Annotation of differential lines used for resistance trials for common bunt

Anders Borgen[1], Magdalena Lunzer[2] and Dennis Kjær Christensen[3]

[1] Agrologica, Houvej 55, DK-9550 Mariager
[2] Institute of Biotechnology in Plant Production, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Straße 20, 3430 Tulln, Austria
[3] Private, Gerding, DK-5020 Skørping

Corresponding author: Dennis Kjær Christensenn
E-mail: dennis@fastcode.dk

Abstract

Based on many years of research in resistance on common bunt (*Tilletia caries* (syn *T.tritici* and *T.leavis* (syn. *T.foetida*)) and dwarf bunt (*T.contraversa* (syn. *T.controversa*), Blair Goates (2012) proposed a differential set of wheat varieties to be used as standards defining the resistance genes Bt1-15 plus BtP. These lines have been used world wide in field trials and for genomic analysis.

New technologies and further research have resulted in new knowledge about some of the lines, and additional resistance genes have been discovered. We therefore argue for a revision of the lines used as the differential set.

Genetic analyses of the differential lines and of crosses with lines having Bt-resistance genes have revealed that some of the differential lines have actually not only a single gene, but multiple resistance factors.

Status of the current knowledge about the Bt-genes and the differential lines:

- **Bt1:** PI554101 is the proposed line by Goates (2012). Genetic analysis has identified the gene at chromosome 2B at the position 755.889.858-772.760.826 bp. (Christensen and Borgen 2023A). Several lines have been well tested for having Bt1 without other Bt-genes and can be used as alternatives, including Starke II Bt1 NIL (selection in NGB11503), 'Albit' (CItr 8275), and 'PG3540' (Agrologica breeding line). For spring wheat trials, 'M83-1531' (PI 554108) can be used.
- **Bt2: '**Selection 2075' (PI 554103) is the proposed differential line by Goates (2012). The gene in PI 554103 is found at chromosome 1D (Unpublished results from BOOST and DIVERSILIENCE). Several varieties behave in field trials as having Bt2 including commercial varieties like Hereward and Bussard, but GWAS analysis indicate that the resistance gene is located at different positions and even at different chromosomes in different varieties, such as Hereward: 41.460.409-70.406.862 bp and Bussard: 278.264.436- 312.620.781 bp (Unpublished results from BOOST and DIVERSILIENCE). Another resistance factor present in the variety 'Quebon' is found at chromosome 7A in the position 674.243.562-675.374.652 bp may be phenotypically associated with Bt2. We believe that it is indeed the same gene with two or three different positions in the genomes. For spring wheat trials, 'M83-1541' (PI554096) can be used.
- **Bt3:** Ridit (CItr 6703) is the proposed differential line by Goates (2012). Genetic analysis has identified the gene at chromosome 1A at the position 498.451.021-506.854.738 bp (Unpublished results from BOOST and DIVERSILIENCE). 'M85-9' (PI 554121) can be used as alternatives for Bt3. For spring wheat trials, M83-1551 (PI 554116) can be used.
- **Bt4:** 'CI 1558' (PI 11610) was proposed as differential line for Bt4 by Goates (2012). We have found Bt4 associated with chromosome 1B at the position 21.384.123-28.019.546 bp. Goates

(2012) analysed the phenotypic reaction of 73 races of common bunt and dwarfbunt, but was unable to separate Bt4 from Bt6. Borgen *et al* (2023) tested 44 European races of common bunt of which only a few indicate a different reaction between Bt4 and Bt6. Both genes are found on the same chromosome, but at two different positions at chromosome 1B. This may lead to debate if there is indeed one or two genes, or maybe copies of the same gene at two different positions at 1B. For spring wheat trials, 'M81-152' (PI 554115) and 'Prins NIL-Bt4' are expected to be a good lines for Bt4.

