Book of Abstracts

XXII International Workshop on Bunt and Smut Diseases

June 13-15, 2023
BOKU Campus TULLN
hybrid event
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XXII International Workshop on Bunt and Smut Diseases

13th – 15th June 2023

Tulln an der Donau, Austria

Edited by:
Hermann Buerstmayr
Magdalena Lunzer

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June 13-15, 2023, BOKU Campus TULLN, Austria
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Organized and hosted by
University of Natural Resources and Life Sciences, Vienna
Institute of Biotechnology in Plant Production & Institute of Plant Breeding
Konrad Lorenz Str. 20, 3430 Tulln, Austria

Co-organized by the H2020 funded project **ECOBREED**

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Many thanks to Richard Buerstmayr for the technical editing of this book.
Many thanks to Maximilian Mehofer and Daniel Fritz for technical support of the workshop.

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Welcome from the Organizers

With great humility and pleasure my team and I took over the responsibility to host the 22nd International Workshop on Bunt and Smut Diseases for the first time in Austria. The 22nd International Workshop on Bunt and Smut Diseases takes place 47 years after the first Workshop on Bunt and Smut Diseases convened during the Pacific Division AAAS meeting in Missoula, Montana in 1976, and has traditionally been held in 2 year intervals.

The workshops on smut and bunt diseases of crop plants have provided opportunities to exchange news, ideas and information and enable networking. The workshop gathers public and private sector researchers, plant-pathologists, geneticists, breeders, extension specialists, farmers and users of cereal products to discuss new findings on bunt and smut diseases of cereals and thus contribute to sustainable control and prevention of these bothersome fungal diseases. Bunt diseases are particularly devastating in organic farming, therefore bunt control for organic cereal production will be emphasized.

Integrated pest management is key to sustainable control of bunt and smut diseases. This means further innovations on cultural, biological and chemical control of bunt diseases are needed together with breeding for durable and efficient resistance.

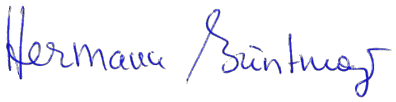
Bunt and smut diseases of cereals have been among the most feared cereal diseases until mid of the last century, but have thereafter been almost forgotten in many cereal growing regions since efficient fungicide seed treatments became available. However, various reasons, including increased popularity of organic farming and low input agriculture, has pushed theses disease back to the priority list. This is also reflected in increased scientific activity, leading to much better knowledge on the genetics of resistance thus enabling targeted and fast-track resistance breeding as well as innovations in cultural and biological control of bunts and smuts.

Resistant cultivars are a key component of integrated disease control. New developments and innovations will deepen our understanding of these important crop diseases and will find their way into knowledge based crop improvement. This requires constant communication between the research and the breeding community. The difficult and complex task to implement innovations in their day to day breeding work remains with the practical breeders. Innovations in technology can only be as good as the germplasm available. Germplasm evaluation and searching for useful alleles in the wheat gene pool, including wild relatives, genebank accessions, and mutant populations will be a major endeavor for future cultivar improvement. Access to germplasm and open germplasm exchange among researchers and breeders must be maintained and stimulated, in order to allow for long term mutual progress in resistance breeding.

It is you who make this workshop a successful and memorable one. My team and I welcome you and thank you for attending this event, either on site or follow the workshop virtually via the life webinar.

Let me thank the local organizing committee, in particular Susanne Weber and Magdalena Lunzer, who contributed to all organizational work.

My sincere gratitude goes to all members of the international organizing committee, who were responsive, supportive and encouraging. Without sponsoring and industry support this Symposium could not be accomplished. Therefore, my sincere appreciation to all supporters.



Hermann Buerstmayr; June 13, 2023; Tulln, Austria
Head of the Local and the International Organizing Committee

Program

Monday, 12 June 2023		
17:00	**Welcome Meet and Greet** (17:00-19:00)	
	Address: Konrad Lorenz Str. 24, 3430 Tulln, Universitäts- und Forschungszentrum Tulln.	
Tuesday, 13 June 2023		
09:00	*Registration Desk Opens*	
10:00	Guided Tour of Plant Breeding Research at BOKU Campus Tulln	
12:00	*Lunch Break*	
12:30	**Opening of the Workshop**	
13:00	**Session 1**	
13:00	Hole, D.	David's Adventures in *Tilletia*-land: with apologies to Lewis Carroll
13:50	Bengtsson, T.	The stinking comeback – measures to understand the cause of the re-emergence of common bunt in Swedish winter wheat?
14:15	Krause, W.	Genomic selection for dwarf bunt resistance in wheat
14:40	Lunzer , M.	Wheat (*Triticum aestivum*) chromosome 6D harbours a major QTL for common bunt resistance present in the Bt11 bunt differential
15:05	Joshi, P.	Assessment of Common Bunt and Dwarf Bunt Resistance in Bt-Differential Lines Grown in Diverse Environments
15:30	*Coffee Break*	
16:10	Borgen, A.	Determination of virulence of European races of common bunt using a differential set of wheat cultivars
16:20	Borgen, A.	Co-evolution of virulence and resistance in heterogeneous wheat populations
16:30	Borgen, A.	Annotation of differential lines used for resistance trials for common bunt
16:40	Borgen, A.	Gene postulation based on phenotyping wheat varieties with a differential set of virulence races of common bunt (*Tilletia caries*)
16:50	Borgen, A.	Registered varieties and Organic Heterogeneous Material (OHM) with resistance to common bunt in Europe
Wednesday, 14 June 2023		
09:00	**Session 2**	
09:00	Dhillon, G. S.	Candidate gene analysis for the 7DS QTL associated with dwarf bunt resistance of winter wheat using targeted capture sequencing technology
09:25	Dumalasová, V.	Reaction of wheat genotypes to Czech common bunt and dwarf bunt samples
09:50	Ciuca, M.	A wheat-rye translocation 1AL.1RS involved in wheat resistance to bunt
10:15	*Coffee Break*	
10:50	Lunzer, M.	How long does it take to develop high performing and common bunt resistant winter wheat lines using organics-compliant methods?

11:15	Fischbach, M.E.	Identification of novel seed treatments and adapted agronomic practices to control common bunt in organic wheat production
11:40	Ren, Z.	Microbiome Signature of Endophytes in Wheat Seed Response to Wheat Dwarf Bunt Caused by *Tilletia controversa* Kühn
12:05	*Lunch Break*	
13:50	***Recreation Tour Wachau Valley***, Bus departure in front of the University building	
19:30	***Workshop Dinner:*** Restaurant **Goldenes Schiff**, at Wiener Strasse 10, 3430 Tulln.	

Thursday, 15 June 2023

09:00	**Poster Session**	
	Christensen, D. K.	Genetic Mapping of Common Bunt Resistance Gene *Bt1*
	Christensen, D. K.	Genetic Mapping of Common Bunt Resistance Gene *Bt7*
	Christensen, D. K.	Genetic Mapping of Common Bunt Resistance Gene *Bt9*
	Christensen, D. K.	Genetic Mapping of Common Bunt Resistance Gene *Bt10*
	Christensen, D. K.	Preliminary Genetic Mapping of Common Bunt Resistance Gene *Bt13*
	Christensen, D. K.	Genetic Mapping of Common Bunt Resistance Gene *BtZ*
	Lunzer, M.	Genome-wide association mapping identifies common bunt resistance loci in a wheat diversity panel
	Rabl, J.	Variation in aggressiveness and virulence among eight common bunt sources collected in Austria
	Steiner, B.	Association genetics of common bunt resistance in *Aegilops tauschii* – preliminary results
	Plūme, S.	Loose smut resistant spring barley breeding for organic farming
	Dumalasová, V.	HealthyMinorCereals spelt diversity panel reaction to rusts, powdery mildew, leaf blotch and common bunt
10:00	*Coffee Break*	
10:30	**Breeders' Workshop: all participants**	Round table discussion on co-ordinated research ideas and efforts to control bunt and smut diseases
13:00	*Lunch Break*	
14:30	***Field Visit*** at BOKU Campus Tulln: Research on resistance to Fusarium head blight and common bunt of wheat	

Friday, 16 June 2023

19:00	**Jazz on Campus** - concert at BOKU Tulln Atrium - free admission

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David's Adventures in *Tilletia*-land: with apologies to Lewis Carroll

David Hole[1], Justin Clawson[1]

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Dwarf bunt, caused by *Tilletia controversa J.G. Kühn [as 'contraversa'], in Rabenhorst, Hedwigia 13: 188 (1874)*, TCK, can be a devastating disease of winter wheat when under certain environmental growing condition. Despite long soil teliospore viability and high levels of infection in certain years, the disease can be difficult to evaluate under field nursery conditions due to varying environmental conditions.

