Bt7 and Bt9 (and not Bt8).
- Differential lines are M82-2123 / PI 554119 (Elgin / PI 166910) and P68-1336-7 / PI 554098 (PI 166910 / Elgin)
- Dimenit / PI 166910, M82-2123 / PI 554119 and P68-1336-7 / PI 554098 do not have Bt7 or Bt9 markers -> extra genes in Dimenit are not Bt7/Bt9
- Has been mapped to 3B by Ciuca, Matilda 2011. Six markers are available
- BOKU RIL population M82-2123 x Rainer segregated for two genes.
- Only one marker (Xwmc231) was weakly associated with resistance.
- 74 (99) lines phenotyped in 2022. Genotyping to be done.

References

Inheritance of resistance in some wheat introductions to selected races of bunt, Tilletia caries (DC) Tul and Tilletia foetida (Wallr) Liro. Abdalla, Osman S. 1984

A Preliminary Report on the Identification of SSR Markers for Bunt (Tilletia sp.) Resistance in Wheat. Czech Journal of Genetics and Plant Breeding. 47. S142-S145. 10.17221/3269-CJGPB. Ciuca, Matilda. (2011).

Breeding for organic agriculture: Evaluation of common bunt resistance in three winter wheat populations derived from M822123, PI178383 and PI560841-bcl. Julia Friederike Hagenguth 2016

Bt12

- Added to the differential set by Blair Goates
- The differential line for Bt12 is PI119333
- Has been mapped to the 7DS interval 6.5–10.8 Mbp by BOKU
- Seven markers for use in MAS found

Marker	Position [Mb]
Kukri_c80931_147	7,07
Ra_c11887_1826	8,05
RAC875_c99892_81	8,19
wsnp_JD_c2734_3667052	8,25
IAAV9104	9,31
D_F5XZDLF02H192C_184	10,72
RFL_Contig1323_544	10,84