- **Bt5:** Hohenheimer is the proposed differential line by Goates (2012). We have found Bt5 associated with chromosome 1B at the position 123.383.762-265.108.595 bp (Unpublished results from BOOST and DIVERSILIENCE), but several references indicate that Hohenheimer has not only Bt5, but also another factor at chromosome 1B (Kanbertay 1982). This is confirmed by phenotypic differences between 'Hohenheimer' and a selected NIL having only Bt5 (Borgen *et al* 2023). Therefore, 'Hohenheimer' is not an ideal differential line for Bt5. Better alternatives are 'Starke II NIL-Bt5' (selection in NGB16106), 'Promesse' (PI 339856), 'M86-65' (PI 554104) or 'Tommi'. For spring wheat trials, 'SegQue-L69' (Breeding line from Agrolgooica) can be used.
- **Bt6:** Rio (CI 10061) is proposed as differential line for Bt6 (Goates 2012). We have found Bt6 associated with chromosome 1B at the position 16.381.367-28.018.966 bp (Unpublished results from BOOST and DIVERSILIENCE). Please see above comments on Bt4. 'Starke II NIL-Bt6' (selection in NGB11504) can be used as alternatives to Rio for Bt6. For spring wheat trials, he lines 'M83-1581' (PI 554117) and can be used.
- **Bt7: '**Sel 50077' (PI 554100) is the proposed differential line by Goates (2012). We have found Bt7 associated with chromosome 2D at the position 621.068.156-624.830.049 bp (Christensen and Borgen 2023B). 'Tambor' can be used as alternative for Bt7. For spring wheat trials, 'M83-1591' (PI554114), 'Fiorina' or 'Quarna' can be used.
- **Bt8:** 'M72-1250' (PI 554120) is the proposed differential line by Goates (2012). Despite being used in several breeding programs, Bt8 has not been mapped yet. 'M83-1601' (PI 554111) can be used as alternatives for Bt8. For spring wheat trials, 'M78-9496' (PI 554110) and 'M83-1601' (PI 554111) can be used.
- **Bt9:** 'R63-6968' (PI 554099) is the proposed differential line by Goates (2012). Bt9 was first found at 6D by Stephan *et al* (2017). We have confirmed Bt9 associated with chromosome 6D at the position 487.432.997-490.336.412 bp (Christensen and Borgen 2023C). 'Starke II NIL-Bt9' (selection in NGB11505) can be used as alternatives for Bt9. For spring wheat trials, M77-1140 (PI 554112) and can be used.
- **Bt10:** 'R63-6982' (PI 554118) is the proposed differential line by Goates (2012). Bt10 was first associated with a position at chromosome 6D by Laroche *et al* (2000). We have confirmed the association with 6D at the position 1.769.916-3.642.206 bp (Christensen and Borgen 2023D). 'Starke II NIL-Bt10' (selection in NGB11506), 'M83-1621' (PI 554109), Tillexus and Tillstop can be used as alternatives for Bt10. For spring wheat trials, M83-1621 (PI 554109) and 'H86-706' (PI 542432), can be used.
- **Bt11:** PI 554119 is the proposed differential line by Goates (2012). Genetic analyses of PI 554119 have shown resistance factors at the distal end of chromosome 6D (Lunzer 2023), but also at 3B (Ciuca et al). PI 554119 also has the resistance conferring alleles for markers indicating Bt7 (unpublished results from BOOST and DIVERSILIENCE). The position of the factor at chromosome 3B is at 498.268.609-523.277.044 bp (unpublished results from BOOST and DIVERSILIENCE). Hence, it is still uncertain if PI 554119 has Bt7 and Bt9 plus Bt11, or if Bt11 is in fact a mixture of two genes different from other known Bt-genes. In any case, PI 554119 is not a good differential lines for the isolated Bt11 gene. Further studies are needed to isolate the gene in a differential line.
- **Bt12:** is the proposed differential line by Goates (2012). Bt12 was first found at chromosome 7D by Müllner *et al* (2017) and the interval of markers associated with the gene has afterwards been reduced to 6.820.874-11.141.495 bp (unpublished results from BOOST and

DIVERSILIENCE). The line PI 119333 has very poor agronomic traits including susceptibility to lodging, and it has also another resistance gene at chromosome 4A (unpublished results from BOOST and DIVERSILIENCE). This line is therefore not a good differential lines for the isolated Bt12 gene. It is still uncertain if Bt12 has been isolated in 'Starke II NIL-Bt12' (selection in NGB16160) or other lines without the factor at 4A.

- **Bt13:** 'Thule-III' (PI 181463) is the proposed differential line by Goates (2012). We have found Bt13 associated with chromosome 6D at the position 6,820,874 – 11,141,495 bp (Christensen and Borgen 2023E). Thule-III has very poor agronomic traits including susceptibility to lodging. For spring wheat trials, 'SegThCia-2' (breeding line from Agrologica) can be used.
- **Bt14:** 'Doubbi' (CI 13711) is the proposed differential line by Goates (2012). Doubbi is a durum wheat, but also the hexaploid line PI 172201 is believes to have Bt14 resistance. Little is known about this gene.
- **Bt15:** 'Carleton' (CI 12064) is the proposed differential line by Goates (2012). Little is known about this gene.
- **BtP:** PI 173437 is the proposed differential line by Goates (2012). PI 173437 has very poor argronomic traits, including susceptibility to lodging. Little is known about this gene.
- **BtZ:** The gene is not among the genes mentioned by Goates (2012), but is a translocation from *Thinopyrum intermedium* via the line 'Hybrid 599'. The cultivar 'Zarya' has 'Hybrid 599' in its pedigree and is the main source of BtZ in European breeding material (Sandukhadze *et al* 2021). We have found BtZ associated with chromosome 3B at the position 3.444.603-4.572.453 bp (Christensen and Borgen 2023F). We propose the variety 'Tilliko' as a differential line for BtZ.
- **Bt-Trintella:** The gene is not among the genes mentioned by Goates (2012) but has been investigated by Dumalasova *et al* (2012) associating the causal gene at 1B near to the centromere and closest to marker Xgwm273 on the short arm. Additionally, in 2008, smaller QTL effects were ascribed to chromosomes 7A and 7B, and another smaller QTL effect to chromosome 5B in 2009 only. Later phenotypic and genotypic studies have not confirmed this association using SNP markers (Unpublished results from BOOST and DIVERSILIENCE)).
- **Bt-Erythrospermum-5221:** The gene is not among the genes mentioned by Goates (2012), but is a translocation from *Agropyrum* (Babayants *et al* 2006).