In the Intermountain Western USA, the Utah State University wheat-breeding program utilizes extensive testing for resistance to TCK. Every cultivar released undergoes evaluation for resistance each year beginning as F_5-derived head rows until final release, and typically testing continues throughout the life of the cultivar.

Since resistance to dwarf bunt typically confers resistance to common bunt with similar *avr* genes, the increase in organic wheat production in Utah has been well served by release of resistant cultivars. The TCK evaluation nursery in Logan Utah has been utilized to evaluate Midwestern regional nurseries, western regional nurseries, and international populations. In addition, other states, such as Idaho and some private breeders submit lines for testing. In some years, lines from the National Plant Germplasm Service in the USA have also been tested. During 30 years of breeding for resistance, changes made over time have managed to maintain reasonable levels of infection while reducing planting and inoculation labor and improving consistency in both. These changes as well as challenges and opportunities in breeding wheat will be presented.

Keywords

winter wheat, dwarf bunt, organic agriculture, resistance breeding

Acknowledgments

Utah Agricultural Experiment Station

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The stinking comeback – measures to understand the cause of the re-emergence of common bunt in Swedish winter wheat?

Therése Bengtsson[1], Fluturë Novakazi[1], Marwan Alamrani[1], Eva Edin[2], Björn Andersson[3], Tina Henriksson[4] & Anna Berlin[3]

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Common bunt (CB), or stinking smut, is a seed-borne disease caused by the two species *Tilletia tritici* and *Tilletia laevis*. CB occurs in all wheat (*Triticum aestivum L.*) producing regions around the world. For conventional production, efficient chemical seed treatments exist, but this is not an option in organic agriculture, which instead is dependent on the availability of resistant cultivars. Over the last few years, CB incidence has increased all over Europe. In 2020, 42% of seeds intended for certification in Sweden were infected with CB. There could be several reasons behind the increase e.g. decreasing number of available seed treatments, increased area of organic production, increased use of farm-saved seed or the evolvement of new or more virulent races.

The two species *Tilletia tritici* and *Tilletia laevis*, are genetically related and biologically similar with identical life cycles, and recent molecular studies even suggest a conspecific status of the two species (Forster et al. 2022). Thus, molecular differentiation has been shown to be difficult. Moreover, identification based on morphology alone is cumbersome and can be further complicated due to the hybridisation of the two species leading to a range of morphological variants. Thus, a molecular diagnosis method is urgently needed.

In 2022 a new project started which aims to understand the cause(s) of the observed increase of CB in Sweden by: 1) studying the occurrence and distribution of *Tilletia* lineages in Sweden; 2) studying their genetic variation and virulence; 3) identifying effective CB resistance genes to be targeted by the breeding program; and 4) developing species-specific markers for molecular identification.

In collaboration with farmers in Sweden, CB-infected wheat spikes were collected from farmer's fields located in Säffle, Havdhem and Visby during the summer of 2022, which complemented an existing collection of CB-infected spikes earlier collected from Svalöv (2020), Alnarp (2021) and Linköping (2021) (Figure 1). The collection will continue in 2023 to cover a wider geographical area. Bunts of the spikes collected from the different regions will be examined under the microscope to identify the causal species based on teliospore morphological features where teliospores of *T. tritici* and *T. laevis* have reticulate and smooth surfaces, respectively.

To confirm the species identification and to understand the distribution of the species complex, a set of Swedish reference isolates of *T. laevis* and *T. tritici* will first be de-novo sequenced. Next, DNA will be extracted from the teliospores of the collected *Tilletia* lineages and sent to the SciLifeLab in Uppsala, Sweden for Illumina sequencing. This data together with the previously published genomes of *T. laevis* and *T. tritici* (Nguyen et al. 2019) should enable reliable species determination and population genetic analysis to better understand the epidemiology of the disease.

The virulence spectrum of the collected *Tilletia* lineages will be tested on a panel consisting of accessions from a CB differential set (*Bt0* to *Bt15* and *BtP*) (Goates, 2012), each with one or more race-specific resistance genes (R-genes), and five Swedish winter wheat cultivars (resistant: Stava, Festival and Hallfreda; susceptible: Brons and Kranich) under greenhouse conditions. The resistance in the three resistant cultivars is based on the same combination of resistance genes, *Bt8*+*Bt9*. A small virulence test was carried out in 2021-2022, using the same wheat panel and *T. tritici* lineages from Svalöv, Alnarp and Linköping. The lineage from Linköping was shown to be the most aggressive of the three included and

virulent against *Bt7*, *Bt8*, *Bt10*, Bt13 and *Bt15*. Interestingly, Hallfreda considered to harbour *Bt8+Bt9* and tolerant to CB had infection rates ranging from 8 to 50%. Currently, the virulence test from 2021-2022 is repeated and expanded to also include *T. tritici* lineages collected in 2022 from two field sites in Visby and one in Säffle. The ongoing virulence test will provide further insights into the virulence of the Swedish lineages, and verify whether there are lineages virulent to the widely deployed combination of *Bt8+Bt9*.

Figure 1: Map showing the Swedish sampling field sites of *Tilletia tritici* lineages collected so far, where 1= Visby (5 fields), 2= Alnarp (1 field), 3= Linköping (1 field), 4= Svalöv (1 field), 5= Säffle (1 field), and 6= Havdhem (1 field). The collection continues in 2023. Map created at https://www.mapcustomizer.com/.

Genomic selection for dwarf bunt resistance in wheat

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Dwarf bunt (DB), caused by the pathogen *Tilletia controversa*, is a fungal disease that poses significant potential risk to winter wheat (*Triticum aestivum*) in the United States and abroad. It reduces grain yield and quality by turning seeds into sori, collections of teliospores commonly referred to as bunt balls. DB can infect small grains and wild grasses, giving it refuge in an area once established. The spores also remain viable in the soil for more than ten years, providing many opportunities to infect hosts. Utah is a large producer of organic wheat, and synthetic fungicides cannot be used in these systems. Host plant resistance is necessary for continued production in these areas. Since a prolonged period of low temperatures and snow cover is required for fungal germination, environmental conditions are not always conducive to achieve consistent DB infection in screening nurseries. The objective of this project is to test genomic selection (GS) approaches for predicting DB resistance. If DB resistance can be predicted accurately, breeding programs can make selections using GS during years with poor infection and/or select for DB resistance among lines that have not been evaluated in screening nurseries.

To complete this study, a population of 384 winter wheat cultivars and breeding lines from the Intermountain West was evaluated for resistance in a DB screening nursery in North Logan, UT during the 2021–2022 growing season. The entries were sown in single-row 1.2m plots on 5 October 2021 in a twice-replicated randomized complete block design. Following emergence, the trial was inoculated with DB spores in a liquid suspension on 17 November 2021. Following physiological maturity of the trial, DB resistance was scored as a percentage of the infected spikes within each plot. The population was also genotyped using a 90K SNP array. The phenotypic data and genotypic data are used to perform GS to determine its efficiency in the context of the first trial year.