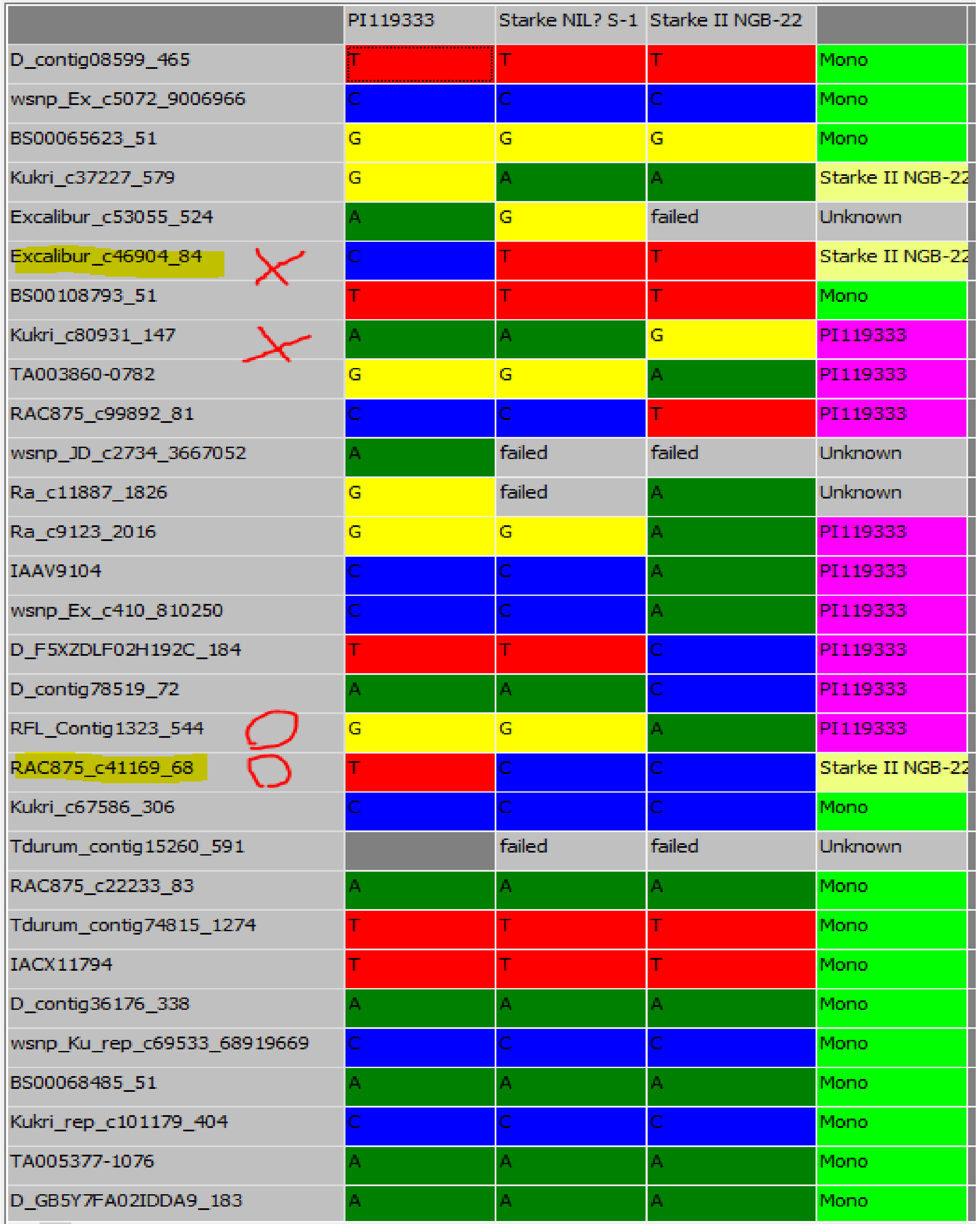
Bt12

- The mapping population as well as the Starke II versus PI199333 has the problem that markers are monomorphic in a large interval above the mapped interval. This makes is impossible to locate the exact interval upper bound.
- A cross between eg. The Starke II Bt12 NIL and a line with maximum marker contrast in the extended interval would be usefull. Proposals are Segor, Bonneville and Marquis.
- If we trust that the marker RAC875_c41169_68 is correctly placed we can detect a recombination event
- The Starke II Bt12 NIL has two detectable recombination events in an extended interval around the mapped interval

If we trust the marker RAC875_c41169_68 !!

The BOKU mapping population has recombination events in the same locations -> recombination hotspots?

