Genetic Mapping of Common Bunt Resistance Gene Bt10

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Abstract

Common bunt is primarily a seed borne disease of wheat. Plant resistance is an important tool to minimize risk of infection in organic farming and could also help reduce the use of seed treatments in conventional farming. Genetic markers are very valuable when breeding new resistant varieties.

Bt10 was identified in the Greek landrace Greece 18 / PI 116301 and in Mocho / PI 116306 (Metzger and Silbaugh 1971). The Bt10 differential line R63-6982 / PI 554118 is a selection from the cross Elgin / PI 178383 (Goates 2012).

Metzger *et al* (1962) established that 6256 / PI 178383 have Bt10 (+ Bt8 and Bt9 and some unknown minor gene) and this line has been a much used donor of Bt10 in breeding programs around the world. In Europe, Bt10 confers good resistance but must be paired with other genes for total immunity, but complete immunity to all known European virulence races will be achieved if Bt10 is combined with Bt1, Bt2, Bt3, Bt5, Bt7 or Bt8 (Borgen et al 2023).

Bt10 has been mapped to 6DS, and a PCR marker is available for use in marker assisted selection. This marker is estimated to be located 1 - 5.5 cM from Bt10 (Laroche *et al* 2000, Menzies et al 2006).

NordGen has 6 genebank accessions developed by MacKay by crossing the variety Starke-II with bunt resistant lines, and backcrossed to Starke-II about 7-8 times while maintaining resistance. The precise protocol is unfortunately lost. The NILs possesses Bt1(NGB-11503), Bt5(NGB-16106), Bt6 (NGB-11504), Bt9 (NGB-11505), Bt10 (NGB-11506) and Bt12 (NGB-16160). The accessions have already been phenotyped, and resistant lines from each accession have been selected (Borgen *et al*. 2018A). In the LIVESEED project, all NILs and Starke II have been genotyped with the TG25K array (Bacanovic-Sisic *et al* 2021).

Our mapping population contains 31 lines from a cross between Weston (Bt7+Bt10) and Xenos (Bt7). Phenotyping with 8 virulence races enables detection of all four combinations of Bt7 and Bt10 in lines from the Xenos x Weston cross.

Table 1: Theoretical infection patterns for lines having Bt0, Bt7, Bt10 and Bt7+Bt10

	Vr-0	Vr-5	Vr-DOT	Vr-3	Vr-2	Vr10	Vr-13	VrZ
Bt0	Bt0	Bt0	Bt0	Bt0	Bt0	Bt0	Bt0	Bt0
Bt7	0,0	Bt7	Bt7	0,0	Bt7	Bt7	0,0	0,0
Bt10	0,0	0,0	0,0	0,0	0,0	Bt10	0,0	Bt10
Bt7+Bt10	0,0	0,0	0,0	0,0	0,0	Bt7+Bt10	0,0	0,0

A red cell means that high infection levels are expected, yellow means low or intermediate infection levels expected and green means no infection expected.

Table 2: Actual infection patterns for a line having Bt7 and one having Bt7+Bt10

	Vr-0	Vr-5	Vr-DOT	Vr-3	Vr-2	Vr10	Vr-13	VrZ
XeWes7D	0,0	50,0	40,0	0,0	37,5	80,0	0,0	0,0
XeWes21	0,0	0,0	0,0	0,0	0,0	75,0	0,0	0,0

Six lines from the Weston x Xenos RIL population not having the expected parents and one being heterozygous at 6DS were excluded, leaving 24 for detailed analysis of recombination events at 6DS

Markers having a unique physical position in a BLAST against RefSeq 2.1 after filtering out alignments with mismatch > 1 in the 6D interval 0 – 25Mbp were used for a detailed analysis.