Keywords

dwarf bunt, genomic selection, resistance breeding, winter wheat, organic agriculture

Acknowledgments

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Wheat (*Triticum aestivum*) chromosome 6D harbours a major QTL for common bunt resistance present in the *Bt11* bunt differential

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Common bunt in wheat has witnessed a renaissance with the conversion of conventional agricultural systems into organically managed areas - a development that started in the 1980s and has been continuing with increasing speed since then. The prohibition of systemic fungicides in organic farming, together with a lack of resistant wheat cultivars, has led to wide-spread problems due to infections with *Tilletia caries* and *T. laevis*. Knowledge about genetic sources for common bunt resistance is still scarce and only a small number of the known range of bunt resistance factors is currently used in breeding. We therefore aimed to map the resistance factor harboured by the Turkish landrace PI 166910, which is the resistance donor for the *Bt11* bunt differential line PI 554119. Four mapping populations (MPs) with 96 to 132 recombinant inbred lines (RILs) were phenotyped for common bunt resistance over two, three or four years with one or two local bunt populations and genotyped with the 25K SNP array. A major bunt resistance locus on the distal end of chromosome 6D designated *QBt.ifa-6DL* was identified in all MPs and experiments. Additional QTL contributing to the durable resistance of many RILs were detected on chromosomes 4B, 1B, 2A and 7B. The locus on 4B (*QBt.ifa-4BS*) was also found in all MPs but its effect on com- mon bunt incidence varied strongly between MPs and years. *Qbt.ifa-6DL* mapped to a region overlapping with the *Bt9* -locus identified in previous studies, but results indicate that *QBt.ifa-6DL* is likely to be different from *Bt9* and rather represents the *Bt11* resistance factor. Markers for the distal region of chromosome 6D between 492.6 and 495.2 Mbp can be used to select for *QBt.ifa-6DL*. This resistance factor from PI 166910 confers high and durable resistance against common bunt and should be integrated into wheat breeding programs for organic and low-input agriculture.

Keywords

common bunt, QTL mapping, *Triticum aestivum*, resistance breeding

Acknowledgments

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Assessment of common bunt and dwarf bunt resistance in *Bt*-differential lines grown in diverse environments

Pabitra Joshi[1], Guriqbal Singh Dhillon[1], David Hole[2], Tyler Gordon[3], Herman Buerstmayr[4], Magdalena Ehn[4], Yaotian Gao[1], Justin Wheeler[1], Will Krause[2], Margaret Krause[2], Jianli Chen[1]*

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Dwarf and common bunt are two destructive race-specific fungal diseases of wheat. Previous research suggested that the two diseases were controlled by the same set of genes, *Bt-1* to *Bt-15*, and *Btp*. Researchers have been using common bunt in initial screening of segregating lines for resistance to dwarf bunt because common bunt can be induced more reliably due to less stringent environmental requirements that are easily managed (Goates, 2012). Studies has also shown that there are differences in the virulence characteristics of the bunt fungi (especially common bunt) between United States and Non-United State isolates (Goates, 2012). The reports solely based on differential lines for common bunt and dwarf bunt are very limited and very less evidence is there to confirm if these diseases are indeed caused by the same set of genes. To confirm this result, the present study assessed dwarf bunt and common bunt resistance in a set of bunt differentials in a collaborative effort in diverse environmental conditions over several years. The set of 15 differential cultivars (*Bt-1* to *Bt-15* & *Btp*) has been used throughout the world to evaluate the virulence characteristics of local wheat bunt isolates and compare it with non-US isolates. Sixteen differential wheat lines that contain the bunt resistance genes *Bt-1* to *Bt-15* and Btp were used in this study (Table 1). The differentials with genes *Bt-1* through *Bt-13* and *Btp* are winter hexaploid wheat, whereas the differentials with *Bt-14* and *Bt-15* are spring tetraploid (durum) wheat. For this study, dwarf bunt resistance of differential lines was assessed in Logan, UT, near campus of Utah State University, USA in 2016 to 2022, in which a composite inoculum of dwarf bunt races was used collected from same place every year. For common bunt resistance data from field nurseries in Austria was used and additional set of dwarf bunt and common bunt data were downloaded from two published papers (Gordon et al., 2020; Ehn et al., 2022). Data were compared with the common bunt resistance recently collected in two greenhouse experiments at the University of Idaho. Differential lines were also characterized based on their head (spike) type in greenhouse. Comparative result shows that differential lines *Bt-8*, *Bt-11*, *Bt-12*, and *Bt-13* had resistant reaction to Europe CB races, Idaho CB races, and Utah DB races; however, *Bt-9* and *Bt-10* showed resistant reaction to Europe CB races, but susceptible reaction to Idaho CB, and variable reactions to Utah DB races over years. While comparing the head type (Fig 1) most of the head type corresponds to what is written in literature. However, surprisingly we found different head types for some differential lines like *Bt-9*. This result shows that there is a need to purify the differential seed source. We also assessed the differential lines with KASP makers that associated with major QTL on the 6DL controlling dwarf bunt resistance using a peak marker that we obtained from a QTL analysis in our DH population (unpublished).

Table 1: CB and DB resistance of differential line across year and different environmental condition (UT=Utah collected data, Aus= Austria collected data, MSGH= Moscow, ID greenhouse data, ABRGH= Aberdeen Greenhouse data, BLUE= Best Linear unbiased estimate across year from published data)

Bt-line	Source	seed_name	16DB_UT	17DB_UT	18DB_UT	19DB_UT	20DB_UT	22DB_UT	21CB_Aus	22CB_Aus	22CB_MSGH	22CB_ABRGH	CB_BLUE_Aus	DB_BLUE_UT
***Bt0*-S-check**	Heines VII	PI209794	50.0	80.0	88.9	95.0	91.3	40.0	NA	NA	NA	0.0	86.6	111.2
***Bt1*-winter**	Selection 2092	PI554101	50.0	85.0	85.6	92.5	92.5	65.0	0.9	3.3	65.0	NA	0.2	104.0
***Bt2*-winter**	Selection 1102	PI554097	70.0	70.0	94.4	96.5	98.0	70.0	33.5	76.1	38.9	12.5	77.5	119.2
***Bt3*-winter**	Ridit	CItr6703	50.0	10.0	23.9	7.5	9.0	10.0	11.1	24.8	13.3	0.0	1.0	51.2
***Bt4*-winter**	CI 1558	PI11610	60.0	98.0	93.4	NA	94.0	65.0	5.2	10.4	82.7	31.6	0.8	120.4
***Bt5*-winter**	Hohenheimer	CItr11458	40.0	70.0	70.0	90.0	90.0	45.0	1.1	8.3	NA	0.0	NA	32.1
***Bt6*-winter**	Rio	CItr10061	85.0	85.0	96.9	99.0	92.5	80.0	3.6	6.4	NA	NA	0.2	67.4
***Bt7*-winter**	Selection 50077	PI554100	90.0	95.0	93.1	87.5	90.0	82.5	35.8	93.1	46.4	43.3	49.9	112.7
***Bt8*-winter**	M72-1250	PI554120	10.0	0.0	1.3	0.5	0.5	2.5	NA	NA	0.0	0.0	13.6	7.5
***Bt9*-winter**	R63-6968	PI554099	10.0	4.0	30.5	60.0	24.0	30.0	NA	NA	60.0	67.7	9.9	55.3
***Bt10*-winter**	R63-6982	PI554118	0.5	4.0	3.5	25.0	17.5	17.5	NA	NA	33.3	45.0	3.1	37.0
***Bt11*-winter**	M82-2123	PI554119	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	NA	0.0	1.0	4.5
***Bt12*-winter**	1696	PI119333	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.6	3.4
***Bt13*-winter**	Thule III	PI181463	0.0	0.0	1.1	0.3	0.3	5.0	28.0	47.3	4.6	0.0	1.6	11.0
***Bt14*-durum-spring**	Doubbi	CItr12064	NA	NA	NA	NA	40.0	NA	NA	NA	NA	NA	NA	3.7
***Bt15*-durum-spring**	Carleton	CItr13711	NA	NA	NA	5.0	NA	NA	NA	NA	NA	NA	NA	14.8
***Btp*-winter**	7838	PI173437	NA	NA	NA	NA	NA	NA	3.6	24.6	0.0	0.0	16.2	0.1
***Bt-Unknown*-winter**	7845	PI476212	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.0	0.1

Figure 1: Head type of differential lines (A=*Bt-0*, B=*Bt-1*, C=*Bt-2*, D=*Bt-3*, E=*Bt-4*, F=*Bt-7*, G=*Bt-8*, H=*Bt-9*, I=B*t-9* (another type of head), J=*Bt-10*, K=*Bt-11*, L=*Bt-12*, M=*Bt-13*, N=*Btp*)

The genotyping result shows that *Bt-1*, *Bt-2*, *Bt-8*, *Bt-9*, *Bt-10* and *Btp* shows the presence of 6DL-QTL on them whereas other differential lines show its absence (Table 2).

Table 2: Marker haplotype of differential lines with 6D peak marker.