Bt 12 is in the interval]Excalibur_c46904_84; RAC875_c41169_68[

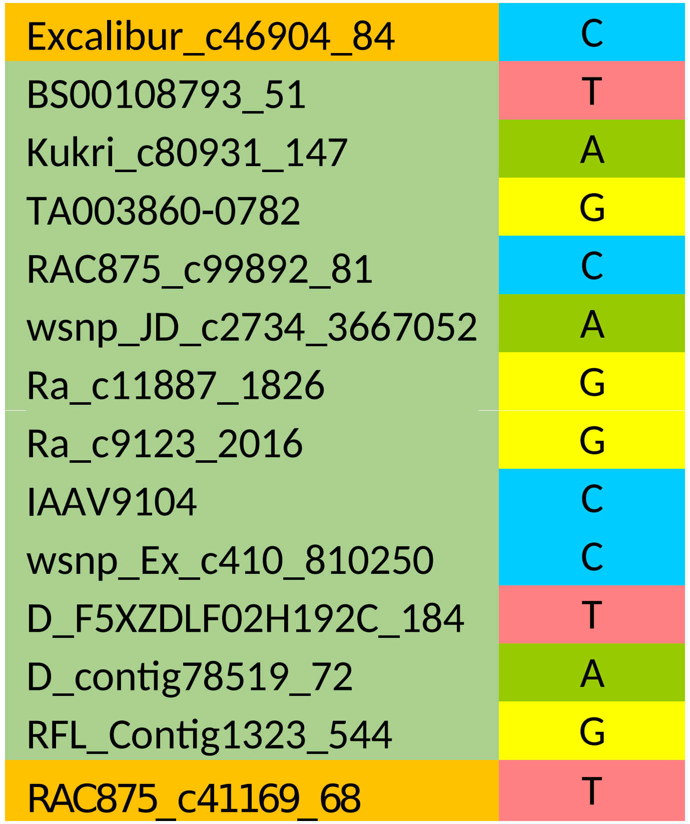


	PI119333	Starke NIL? S-1	Starke II NGB-22	
D_contig08599_465	T	T	T	Mono
wsnp_Ex_c5072_9006966	C	C	C	Mono
BS00065623_51	G	G	G	Mono
Kukri_c37227_579	G	A	A	Starke II NGB-22
Excalibur_c53055_524	A	G	failed	Unknown
Excalibur_c46904_84	C	T	T	Starke II NGB-22
BS00108793_51	T	T	T	Mono
Kukri_c80931_147	A	A	G	PI119333
TA003860-0782	G	G	A	PI119333
RAC875_c99892_81	C	C	T	PI119333
wsnp_JD_c2734_3667052	A	failed	failed	Unknown
Ra_c11887_1826	G	failed	A	Unknown
Ra_c9123_2016	G	G	A	PI119333
IAAV9104	C	C	A	PI119333
wsnp_Ex_c410_810250	C	C	A	PI119333
D_F5XZDLF02H192C_184	T	T	C	PI119333
D_contig78519_72	A	A	C	PI119333
RFL_Contig1323_544	G	G	A	PI119333
RAC875_c41169_68	T	C	C	Starke II NGB-22
Kukri_c67586_306	C	C	C	Mono
Tdurum_contig15260_591		failed	failed	Unknown
RAC875_c22233_83	A	A	A	Mono
Tdurum_contig74815_1274	T	T	T	Mono
IACX11794	T	T	T	Mono
D_contig36176_338	A	A	A	Mono
wsnp_Ku_rep_c69533_68919669	C	C	C	Mono
BS00068485_51	A	A	A	Mono
Kukri_rep_c101179_404	C	C	C	Mono
TA005377-1076	A	A	A	Mono
D_GB5Y7FA02IDDA9_183	A	A	A	Mono

Bt12

Markers in green are usefull for MAS.

Markers in brown define the interval.



Excalibur_c46904_84	C
BS00108793_51	T
Kukri_c80931_147	A
TA003860-0782	G
RAC875_c99892_81	C
wsnp_JD_c2734_3667052	A
Ra_c11887_1826	G
Ra_c9123_2016	G
IAAV9104	C
wsnp_Ex_c410_810250	C
D_F5XZDLF02H192C_184	T
D_contig78519_72	A
RFL_Contig1323_544	G
RAC875_c41169_68	T

References

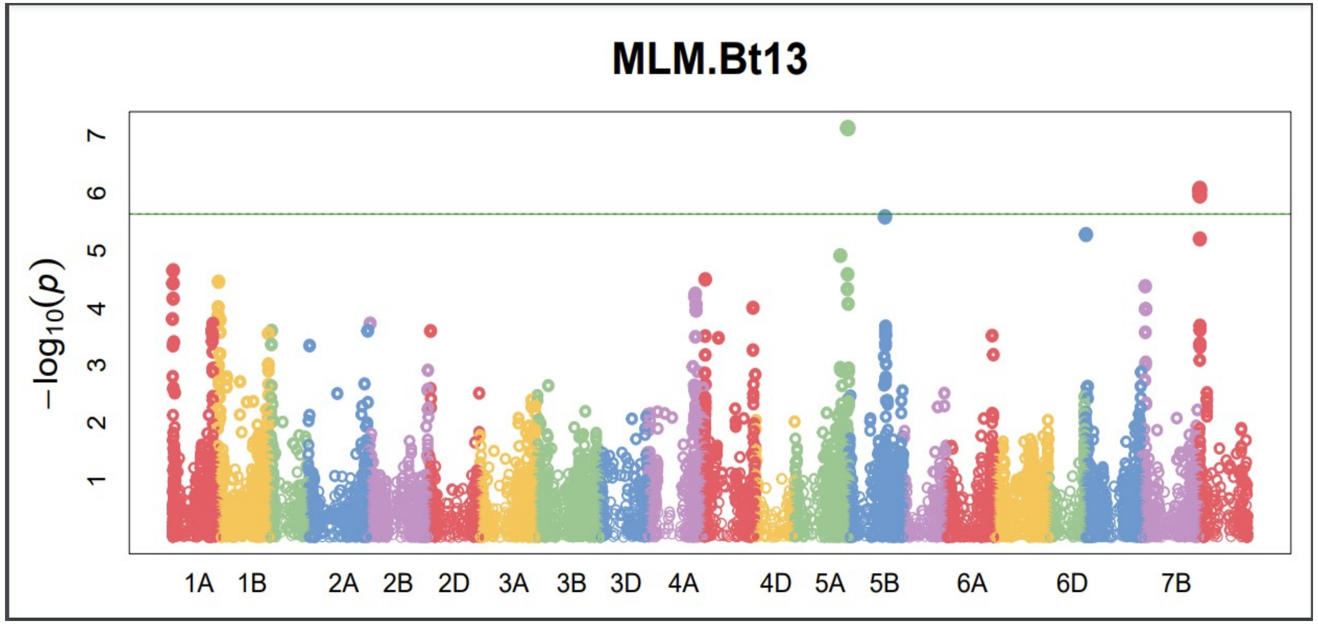
Muellner, A.E., Eshonkulov, B., Hagenguth, J. *et al.* Genetic mapping of the common and dwarf bunt resistance gene *Bt12* descending from the wheat landrace PI119333. *Euphytica* **216,** 83 (2020). https://doi.org/10.1007/s10681-020-02614-w

Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter wheat Landraces in the USDA-ARS National Small Grains Collection. Blair J. Goates* and Harold E. Bockelman.