Table 3: XeWes5A inheritance pattern

	Weston	XeWes5A	Xenos	
TA002853-0110-w	A	A	A	Mono
Kukri_c55362_75	A	A	C	Weston
AX-108746724	C	C	C	Mono
Excalibur_c7731_2743	A	A	A	Mono
AX-158531240	C	C	C	Mono
BS00011513_51	A	A	failed	Unknown
AX-95159175	G	A	A	Xenos
BS00065960_51	C	C	C	Mono
AX-94880114	G	G	G	Mono
Kukri_c73802_205	G	G	G	Mono
RFL_Contig2163_1769	C	C	C	Mono
RFL_Contig2163_1080	C	C	C	Mono
BobWhite_rep_c52808_186	T	T	T	Mono
RAC875_rep_c109653_409	A	A	A	Mono
AX-94433248	T	G	G	Xenos
RAC875_rep_c85994_258	M	C	A	Unknown
RAC875_c68978_220	C	C	C	Mono
TA005787-0140	T	T	T	Mono
AX-158531809	C	C	C	Mono
wsnp_Ku_c2637_5009091	C	C	C	Mono
TG0135	T	T	T	Mono
TGWA25K-TG0135	T	T	T	Mono
BobWhite_c11808_975	A	A	A	Mono
RFL_Contig5885_435	G	G	G	Mono
AX-94570446	G	G	G	Mono
AX-94573105	A	A	A	Mono
AX-94647124	G	G	G	Mono
IAAV2577	C	C	C	Mono
Excalibur_c24288_548	T	C	C	Xenos
TA001144-0714	T	C	C	Xenos

Table 4: XeWes7A-A inheritance pattern

	Weston	XeWes7A-A	Xenos	
TA002853-0110-w	A	A	A	Mono
Kukri_c55362_75	A	C	C	Xenos
AX-108746724	C	C	C	Mono
Excalibur_c7731_2743	A	A	A	Mono
AX-158531240	C	C	C	Mono
BS00011513_51	A	A	failed	Unknown
AX-95159175	G	G	A	Weston
BS00065960_51	C	C	C	Mono
AX-94880114	G	G	G	Mono
Kukri_c73802_205	G	G	G	Mono
RFL_Contig2163_1769	C	C	C	Mono
RFL_Contig2163_1080	C	C	C	Mono
BobWhite_rep_c52808_186	T	T	T	Mono
RAC875_rep_c109653_409	A	A	A	Mono
AX-94433248	T	T	G	Weston
RAC875_rep_c85994_258	M	C	A	Unknown
RAC875_c68978_220	C	C	C	Mono
TA005787-0140	T	T	T	Mono
AX-158531809	C	C	C	Mono
wsnp_Ku_c2637_5009091	C	C	C	Mono
TG0135	T	T	T	Mono
TGWA25K-TG0135	T	T	T	Mono
BobWhite_c11808_975	A	A	A	Mono
RFL_Contig5885_435	G	G	G	Mono
AX-94570446	G	G	G	Mono
AX-94573105	A	A	A	Mono
AX-94647124	G	G	G	Mono
IAAV2577	C	C	C	Mono
Excalibur_c24288_548	T	T	C	Weston
TA001144-0714	T	T	C	Weston

Four different haplotypes were present in the investigated interval: The Weston haplotype, the Xenos haplotype and two representing recombined haplotypes. The recombined haplotype represented by XeWes7A-A was postulated to have Bt7 and not Bt10 and the haplotype represented by XeWes5A was postulated to have Bt7+Bt10.

Weston and Xenos are monomorphic for most markers at 6DS and we therefore get no information for large intervals. Assuming one recombination event per line located between markers Kukri_c55362_75 and AX-95159175 we get the candidate interval 0 - AX-95159175 (0 – 4,108,252 bp). XeWes7A-A is postulated to not having Bt10 and it has inherited from Xenos in the interval. For XeWes5A and the remaining lines postulated to have Bt10, we see that they as expected have inherited from Weston in the candidate interval. These conclusions rest on the assumption that the physical position for Kukri_c55362_75 is in that interval. The BLAST for Kukri_c55362_75 gives three hits at 6D, 6A and 3B with 1, 3 and 6 mismatches. Linkage analysis strongly indicates 6D as the correct position.