Line	6DL_peak_marker
Bt-0-S-check (PI 209794)	-
Bt-1-winter (PI 554101)	+
Bt-2-winter (PI 554097)	+
Bt-3-winter (CItr 6703)	-
Bt-4-winter (PI 11610)	-
Bt-5-winter (CItr 11458)	-
Bt-6-winter (CItr 10061)	-
Bt-7-winter (PI 554100)	-
Bt-8-winter (PI 554120)	+
Bt-9-winter (PI 554099)	+
Bt-10-winter (PI 554118)	+
Bt-11-winter (PI 554119)	-
Bt-12-winter (PI 119333)	-
Bt-13-winter (PI 181463)	-
Btp-winter (PI 173437)	+
Bt-Unknown-winter (PI 173438)	-

Keywords

Common bunt, dwarf bunt, Differential, Spike type, KASP, QTL

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Determination of virulence of European races of common bunt using a differential set of wheat cultivars

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The development and increased use of wheat cultivars resistant against common bunt (*Tilletia caries/T. leavis*) could contribute to a reduction of fungicide seed treatment in conventional agriculture and reduce the impact of these pathogens in organic agriculture. In order to choose the optimal resistance genes for breeding programs, knowledge is needed about the occurrence of virulence of the pathogen against the different resistance genes of the host against common bunt in wheat. The purpose of the study was to determine the virulence patterns of common bunt present in Europe and Iran.

Part of the spores used in this study were collected in different places in Denmark about 20 years ago in the ORGSEED project, maintained on susceptible varieties and used for screening in variety trials. From 2010 onward, spores were collected and maintained on resistant varieties in the COBRA and LIVESEED project, and thereby a collection of races was established with specific virulence to the resistance of the varieties on which they were maintained (Borgen et al 2018).

At Julius Kühn Institute, another set of spores were collected mainly from different sources in Europe to develop identification tools for the differentiation of common and dwarf bunt (Sedaghatjoo et al. 2021; Forster et al. 2022). In 2019 these spores were provided to Agrologica and multiplied on the susceptible winter wheat variety Creator for two years. In 2021, spores from each spore sample were applied to seed of Creator and 24 resistant winter wheat varieties representing 14 different resistance genes. 50 seeds of each spore – seed combination were sown without replication. The trial was assessed by visual inspection in 2022.

Discussion

The wheat variety Creator is susceptible to almost all bunt races. Accordingly, all but one of the bunt strains sampled were able to infect this variety. The other varieties of the field trial displayed anything from high to medium (and lower) infection rates indicating that the respective bunt sample is virulent toward the resistance gene in the given variety, while other varieties are not infected at all by a given spore sample indicating that the variety has one or more functioning resistance genes against the respective bunt strain. Very low infections rates in some varieties could be caused by different factors: Either the variety is not 100% genetically homogeneous with respect to the resistance gene, or the spore sample is in fact a mixture of different races with different virulences, with some virulences only present in small amounts. Based on this study a definite conclusion of these two possible causes cannot be provided. In particular, Thule III(*Bt13*) and maybe also Rio(*Bt6*) and Pi554115(*Bt4*) seem to be infected at low levels by a range of common bunt samples, and it is therefore likely that the seed samples of the used varieties were to some degree heterogeneous.

Table 1: Wheat varieties with resistance to common bunt infected by 44 spore collections of common bunt from Europe and Iran. The spore samples can be grouped into 14 different races based on the differential lines reactions to infection.

	Blizzard	Tilliko	Thule III	IFA-P106.30	NIL-12	Pi-554098	NIL-10	Tillexus	Pi-554099	NIL-9	Magnifik	Pi554120	Pi554100	Rio	Hohenheimer	NIL-5	Pi554115	Nebred	pi554121	Ridit	Busaard	Pi554097	PG3540	NIL-1	Creator	
	Bt3 +Bt6 +7A	BtZ	Bt13	Bt12		Bt11	Bt10		Bt9		?	Bt8	Bt7	Bt6	Bt5 +1B	Bt5	Bt4		Bt3		BtH	Bt2	Bt1		Bt0	
Pan-35, Germany	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	
Pan-9, Germany	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	3	0	0	0	83	
Vr-13, Denmark	2	0	2	0	0	0	0	0	4	0	0	6	0	0	0	0	2	0	0	0	0	0	0	0		avirulent
Hansa, Sweden	0	0	19	0	0	0	0	0	0	0	0	3	0	0	0	0	3	3	0	0	5	0	0	0	50	
Vr-0, Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	11	0	0	11	7	0	0	0		
Pan-34 Sweden	0	0	3	0	0	0	0	0	0	0	3	0	0	0	0	0	19	3	0	0	9	0	0	0	89	
Pan-19, Germany	0		9	0	0	0	0	0	0	0	0	0	0	3	0	0	33	31	17	0	12	14	0	0	33	Bt4
Pan-24, Schweiz	0	0	9	0	0	0	0	0	9	0	0	33	20	9	0	0	9	9	50	9	17	12	0	0	33	Bt8 Bt3
Pan-25, Italy	0	0	0	0	0	0	0	0	0	0	0	64	9	3	0	0	14	0	0	0	0	0	0	0	35	
Pan-26, Italy	0	0	3	0	0	0	0	0	0	0	0	23	27	0	0	0	0	9	0	0	11	0	0	0	67	
Pan-22, Germany	0	0	12	0	0	0	3	0	0	0	0	43	25	6		12	3	0	0	0	0	9	0	0	71	Bt7 Bt8
Pan-23, Germany	0	0	24	0	0	0	0	0	0	0	0	77	29	0	0	3	7	0	0	0	5	0	0	0	60	
Wiik-Emmer	0	3	0	0	0	0	0	0	0	0	0	25	14	0	0	0	0	0	9	0	3	12	0	0	50	
Vr10, Denmark	0	43	0	###	0	0	38	98	0	0	0	0	22	2	0	2	2	0	0	0	2	0	0	7		BtZ Bt10 Bt7
Pan-7, Germany	0	0	14	0	0	0	3	0	9	0	0	0	0	0	0	0	0	0	0	0	0	91	97	0	50	Bt2 Bt1???
Pan-17, Germany	0	0	12	0	0	0	0	0	12				6	3	0	0	12	3	0	0	0	0	88	94	57	Bt1
Vr-5, Denmark	0	0	2	2	0	0	0	0	0	0	0	0	2	2	17	83	20	0	0	0	75	2	4	4		Bt5 Bt2???
Pan-1, Austria	0	0	0	0	0		0	0	3	0	0	0	3	3	0	3	23	0	3	0	86	60	0	0	40	Bt2 Bt4?
Pan-6, Austria	0	0	0	6	0	0	0	0	9	0	0	23	0	0	0	0	6	0	0	0	50	14	0	0	50	
Vr-DOT, Denmark	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	11	6	0	0	2	94	26	7	7		
Pan-18, Germany	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	60	50	0	0	75	
Pan-11, Germany	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	91	50	0	0	83	
Pan-21, Germany	0	5	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	88	33	0	0	50	Bt2
Pan-4, Austria	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	3	14	0	0	0	86	91	0	0	67	
Pan-10, Germany	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	100	86	0	0	75	
Pan-20, Germany	0	0	33	0	0	0	0	0	0	0	0	6	6	0	0	0	0	0	3	0	86	97	0	0	75	
Pan-8, Germany	0	0	19	11	0	0	0	0	0	0	0	0	0	3	0	0	6	0	0	9	94	87	0	0	67	
Pan-13, Germany	0	0	25	0	0	0	0	0	6	0	0	0	0	0	0	0	3	0	0	0	97	87	0	0	50	
pan-Veron, Tjeck Rep.	0	0	11	0	0	0	0	0	6	0	0	3	50	23	0	0	50	33	38	0	83	0	3	60	67	Bt7 Bt4 Bt3?? Bt2?? Bt1???
Pan-3, Austria	0	0	6	0	0	0	0	0	5	0	0	0	67	0	0	15	0	0	0	0	67	91	0	0	50	
Pan-Aros	0	0	12	0	0	0	0	0	0	0	0	2	57	0	0	6	14	3	0	3	86	86	0	24	57	
Pan-2, Austria	0	0	9	0	0	0	0	0	0	0	0	0	57	0	0	0	5	0	3	0	86	100	0	0	83	Bt7 Bt2
Pan 30, Iran	0	0	17	0	0	0	0	0	0	0	0	0	57	6	0	6	0	0	6	6	94	100	0	0	67	
Pan-16, Germany	0	0	0	0	0	0	0	0	14	0	3	17	50	3	0	0	14	3	13	11	86	100	0	0	86	
Pan-29, Iran	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	50	3	0	0	97	98	0	0	50	Bt4 Bt2
pan-31, Iran	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	30	2	0	7	95	100	0	0	94	
Pan-27, Latvia	0	6	0	0	0	0	0	0	0	0	0	0	14	12	0	0	6	###	0	0	83	67	50	44	0	
Vr-2, Denmark	0	0	0	0	0	0	0	0	0	0	0	0	14	2	0	0	8	0	0	0	94	91	60	75		Bt7 Bt2 Bt1
Pan-Stava, Sweden	0	0	3	0	0	0	0	0	0	0	0	0	26	3	0	0	0	3	0	0	91	97	83	75	86	
Vr-3, Denmark	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	75	80	100	89	0	0		Bt3 Bt2
VrZ, Denmark	0	57	6	0	0	0	38	50	0	0	0	0	0	33	0	0	75	26	0	0	4	0	0	0		BtZ Bt13 Bt10 Bt4 Bt6
Pan-28, Iran	0	43	71	3	0	0	33	44	14	0	0	0	0	14	0	0	86	40	20	0	12	0	0	0	50	
Pan-32, Iran	0	0	86	0	0	0	0	0	0	0	0	0	0	50	0	0	94	81	67	89	88	50	0	0	26	Bt13 Bt6 Bt4