Bt13

- Added to the differential set by Blair Goates.
- The differential line for Bt13 is Thule III , not to be confused with the swedish cultivar Thule III (NGB6714).
- Thule III and PI119333 (Bt12) has identical haplotypes across the Bt12 interval.
- All Bt13 lines in the mapping population descend directly from Thule III -> strong population structure

Bt13

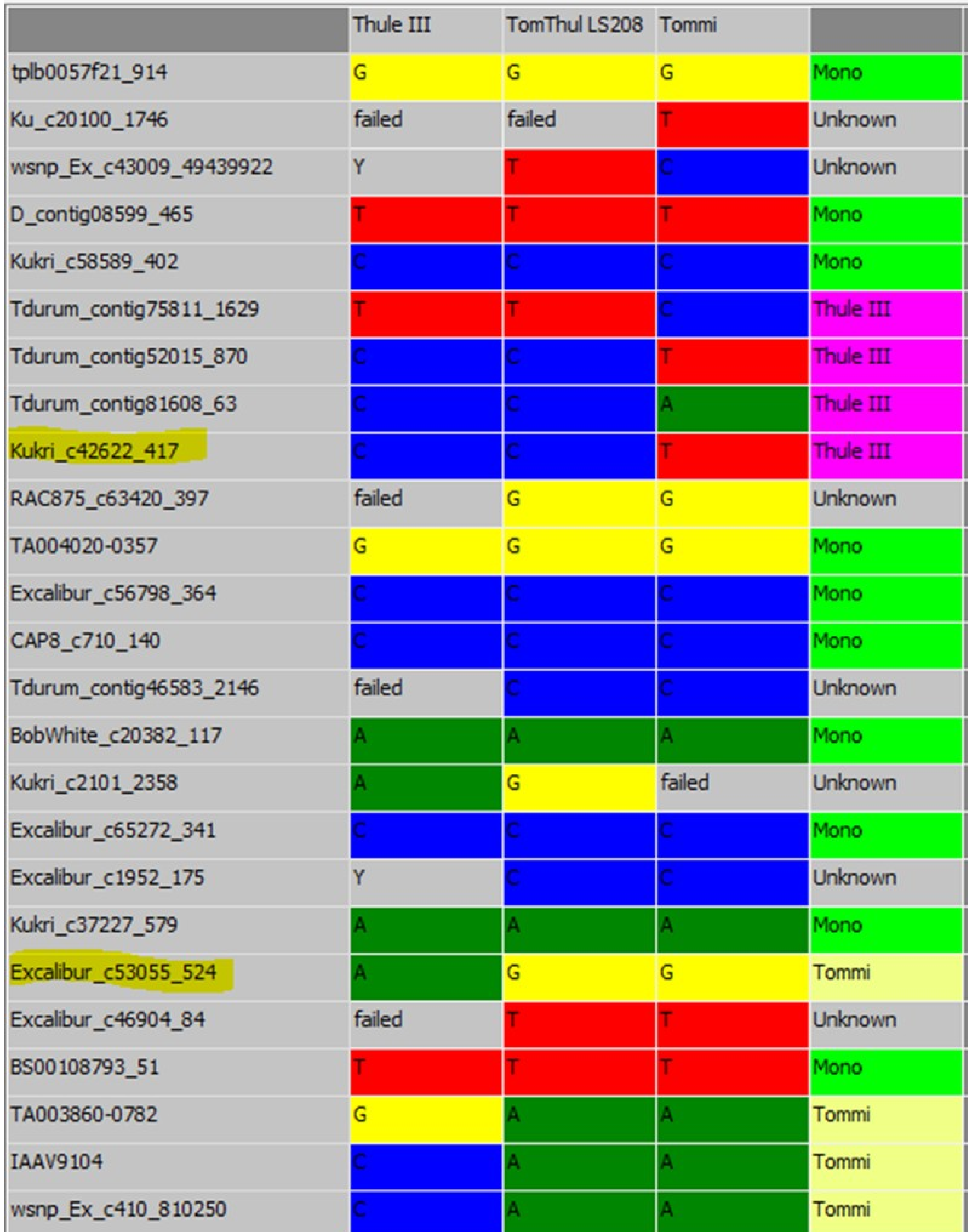


Two (weak) signals – one at 5A and one at 7D

SNP	Chromosome	Position	FDR_Adjusted_P-values
Excalibur_c49550_97	5A	597660574	0,033161345
wsnp_Ku_rep_c72362_72059764	5A	694973053	0,001588628
Ku_c8733_458	5B	471128091	0,011219993
RAC875_c23140_909	7A	8691377	0,018977653
TA003860-0782	7D	7447604	0,005999824
RAC875_c99892_81	7D	8185556	0,005999824
IAAV9104	7D	9309621	0,019398405
wsnp_Ex_c410_810250	7D	9312209	0,005999824