The Starke II Bt10 NIL (NGB 11506) has Selection M66-23 as Bt10 donor. Selection M66-23 is from a PI 178383 x Elgin cross, but it has not been genotyped. In the hope that the NIL has inherited from 6256/PI 178383 via Selection M66-23 in the interval we investigate, 6256/PI 178383 is used as a stand-in for Selection M66-23 for a detailed analysis of recombination events. Starke II and 6256/PI 178383 are also monomorphic in intervals too large to be ignored, but the 0 – 3,642,156 bp interval seems plausible.

Table 5: Starke II Bt10 NIL inheritance pattern

	6256	Starke NIL Bt10	Starke II NGB-22	
Excalibur_c4789_2748	A	G	G	Starke II NGB-22
wsnp_Ex_c18664_27540364	G	G	A	6256
Excalibur_c10358_1800	G	G	G	Mono
GENE-3775_326	T	T	T	Mono
RAC875_c7178_404	C	C	C	Mono
wsnp_Ku_c19587_29102203	G	G	A	6256
CAP7_c1208_150	T	T	T	Mono
wsnp_Ex_c14439_22426200	C	C	T	6256
TA002853-0110-w	A	A	A	Mono
Kukri_c55362_75	A	A	C	6256
AX-108746724	C	C	C	Mono
Excalibur_c7731_2743	A	A	G	6256
AX-158531240	C	C	T	6256
BS00011513_51	G	A	A	Starke II NGB-22
AX-95159175	A	G	G	Starke II NGB-22
BS00065960_51	C	C	C	Mono
AX-94880114	A	A	A	Mono
Kukri_c73802_205	G	G	G	Mono
RFL_Contig2163_1769	C	C	C	Mono
RFL_Contig2163_1080	C	C	C	Mono
BobWhite_rep_c52808_186	T	T	T	Mono
RAC875_rep_c109653_409	A	A	A	Mono
AX-94433248	G	T	T	Starke II NGB-22
RAC875_rep_c85994_258	C	C	C	Mono
RAC875_c68978_220	C	T	T	Starke II NGB-22
TA005787-0140	T	C	C	Starke II NGB-22

The marker Excalibur_c4789_2748 is most likely located at 6D 1,405,354 bp by BLAST, but 6B is also possible. From linkage analysis, it appears to be at 6D. If it is at 6D, the interval will be 1,405,354 – 3,642,156 bp based on Starke II NIL analysis.

The marker GENE-3775_326 at 1,769,916 bp has both C and T allels in Bt10 postulated lines. This could be because it is misplaced, or because it is outside the interval. Based on BLAST and linkage analysis, it seems to be correctly placed and most likely the candidate interval is 1,769,916 - 3,642,156 bp, but due to lack of marker polymorphism, it is hard to give a definite answer.

Table 1: Markers usable for Bt10 MAS

GENE-3775_326	T
RAC875_c7178_404	C
wsnp_Ku_c19587_29102203	G
CAP7_c1208_150	T
wsnp_Ex_c14439_22426200	C
TA002853-0110-w	A
Kukri_c55362_75	A
AX-108746724	C
Excalibur_c7731_2743	A
AX-158531240	C
BS00011513_51	A

The nine markers in green can be used to track the presence of Bt10. The two in brown define the interval.

The markers has been tested in a mapping population with 1192 lines with phenotypic and genotypic data available. 38 of these lines contained Bt10 (Borgen *et al* 2018B, Borgen and Christensen 2023). The hit rate for MAS markers in this mapping population is 86%. The five lines AC Taber, M83-1621, H86-706, Ark and PI 554113 are postulated to have Bt10, but markers do not match in them. The reasons why are currently unknown. False positive rate is 10% in the mapping population.

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