After assessment of the field trials, spores were collected from resistant varieties with low infections, and reinoculated on the same variety. The result of this reinoculation is not available yet. If this reinoculation leads to increased infection, it will indicate that virulence has been present in the spore sample, whereas if it leads to a similar low or lower infection, it will indicate that the infection was caused by impurity of the differential line.

Bunt samples that show a similar infection level to the distinct resistant varieties may be of the same race. Common bunt is caused by two closely related species, *Tilletia caries* (syn. *T. tritici*) and *T. laevis* (syn *T. foetida*). Goates (2012) described 57 races of bunt by their reaction to a similar set of differential lines. Only few races from our study have the same combination of virulences and avirulences as the races described by Goates (2012).

Although the collection of spores does represent primarily Germany, Sweden and neighboring countries it provides a first impression of common virulences. Our study suggests that the virulence against *Bt2* and *Bt7* is most common in this region, whereas virulences against *Bt1*, *Bt3*, *Bt5*, *Bt9*, *Bt10* and *BtZ* is more rare. As mentioned above, it is likely that the differential line of *Bt13* is impure, and that virulence against this gene is more rare than the results indicate. There are no races with virulence against *Bt11* and *Bt12* and the variety Blizzard. Genetic studies have shown that these genes are not single genes but are combinations of multiple genes (Unpublished).

These conclusions are in line with previous surveys made by Mascher et al (2018), but represent a broader range of spore samples. Babayants et al (2006) found virulence in Ukraine to all *Bt*-genes *Bt1-Bt7*, but not to *Bt8-Bt15*.

Keywords

winter wheat, common bunt, organic agriculture, resistance breeding, virulence survey

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Co-evolution of virulence and resistance in heterogeneous wheat populations

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The pathogenesis of common bunt caused by *Tilletia caries* or *T. leavis* is that spores are introduced to a crop via farm equipment (Kristensen and Borgen 2001) or soil contamination (Borgen 2000A). Hereby, the new crop will be infected, and secondary infection will happen from year to year during threshing, as more plants will be infected, and the infection rate is proportional with the amount of spores in the seed lot (Heald 1921). The speed of multiplication from year to year will depend on many factors, including equipment used for threshing (Kristensen and Borgen 2001) and seed cleaning (Borgen 2005), climatic conditions in particular in the period after sowing and the resistance of the variety and the virulence of the spores (Borgen 2000B).

Varieties with a single dominant resistance gene will according to the gene-for-gene relationship discovered by Harold Henry Flor (1942) either not be infected, if the spores are avirulent to the resistance gene, or it will be fully susceptible if the spores are virulent. This system is widely accepted as the dominant mechanism for pure line varieties with the known resistance genes summarized by Blair Goates (2012). However, little is known about what happens if a crop is not a pure line variety with a single resistance gene, or if the spores are not a single race with a specific virulence.

In the new EU Regulation for organic farming (EU 2018), a new term is introduced called Organic Heterogeneous Material (OHM), which is not a single variety but a mixture of plants with diverse genetic background. As OHM is introduced for organic farming, and common bunt is a main issue for organic wheat production, organic breeders of OHM often attempt to introduce resistance to common bunt in the populations. Among the OHM accepted in EU so far are Brandex and Liocharls from Dottenfelder Hof and Mariagertoba and Popkorn from Agrologica. These populations all have resistance to common bunt with *Bt7* dominant in Brandex, Liocharls and Mariagertoba and with a broader mixture of resistances in Popkorn.

The aim of this trial is to study to dynamic of the epidemiology of common bunt over years in populations with multiple resistances.

8 varieties were used in the trial with differences in resistance genes:

1. NIL1 (*Bt1*)
2. NIL5 (*Bt5*)
3. Promesse (*Bt5*)
4. NIL6 (*Bt9*)
5. NIL9 (*Bt9*)
6. NIL10 (*Bt10*)
7. Magnifik (*Bt9*(+?))
8. Pi554121 (*Bt3*)

race		Variety/mixture	Frequency of avirulent genes	Actual Infection **2022** % in the plot	Actual Infection **2021** % in the plot	Expected infection in the mixture based on the infections in the components when tested alone
Vr10	2	NIL 1+10	50	57,4	15,0	23,5
					1,2	0,0
Vr10	2	NIL 5+10	50,0	33,3	22,1	23,5
Vr10	3	NIL 1+5+10	66,7	27,6	10,0	15,7
Vr10	4	NIL 1+5+10+6	75,0	12,1	6,1	11,8
Vr10	4	NIL 1+5+9+10	75,0	15,3	7,4	11,8
Vr10	6	NIL 1+5+10+6+9+Promesse	83,3	13,6	5,4	7,8
					6,5	6,7
Vr10	8	NIL 1+5+10+6+9+Promesse+Magnifik+Pi554121	87,5	14,6	5,1	5,9
Vr2	2	NIL 1+10	50,0	17,8	10,9	23,5
Vr2	2	NIL 1+5	50,0	17,4	8,0	23,5
Vr2	3	NIL 1+5+10	66,7	18,1	12,3	15,7
Vr2	4	NIL 1+5+10+6	75,0	15,5	6,1	11,8
Vr2	4	NIL 1+5+9+10	75,0	11,4		
Vr2	6	NIL 1+5+10+6+9+Promesse	83,3	19,6	0,0	7,8
Vr2	7	NIL 1+5+10+6+9+Promesse+Magnifik	85,7	18,9	2,5	7,8
Vr2	8	NIL 1+5+10+6+9+Promesse+Magnifik+Pi554121	87,5	5,5	0,0	6,7
					1,7	6,7
					3,9	5,9
Vr5	2	NIL 1+5	50,0	47,6	12,3	23,5
Vr5	3	NIL 1+5+10	66,7	21,6	16,4	15,7
Vr5	4	NIL 1+5+10+6	75,0	15,0	7,0	11,8
Vr5	4	NIL 1+5+10+9	75,0	23,9	5,2	11,8
Vr5	6	NIL 1+5+10+6+9+Promesse	66,7	12,5	10,5	15,7
Vr5	7	NIL 1+5+10+6+9+Promesse+Magnifik	71,4	9,3	2,4	13,4
Vr5	8	NIL 1+5+10+6+9+Promesse+Magnifik+Pi554121	75,0	10,5	6,6	11,8
Vr5+10	3	NIL 1+5+10	33,3	48,2	20,9	31,3
Vr2+10	3	NIL 1+5+10	33,3	32,0	21,1	23,5
Vr2+5	3	NIL 1+5+10	33,3	27,2	15,8	23,5
Vr2+5+10	3	NIL 1+5+10	0,0	29,7	13,0	23,5

The varieties were selected with the hope that they all had different resistance genes, but later trials have shown that this was not quit true. Promesse, NIL6 and Magnifik was included as they were expected to have *Bt4*, *Bt6* and *Bt8* respectively, but it turned out that they did not. The NIL's (described in Borgen et al 2018) were used as these being closely related are expected to compete in the population mainly based on their differences in response to common bunt and not on agronomic performance in the field.