Bt13

- Most lines (75%) having Bt13 matched markers in the interval
- Detailed analysis with “high quality” markers revealed that SegThul LS168, SegThul LS169, SegThul LS173, SegThul LS180 had inherited from Segor in the entire interval 9,312,258 – 101,558,992 bp and ruling it out as candidate interval.
- TomThul LS208 has an interesting recombination event

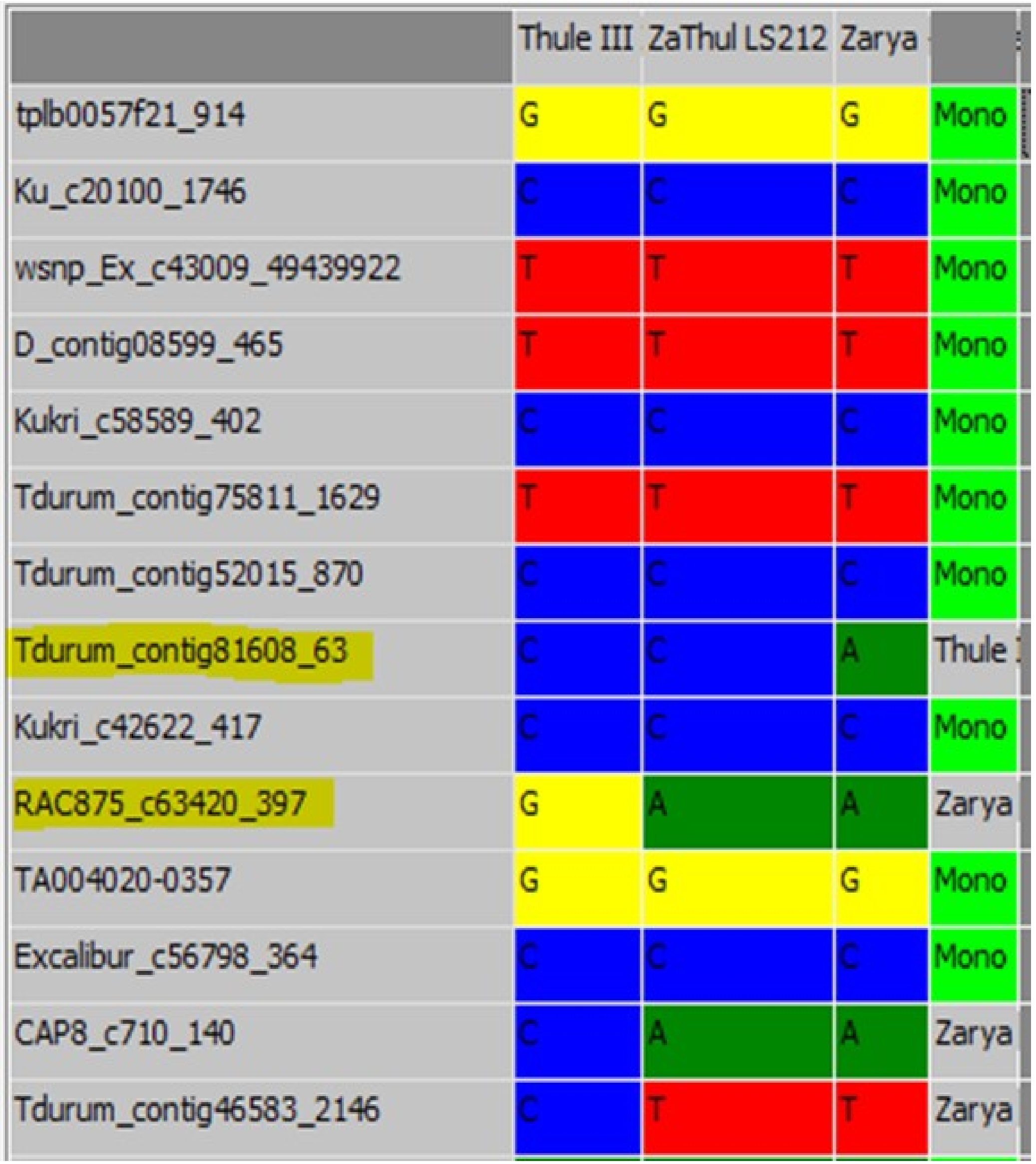


	Thule III	TomThul LS208	Tommi	
tplb0057f21_914	G	G	G	Mono
Ku_c20100_1746	failed	failed	T	Unknown
wsnp_Ex_c43009_49439922	Y	T	C	Unknown
D_contig08599_465	T	T	T	Mono
Kukri_c58589_402	C	C	C	Mono
Tdurum_contig75811_1629	T	T	C	Thule III
Tdurum_contig52015_870	C	C	T	Thule III
Tdurum_contig81608_63	C	C	A	Thule III
Kukri_c42622_417	C	C	T	Thule III
RAC875_c63420_397	failed	G	G	Unknown
TA004020-0357	G	G	G	Mono
Excalibur_c56798_364	C	C	C	Mono
CAP8_c710_140	C	C	C	Mono
Tdurum_contig46583_2146	failed	C	C	Unknown
BobWhite_c20382_117	A	A	A	Mono
Kukri_c2101_2358	A	G	failed	Unknown
Excalibur_c65272_341	C	C	C	Mono
Excalibur_c1952_175	Y	C	C	Unknown
Kukri_c37227_579	A	A	A	Mono
Excalibur_c53055_524	A	G	G	Tommi
Excalibur_c46904_84	failed	T	T	Unknown
BS00108793_51	T	T	T	Mono
TA003860-0782	G	A	A	Tommi
IAAV9104	C	A	A	Tommi
wsnp_Ex_c410_810250	C	A	A	Tommi

Placing Bt13 below Excalibur_c53055_524 at 5,167,956 bp (TraitGenetics position)

Bt13

- ZaThul LS212 / ZaThul LS 213 are exceptions



	Thule III	ZaThul LS212	Zarya	
tplb0057f21_914	G	G	G	Mono
Ku_c20100_1746	C	C	C	Mono
wsnp_Ex_c43009_49439922	T	T	T	Mono
D_contig08599_465	T	T	T	Mono
Kukri_c58589_402	C	C	C	Mono
Tdurum_contig75811_1629	T	T	T	Mono
Tdurum_contig52015_870	C	C	C	Mono
Tdurum_contig81608_63	C	C	A	Thule
Kukri_c42622_417	C	C	C	Mono
RAC875_c63420_397	G	A	A	Zarya
TA004020-0357	G	G	G	Mono
Excalibur_c56798_364	C	C	C	Mono
CAP8_c710_140	C	A	A	Zarya