Conclusion

- We can confirm that the current use of the differential lines for Bt1, Bt3, Bt4, Bt6, Bt7, Bt9, Bt10 and Bt13 does indeed represent the genes in question, but we have proposed alternative with improved agronomic traits, including lines without vernalisation requirement for spring sowing.
- The differential lines for Bt2 does represent Bt2, but it should be noted that the gene may be positioned at different locations in different varieties having Bt2. For genetic analysis, different varieties should be used to represent the different positions.
- The differential line for Bt5, 'Hohenheimer', should be exchanged with 'Starke II NIL-Bt5' or another lines having only Bt5.
- The differential lines for Bt11 and Bt12 are believed to have more than one resistance gene each. Work is ongoing to identify lines with only a single resistance gene that can be used to replace them.
- Too little is known about Bt8, Bt14, Bt15, BtP, Trintella-resistance and Erythrospermum-5221 to evaluate the lines representing the resistance genes.

Keywords

winter wheat, common bunt, variety trial

Acknowledgments

Results are compiled from trials made in the BOOST projekt funded by Organic RDD and DIVERSILIENCE funded by CoreOrganic Cofund and ECOBREED project funded by HORIZON2020.

References

Borgen, A., M.K. Forster, S.Sedaghatjoo, D.K. Christensenn, W. Maier 2023. Determination of virulence of European races of common bunt using a differential set of wheat cultivars. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023A. Genetic mapping of Common Bunt Resistance Gene Bt1. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023B. Genetic mapping of Common Bunt Resistance Gene Bt7. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023C. Genetic mapping of Common Bunt Resistance Gene Bt9. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023D. Genetic mapping of Common Bunt Resistance Gene Bt10. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023E. Genetic mapping of Common Bunt Resistance Gene Bt13. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Christensen, D and A. Borgen 2023F. Genetic mapping of Common Bunt Resistance Gene BtZ. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

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

Goates, B., 2012: Identification of New Pathogenic Races of Common Bunt and Dwarf Bunt Fungi, and Evaluation of Known Races Using an Expanded Set of Differential Wheat Lines. Plant Dis. **96**(3):361-369. doi: 10.1094/PDIS-04-11-0339.

Babayants,L.T. , O.V. Babayants, V.L. Baranovskaya and L.A. Dubinina 2006. Tilletia caries and Resistance of Wheat to this Pathogen in Ukraine Proc. XVth Biennial Workshop on the Smut Fungi, Prague, June 11–14, 2006 33 / Czech J. Genet. Plant Breed., 42, 2006 (Special Issue)

Dumalasová, V., J.R. Simmonds, P. Bartoš, J. Snape 2012. Location of genes for common bunt resistance in the European winter wheat cv. Trintella. Euphytica 186(1). DOI: 10.1007/s10681-012-0671-7

Kanbertay, M. 1982: Inheritance of resistance to common bunt races T-23 and L-16 and association of bunt resistance with selected agronomic characterizing a hybrid og Hohenheimer x Stephan wheat. MSc Thesis, Oregon State University.

Laroche, A. T.Demeke, D.A. Gaudet, B.Puchalski, M.Frick, and R. McKenzie 2000. Development of a PCR marker for rapid identification of the Bt-10 gene for common bunt resistance in wheat. Genome Vol. 43.

Lunzer, M. 2023. Wheat chromosome 6D harbours a major locus for common bunt resistance present in the Bt11 bunt differential. Proceedings of the XXII International Workshop on Bunt and Smut Diseases. BOKU, Austria.

Sandukhadze, B.I., R.Z. Mamedov, M.S. Krakhmalyova, and V.V. Bugrova, 2021. Scientific breeding of winter bread wheat in the Non-Chernozem zone of Russia: the history, methods and results. Vavilovskii Zhurnal Genet Selektsii. 2021 Jul; **25**(4): 367–373. doi: 10.18699/VJ21.53-o

Steffan, P.M, A.M. Torp, A. Borgen, G. Backes, S.K. Rasmussen 2017. Mapping of common bunt resistance gene Bt9 in wheat. Theoretical and Applied Genetics 130(5). DOI: 10.1007/s00122-017-2868-6