All varieties were tested for response to bunt of 3 different races of bunt.

Mixtures were made by mixing the varieties in equal amounts with increasing degree of diversity regarding resistance genes, or rather increasing expected degree of resistance as some varieties did not truly have the resistance genes expected at the time of experimental design.

Each mixture were then infected with one of the three races of bunt spores, and with a combination of races. After assessment and at maturity of the wheat, healthy and infected plants were harvested separately, and spores of the infected plants were then applied to the seed of the healthy plants for regrowing in the following season. This design was chosen to avoid the problem of contaminating threshing equipment and thereby mixing up the spores in the different seed lots. The design however has the limitation that it does not represent true agricultural practice, which may affect the epidemiology of the disease.

Based on the infection of the pure line varieties by each race, an expected infection rate can be calculated by the proportion of the different varieties in the mixtures. In some cases, the actual infection differed from the expected infection (Table 1). These differences can be affected by epidemiological effects of the mixtures.

In the first year (2021), the actual infection in the mixtures was generally slightly lower what was expected from the infection in the pure lines and the proportion of the varieties in the mixtures. In the second year (2022) it was higher.

When a plant is infected with common bunt, the grain yield of the infected plant will be reduced by 50-100% as infected heads will produce no seed. Some plants of a susceptible variety will not be infected.

In conclusion, it is expected that selection pressure in disfavor of susceptible varieties will reduce these by some 50% in comparison with the resistant varieties within the mixtures. From year to year, it would therefore be expected that the mixtures get more and more dominated by resistant varieties and the frequency of susceptible varieties will decrease. After two years, this effect seems not to be recorded in the actual infection rate. There is a possibility that spores from other races have contaminated the mixtures by mistake, or that the fungal races develop and adapt within the mixtures faster than the dynamic between wheat varieties. The most dominant effect however may be that each year spores are re-applied to the mixtures by artificial spore application. In real life, the amount of spores will depend on the frequency of infected plants in the field. It is possible that the actual infection rate under farm conditions will be so low that the amount of spores will be limiting the infection rate, whereas in this experiment, that same amount of spores are applied to all mixtures, disregarding the differences in infection rate in the previous season. The chosen design has the presumption that a few percent of infected plants will produce so many spores that it will matter little if there are more than a few percent infected plants, but maybe this is not really the case.

Even though the infection rate is still high after selection pressure, this means that secondary infection is still able to maintain infection. It is likely the composition of the mixtures has indeed changed and increased resistance, and that this will give increased protection against new primary infection, if the secondary infection is eliminated by seed treatment.

The experiment will continue for another year to follow the development.

Keywords

winter wheat, common bunt, organic agriculture, population dynamics, Organic Heterogeneous Material.

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Annotation of differential lines used for resistance trials for common bunt

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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 worldwide 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) analyzed the phenotypic reaction of 73 races of common bunt and dwarf bunt, 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 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 line 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 line 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 agronomic 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 vernalization 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.

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Keywords

winter wheat, common bunt, variety trial

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Gene postulation based on phenotyping wheat varieties with a differential set of virulence races of common bunt (*Tilletia caries*)

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Knowledge about the resistance genes in wheat varieties is crucial for predicting the actual field resistance against plant diseases, and also as a basic knowledge for development of genetic markers. As a basis for development of genetic markers for resistance to common bunt (*Tilletia caries* and *T. leavis*), and as a screening trial for breeding lines, a field trial was set up to evaluate wheat germplasm.

Based on a design described in Borgen et al (2018), a trial was set up using 8 races of common bunt (*Tilletia caries*) to infect 850 wheat varieties and breeding lines.

Approximately 50 seed of each line were applied with dry spores in a paper bag and shaken. Enough spores were applied to cover the seed surface and leave a few spores in surplus in the bottom of the bag. Seed was hand sown in rows directly from the seed bag to avoid mixtures of spores in sowing equipment. Symptoms of infection was done by visual assessment 4-5 weeks after heading.

Breeding lines and approved varieties for the experiment was supplied by Dottenfelder Hof, Cultivari, Saatzucht Donau and Agrologica. Genebank accessions was supplied by NordGen, John Innes Institute and USDA National Small Grain Collection.

Goates (2012) described 16 resistance genes denominated *Bt1-15* plus *BtP*. Additional resistance genes *BtZ* (Blažková and Bartoš 2002), Trintella-resistance (Dumalasová et al 2012) and Blizzard-resistance (Wang et al. 2009) has been described.

The races used in the experiment was:

- Vr-2 Virulent to *Bt1*, *Bt2* and *Bt7*
- Vr10 Virulent to *Bt7*, *Bt10* and *BtZ*
- VrZ Virulent to *Bt4*, *Bt6*, and *Bt10*
- Vr-5 Virulent to *Bt5* and low infection in *Bt7* and *Bt4*,
- Vr-3 Virulent to *Bt2* and *Bt3*,
- Vr-DOT Virulent to *Bt2* and low infection in *Bt7* and *Bt1*,
- Vr-0 Not virulent to any *Bt*-genes,
- Vr-13 Low infection in *Bt13*.

None of the races were virulent to *Bt8*, *Bt9*, *Bt11*, *Bt12*, Blizzard or *BtP*. Also, no virulence was found against Erythrospermum 5221, which has a resistance gene translocated from *Agropyron* (Baranovskaya et al. 2003 cited in: Babayants 2006). Germplasm containing any of these genes came out as being resistant to all races.

There seems to be no additive effect of gene combinations. In contrast, the resistance of a variety is governed by the most effective gene, and if a bunt race is virulent to all the genes in a variety, then the variety will be susceptible to the race. This confirms that the *Bt*-genes follows the gene-to-gene relationship (Flor 1942).

Based on the phenotyping, it was possible to postulate the *Bt*-resistance genes in most lines having a single or a combination of few genes to which virulence was present. Some two-gene combination were

resistant to all races, and the resistance genes can therefore not be determined by this experiment alone. However, if the parents and the resistance of the parents are known, then phenotyping combined with parental information can often predict the resistance.

Based on parental information in combination with this experiment, and in combination with genotyping of the involved germplasm, it has been possible to perform GWAS and develop a range of genetic markers associated with the *Bt*-genes (Christensen and Borgen 2023A-F, Borgen and Christensen 2023).

Keywords

winter wheat, common bunt, breeding resistance, plant diseases.

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Registered varieties and Organic Heterogeneous Material (OHM) with resistance to common bunt in Europe

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Breeding wheat varieties with resistance to common bunt is one of the most effective and cheap measures that can be used to reduce fungicide use for seed treatment in conventional agriculture and to prevent the risk of disease infections in organic agriculture. However, few varieties are brought to the market with resistance and few adapted varieties are available for future plant breeding.

In the LIVESEED, ECOBREED, DIVERSILIENCE and BOOST projects, and in the annual field trials by private breeders, some varieties have been identified with resistance to common bunt.

The purpose of this study is to bring an overview of varieties with resistance to common that has been confirmed to be resistant and are available for farmers, seed companies and plant breeders.