Tdurum_contig46583_2146	C	T	T	Zarya

If marker positions are to be trusted we see a recombination event and this places Bt13 below RAC875_c63420_397 at 4631914..4632014 bp in JBrowse - RefSeq 1.0 position

Trusting one marker to detect a recombination event is risky

Lines from crosses with good marker contrast in the interval 0 – 10 Mbp are needed

Bt13

tplb0057f21_914	G
Ku_c20100_1746	C
wsnp_Ex_c43009_49439922	T
D_contig08599_465	T
Kukri_c58589_402	C
Tdurum_contig75811_1629	T
Tdurum_contig52015_870	C
Tdurum_contig81608_63	C
Kukri_c42622_417	C
RAC875_c63420_397	G

Marker highlighted in pink define the interval upper bound.
Markers in green define the Bt13 haplotype.

These markers are not effective for MAS due to a high number of false positives (in our population)

Markers in the Bt12 interval are more effective as this haplotype is rare, but they are less tightly linked to the gene (wrong interval)

References

Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter wheat Landraces in the USDA-ARS National Small Grains Collection.
Blair J. Goates* and Harold E. Bockelman.

Mapping Common Bunt Resistance Gene Bt13. Dennis Kjær Christensen, Anders Borgen, Gunter Backes, Carl Vollenweider and Karl-Josef Müller. 2022.

Bt14

- The Durum wheat Doubbi is the differential line
- The line 186/ PI 172201 is a cross between Doubbi and the hexaploid wheat variety Ranee
- Erythrospermum 5221 is claimed to have Bt14, but it is unclear whether it actually has the same gene as Doubbie. Let's call it Bt14b
- Judged from the pedigree it appears to have inherited its resistance from Secale Cereale

References

Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter wheat Landraces in the USDA-ARS National Small Grains Collection. Blair J. Goates* and Harold E. Bockelman.