Table 1: Wheat varieties listed in EU Seed Catalogue 2023

Variety	Type	Breeder	Year of release	Seed Company	Resistance Gene(s)	
Apostel	Winter	IG-Pflanzenzucht			Bt5	(*)
Aristaro	Winter	Landbauschule Dot enfelderhof eV	2016	Bioland Handelsgesellschaft	Bt9+	(**)
Axano JB Asano???	Winter	Saatzucht Donau	2020	RWA Austria	Bt5	(*)
Bosporus	Winter	Breun			Bt5	(*)
Brandex (OHM)	Winter	Landbauschule Dot enfelderhof eV	2022	Bioland Handelsgesellschaft	Bt7	(*)
Bussard	Winter	KWS	1990		Bt2	(***)
Butaro	Winter	Landbauschule Dottenfelderhof eV	2009	Bioland Handelsgesellschaft	Bt2	(***)
Curier	Winter	Landbauschule Dottenfelderhof eV	2019	Bioland Handelsgesellschaft		
Festival	Winter	Lantmännen			Bt8+Bt9?	(*)
Fiorina	Spring	Agroscope	2001	Delley Samen und Pflanzen AG	Bt7	(*)
Florian	Winter	SaatenUnion			??	(**)
Fritop	Winter	Cultivari		Nordic Seed	BtZ+?	(***)
Genius	Winter	SaatenUnion		Nordsaat Saatzuchtgesellschaft GmbH	Bt5	(*)
Grannosos	Winter	Landbauschule Dot enfelderhof eV	2020	Bioland Handelsgesellschaft	Bt2	(*) (**)
Graziaro	Winter	Landbauschule Dot enfelderhof eV	2016	Bioland Handelsgesellschaft	BtZ	(**)
Hallfreda	Winter	Lantmännen			Bt8+Bt9?	(*)
LG Initial	Winter	Limagrain Europe S.A.			Bt5	(*)
Liocharls (OHM)	Winter	Landbauschule Dottenfelderhof eV	2022	Bioland Handelsgesellschaft	Bt7	(*)
Mariagertoba (OHM)	Spring	Agrologica	2022	Landsorten	Bt7	(****)
Popkorn (OHM)	Winter	Agrologica	2022	Landsorten	Mixed resistance	(****)
Quarna	Spring	Agroscope	2002	Delley Samen und Pflanzen AG	Bt7	(*)
Roderik	Winter	Cultivari		Oeko-Korn-Nord, Germany	Bt7	(****)
Sailor	Winter	Agroscope	2015	Delley Samen und Pflanzen AG	Bt7	(*)
Sarastro	Winter	Cultivari		Oeko-Korn-Nord, Germany	BtZ	(****)
Segor	Spring	Agroscope	2002	Delley Samen und Pflanzen AG	Bt7	(***)
Spontan	Winter	Limagrain Europe S.A.			Bt5	(*)
Stava	Winter	Lantmännen	1990		Bt8+Bt9	(*)
SW Magnifik	Winter	Lantmännen			??	(*)
Thomaro	Winter	Landbauschule Dottenfelderhof eV	2018	Bioland Handelsgesellschaft	Bt7	(**)
Tillexus	Winter	Saatzücht Donau	2018	Saatbau Linz	Bt10	(*)
Tilliko	Winter	Cultivari		RWA Austria	BtZ	(*)
Tillsano	Winter	Saatzücht Donau	2020	Probstdorfer Saatzucht	Bt5	(*)
Tillstop	Winter	Saatzücht Donau		Probstdorfer Saatzucht	Bt10	(*)
Trebelir	Winter	Cultivari		Oeko-Korn-Nord, Germany	Bt7	(****)
WPB Calgary	Winter	Wiersum PB			Bt5	(*)

(*) results from BOOST and DIVERSILIENCE projects (Borgen and Christrensen 2023)
(**) Rwsults from the ECOBREED project.
(***) Results from the LIVESSED project (Borgen et al 2018)
(****) Breeders information based on own results

Keywords

winter wheat, common bunt, organic agriculture, resistance breeding

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Candidate gene analysis for the 7DS QTL associated with dwarf bunt resistance of winter wheat using targeted capture sequencing technology

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The wheat dwarf and common bunt are two destructive diseases causing significant yield losses and quality deterioration in epidemic conditions. Development of resistant cultivar is the sustainable practice but requires a thorough understanding of the underlying genetic mechanisms. Two major QTLs on chromosome 7DS associated with the bunt resistance were previously identified in two independent studies. To identify the candidate genes for the two 7DS QTLs, the present study used a targeted sequencing approach with Illumina PE151 paired-end sequencing. A 3-11 Mb region of the 7D chromosome flanking the two QTLs was targeted for capture in one resistant parent 'IDO444' and susceptible parent 'RioBlanco', nine resistant and eight susceptible recombination inbred lines derived from the two parents, as well as three bunt differentials (*Bt0*, *Bt12*, and *Bt13*). More than 10 million raw reads per sample were obtained. Variant calling in the targeted region of the parental genotypes identified that these variants spanned genomic regions of 145 genes from 171 genes of the reference genome while covering exomic regions of only 29 genes. Based on the mutation type and position fifteen genes were used in further analysis, including three genes with gain of stop codon mutations. These genes were functionally classified as: six NBS-LRR-like protein, four protein kinase, three F-box protein, and one Cytochrome P450 coding genes. Gene specific primers were designed for the fifteen genes and have been analyzed using nulli-tetrasomic lines, genotypes with contrasting phenotypes, and mutation lines of the resistant parent showing loss of function. Preliminary results on the candidate gene analyses will be discussed in the present study and selected few candidate genes will be used in gain of function and loss of function studies in the ongoing projects.

Keywords

winter wheat, dwarf bunt, candidate gene, QTL, targeted capture sequencing

Acknowledgments

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Reaction of wheat genotypes to Czech common bunt and dwarf bunt samples

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Common bunt (*Tilletia caries*, syn. *T. tritici*; *T. laevis*, syn. *T. foetida*) and dwarf bunt (*T. controversa*) occur on wheat in the Czech Republic. In conventional agriculture, fungicide seed treatments provide protection against common bunt and dwarf bunt. However, with the spread of organic agriculture, interest in breeding for resistance is increasing.

At the Crop Research Institute in Prague – Ruzyně, field experiments with artificial inoculation of winter wheat varieties and lines with common bunt and dwarf bunt are ongoing. In 2022, 88 varieties and lines of winter wheat were tested for resistance with the ´RUKR´ inoculum mixture, containing teliospores of *T. caries* and *T. laevis*. Common bunt incidence in the susceptible control, variety ´Heines VII´, reached 38.9 % of ears. The highest infestation, 71.1 %, was recorded in the variety ´LG Atelier´. The bunt incidence exceeding 10 % of the ears is considered to be a manifestation of a susceptible reaction. A resistant reaction with a bunt incidence of less than 10 % of the ears was detected in 15 varieties and in 36 lines this year. In the varieties ´Aristaro´, ´Bonneville´, ´Deloris´, ´Genius´, ´UI SRG´, ´Blizzard´ and ´Spontan´, a resistant reaction was detected both in 2022 and in previous years. The varieties ´Unitar´ and ´Campesino´ showed a resistant response, but so far only in the first year of testing. The varieties ´Tillexus´, ´Tilliko´, ´Tillstop´, ´Graziaro´, ´Thomaro´ and ´Butaro´, carrying genes for resistance to bunt, had low infestation with the RUKR inoculum mixture in 2022. However, there are physiological races overcoming the *Bt10* and *BtZ* resistance genes in Europe.

In 2022, first year results were obtained using six different Czech inoculum samples of common bunt on a set of 9 winter wheat varieties carrying resistance genes. Varieties ´Aristaro´ (*Bt*?), ´SW Magnifik´ (*Bt8*+*Bt9*?), ´Genius´ (Bt5) and ´Spontan´ (*Bt5*) were resistant to all tested samples. No virulence to the resistance genes in the tested set of varieties was detected in the inoculum mixture ´RUKR´. Three inoculum samples (´AM´, ´MH´, ´ST´) were able to cause infestation only in the ´Butaro´ variety (*Bt2*). The last two inoculum samples (´BK´, ´MK´) showed virulence to the resistance genes of the varieties ´Tillexus´ (*Bt10*), ´Tillstop´ (*Bt10*), ´Graziaro´ (*BtZ*) and ´Tilliko´ (*BtZ*).

On the basis of available data, virulence to most of the known resistance genes to bunt can be assumed in Europe. From the point of view of durability of resistance, a combination of several specific resistance genes, or a combination of specific resistance genes and genes for quantitatively based resistance, is therefore needed for resistant varieties.

In 2021 and 2022 the incidence of dwarf bunt on a set of sources of resistance was evaluated. The level of dwarf bunt infection is low in Prague-Ruzyně in most years. The susceptible varieties ´Penelope´ and ´Bernstein´ showed mean bunt incidence 23.6 % and 20.3 %, respectively. The susceptible control ´Heines VII´ had only 9.2 % infected ears. However, the sources of resistance ´Cardon´, ´Crest´, ´Deloris´, ´Franklin´, ´Hansel´, ´Lewjain´, ´Manning´, ´Meridian´, ´Promontory´, ´Sprague´, ´UI SRG´, ´Ute´ and ´Winridge´ were completely free of infestation in both years.

Other wheat species were also studied. The presence of effective resistance genes was assumed in the Czech einkorn wheat variety ´Rumona´. It was resistant both to common bunt and dwarf bunt in 2021 and 2022.