Bt15

- The Durum spring wheat Carleton is the differential line.
- One of the Carleton parents is Vernal Emmer
- The parents of Hope is Vernal Emmer and Marquis. Marquis does not have Bt15.
- Is Vernal Emmer the donor of Bt15 in Carleton and does Hope have Bt15?

References

Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter wheat Landraces in the USDA-ARS National Small Grains Collection. Blair J. Goates* and Harold E. Bockelman.

BtP

- Added to the differential set by Blair Goates

References

Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter wheat Landraces in the USDA-ARS National Small Grains Collection. Blair J. Goates* and Harold E. Bockelman.

BtZ

- Is not part of the differential set
- Commonly used source is Zarya
- Its orginal source is PPG 599 which is a Hybrid between a hexaploid wheat / wheatgrass
- Phenotyping cannot distinguish Bt10 and BtZ
- No GWAS signal yet, but plenty of phenotyped material

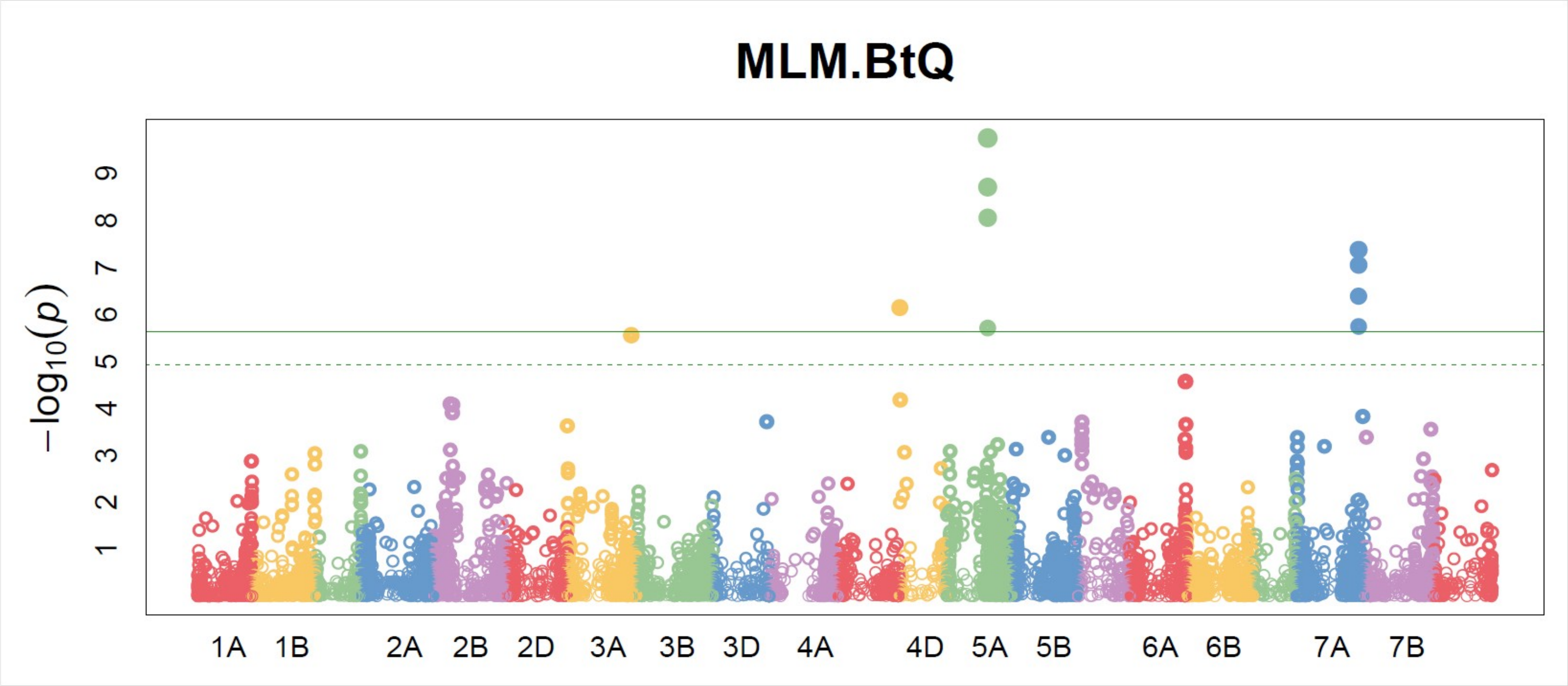
References

Scientific breeding of winter bread wheat in the Non-Chernozem zone of Russia: the history, methods and results. B I Sandukhadze, R Z Mamedov, M S Krakhmalyova, V V Bugrova. 2021

BtQ

- BtQ is not a recognized gene. It is Quebon resistance.
- Lines from a Segor and Quebon were phenotyped to have Bt2, Bt5 and Bt7 or combinations of this
- VrG can infect Bt2 and Bt5 lines.
- BtQ can have low infection levels with VrG
- Bt5 markers match in all BtQ postulated lines.
- A part of Quebon resistance is Bt5

BtQ



Bt5 lines present in GWAS control group -> no signal for Bt5

Two signals and a few markers to be investigated

BtQ

SNP	Chromosome	Position	FDR_Adjusted_P-values
Tdurum_contig102312_245	5A	444891150	3,78661E-06
BS00005311_51	5A	444596309	2,09939E-05
BS00022003_51	5A	444849939	6,28659E-05
JD_c149_1700	7A	670768834	0,000227052
Kukri_c24408_743	7A	670765573	0,000383043
RAC875_c37085_317	7A	670245194	0,001473717
RAC875_rep_c76650_164	4D	1243635	0,002213336
BobWhite_c1201_384	7A	670780740	0,004671187
wsnp_Ex_c43578_49857984	5A	444943632	0,004671187
BS00067015_51	3A	694977066	0,005977334

But where are the markers?

	TG		**RefSeq 1.0**		**RefSeq 2.1**		**Jbrowse RefSeq 1.0**	
BS00067015_51	3A	694977066	3D	558566197	3B	756435306	3A/3B/3D	
RAC875_rep_c76650_164	4D	1243635	4D	1243612			4D	1243562..1243662
BS00005311_51	5A	444596309	Un		Un			
BS00022003_51	5A	444849939					5A/5B/5D	
Tdurum_contig102312_245	5A	444891150	5A	444891174	5D	349692123	5A/5D	
wsnp_Ex_c43578_49857984	5A	444943632	5A	444943584			5A	444943484..444943684
RAC875_c37085_317	7A	670245194	7A	670245171	7A	674243512	7A	670245121..670245221
Kukri_c24408_743	7A	670765573	7A	670765552	7B	599850061	7A/7B/7D	
JD_c149_1700	7A	670768834	7A	670768858	7A	675408761	7A/7A	
BobWhite_c1201_384	7A	670780740	7A	670780764	7A	675374602	7A	670780714..670780814

The 7A signal is the most trustworthy, but 5A should be investigated also.

Detailed analysis is postponed until further data is available

Overview (Not fully updated)

		Interval				MAS	
Gene	Chromosome	Min [bp]	Max [bp]	Size [bp]	Quality of Mapping	False Negatives [%]	False Positives [%]
Bt1	2B	755889858	772760826	16870968	Poor	NA	NA
Bt2	Unknown	Unknown	Unknown	Unknown	NA	Unknown	Unknown
Bt3	1A	498451021	506854738	8403717	Medium	Unknown	Unknown
Bt4	1B	Unknown	Unknown	Unknown	NA	Unknown	Unknown
Bt5	1B	92540335	319569717	227029382	Medium	Unknown	Unknown
Bt6	1B	9555825	32766608	23210783	Good	Unknown	Unknown
Bt7	2D	621068156	624830049	3761893	Good	Unknown	Unknown
Bt8	Unknown	Unknown	Unknown	Unknown	NA	Unknown	Unknown
Bt9	6D	490337515	491431473	1093958	Good	Unknown	Unknown
Bt10	6D	0	Unknown	Unknown	Unknown	Unknown	Unknown
Bt11	3B	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Bt12	7D	5198984	11021649	5822665	High	Unknown	Unknown
Bt13	7D	0	4631914	4631914	Medium	Unknown	Many
Bt14	Unknown	Unknown	Unknown	Unknown	NA	Unknown	Unknown
Bt15	Unknown	Unknown	Unknown	Unknown	NA	NA	NA
Btp	Unknown	Unknown	Unknown	Unknown	NA	NA	NA
BtZ	Unknown	Unknown	Unknown	Unknown	NA	NA	NA
BtQ_5A	5A	Unknown	Unknown	Unknown	Good	Unknown	Unknown
BtQ_7A	7A	671343067	676631073	5288006	Medium	Unknown	Unknown

Thanks

- Anders Borgen for many things
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- Jianli Chen for information exchange
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