The spelt wheat genotype ´Sofia 1´ was resistant to common bunt in five different years (2015-2017 and 2019-2020) and in four years (2017-2020) to dwarf bunt. It was crossed and F3 generation of the crosses spelt wheat ´Sofia 1´ x winter wheat ´Genius´ and F4 generation of spelt wheats ´Tauro´ x ´Sofia 1´ were raised.

Table 1: Response of wheat varieties carrying different resistance genes to six Czech common bunt samples

	Common bunt incidence (% of ears)					
Variety	´AM´	´BK´	´MH´	´MK´	´RUKR´	´ST´
Heines VII	56,7	54,5	67,4	74,2	68,5	70,9
Aristaro	0,0	7,9	0,0	0,0	0,0	0,4
Butaro	18,8	8,0	12,9	0,5	3,5	24,6
Genius	0,8	3,1	0,7	0,3	0,9	0,5
Graziaro	0,0	32,2	0,0	18,6	1,4	0,0
Spontan	1,8	6,4	1,1	2,8	2,1	2,8
SW Magnifik	0,0	5,3	0,0	0,0	0,0	0,0
Tillexus	0,0	29,6	0,0	45,5	0,0	0,0
Tilliko	0,0	11,1	0,0	19,2	0,0	2,3
Tillstop	0,0	17,5	0,0	28,6	0,0	0,0

Keywords

winter wheat, einkorn wheat, spelt wheat, common bunt, dwarf bunt

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A wheat-rye translocation 1AL.1RS involved in wheat resistance to bunt (preliminary results)

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The fundamental changes in UE agricultural production systems, such as the reduction or the lack of chemical will lead to resurgence of many seed-borne diseases including bunts which were previously controlled with chemicals. To transpose the European Green Deal into reality, a strategic step is represented by access to wheat cultivars resistant to diseases. One difficult disease for wheat is bunt produce by *Tilletia sp.* The obtaining wheat cultivars resistant to *Tilletia sp.* is a big challenge for breeders and researchers from other areas. This work reports partial results obtained on 85 DH lines, after one-year artificial inoculation with mixture of teliospors (2021-2022). Inoculated seeds were planted on 1metre long rows, using as susceptible cultivars Doina and Izvor. At maturity, the lines were classified in bunted and non-bunted. Infected spikes (where at least one grain was replaced by bunt balls) were counted and expressed as percentage from total number of spikes.

The 85 doubled haploid (DH) lines were obtained from the cross Izvor/F000628G34-M, using the protocol described by Giura A. (2011). The F00628G34-M line created at NARDI Fundulea by crosses between triticale and wheat showed resistance to bunt in artificial infections, both in the tests done at Fundulea (Ittu et al., 2006) and Simnic (Oncica and Saulescu, 2008) in Romania, but also, in international tests from the European project "*Tilletia* Ring Test" (Saulescu et al., 2010). Based on the study by Ciuca et al. (2015) the F00628G34-M line carries wheat-rye translocation 1AL.1RS. In that study, based on sixty-eight randomly extracted F4 lines from a cross between F000628G34-M and cultivar Litera, was found that the Chi square test showed significant deviation ($P < 5\%$) from the expected Mendelian monogenic segregation, suggesting that resistance gene is recessive or partially dominant and/or the resistance is affected by suppressing factors from wheat genome (Ciuca et al., 2015). Also, that study found the gene is close to Xgwm1223 microsatellite locus (P=0,0004).

In the present study on 85 DH lines (Izvor/F000628G34-M) molecular assay with TSM106, TSM592, GWM1223, SCM9 microsatellite markers showed that 33 lines carry rye translocation (1RS.1AL). Phenotype observations realized in 2022, showed that the lines with rye translocation, presented between 0-9% infected spikes, while Izvor showed 53% infected spikes and F000628G34-M showed 7% of infected spikes.

However, we ask ourselves: Way does not all rye translocation show resistance to bunt? Is this resistance affected by the rye type or the line F000628G34-M carries an avoidance mechanism of *Tilletia sp.* infection?

To answer of these questions, we started to use the resistance gene analogs (RGAs). At present, the results obtained using RGA 5a marker showed a distinct PCR product for F000628G34-M. This PCR product was observed in lines with 1RS.1AL translocation and resistance to bunt.

Based on these results, we will continue the molecular assay with RGA 5a and phenotypic testing to find the best marker for 1RS.1AL translocation associated with resistance to *Tilletia sp*.

Keywords

Wheat-rye translocation, common bunt, organic agriculture, resistance breeding, marker-assisted selection

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How long does it take to develop high performing and common bunt resistant winter wheat lines using organics-compliant methods?

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Once among the most devastating wheat diseases, common bunt caused by *Tilletia tritici* and *T. laevis* was successfully banned from most fields by the invention of seed dressings with hexachlorobenzenes (HCBs) in the 1950s. During the past decades, a continuously increasing area of agricultural land has been converted to organic management, refraining from the use of chemical pesticide applications and common bunt as a primarily seed-borne disease is experiencing a come-back. The most sustainable and efficient way to avoid yield and quality losses due to bunt infections is the use of resistant cultivars. Although 17 different resistance genes have been postulated so far, only very few have been mapped and are available for applied breeding. In consequence, the development of bunt resistant cultivars is slow and very few varieties with high resistance levels are currently available. In this study, we therefore aim to determine how fast breeding lines can be selected that unite bunt resistance and good agronomic performance.

For this purpose, we developed pseudo-back-cross populations with bunt resistance alleles introgressed from exotic donor lines. Resistance QTL in these donors were mapped in previous projects at IFA Tulln, enabling marker-assisted selection (MAS) via KASP-markers. The three resistance donors 'Blizzard', 'Bonneville' (north-American cultivars registered in the 1990s) and PI119333 (differential line for the bunt resistance gene *Bt12*) were initially crossed to the susceptible cultivar 'Rainer'. During population development, three back-crossing steps were carried out, each with a different back-crossing parent that was either a variety or a breeding line adapted to Austrian growing conditions. After each back-crossing step, F_1-progeny was screened for the presence of one to three different resistance QTL inherited from the donors using KASP-markers. In generation BC_3F_1, genomics-assisted selection (GAS) based on genomic estimated breeding values (GEBVs) was applied to select lines with promising genetic backgrounds based on genome-wide marker data from genotyping by sequencing (GBS). Selected progeny was self-pollinated and lines with QTL fixed in a homozygous allelic state were identified by MAS. Field testing for common bunt resistance was carried out for selected lines together with a control panel of negatively selected genotypes. Data from two seasons of common bunt testing in artificially inoculated field trials in Austria and one season of dwarf bunt testing with artificial inoculation in Logan, Utah, is available to determine disease resistance levels in the population. In addition, a replicated yield trial was conducted in 2022.

The number of lines undergoing propagation in the greenhouse or field testing was greatly reduced by the MAS and GAS steps. Selected lines comprised 33.6%, 8.8% and 9.1%, respectively, of all lines in the individual MAS cycles. Thereby, not only resources required for field testing were kept low, but also the time from the initial cross to the first homozygous resistant lines in generation BC_3F_2 was reduced. Of all lines selected to harbor one or several of the introgressed resistance QTL, 35% (69 lines) were fully or highly resistant (<= 5% incidence) to common bunt across two years. The high proportion of lines showing mild to severe infections can be explained by the following factors: markers applied for MAS were not diagnostic but only flanking the chromosomal regions conferring resistance and marker polymorphisms were scarce due to the complex pedigrees with five different parents for each line, lowering selection accuracies. As some of the intervals flanked by the applied markers are relatively large, also recombination events might have occurred in these regions that could not be tracked with the markers and that led to a loss of resistance in positively selected lines.

We also observed that common and dwarf bunt resistance are not conferred by the same genes in our experimental population. While lines harboring the resistance locus on chromosome 1B showed high resistance against common bunt, they were to a large extent infected by dwarf bunt. The opposite pattern was observed for lines with the *Bt12*-locus on chromosome 7D where recombination events in the chromosomal region may have been responsible for a loss of resistance against common bunt but not against dwarf bunt. Common bunt incidence was uncorrelated with yield and quality traits. We found experimental lines with complete resistance against common bunt that performed equally well or slightly better in terms of yield and quality than the highly susceptible registered varieties used as standards (Figure 1). Cultivars registered as bunt tolerant in Austria and Germany that were included as check varieties were moderately to highly infected with common bunt in our trials.

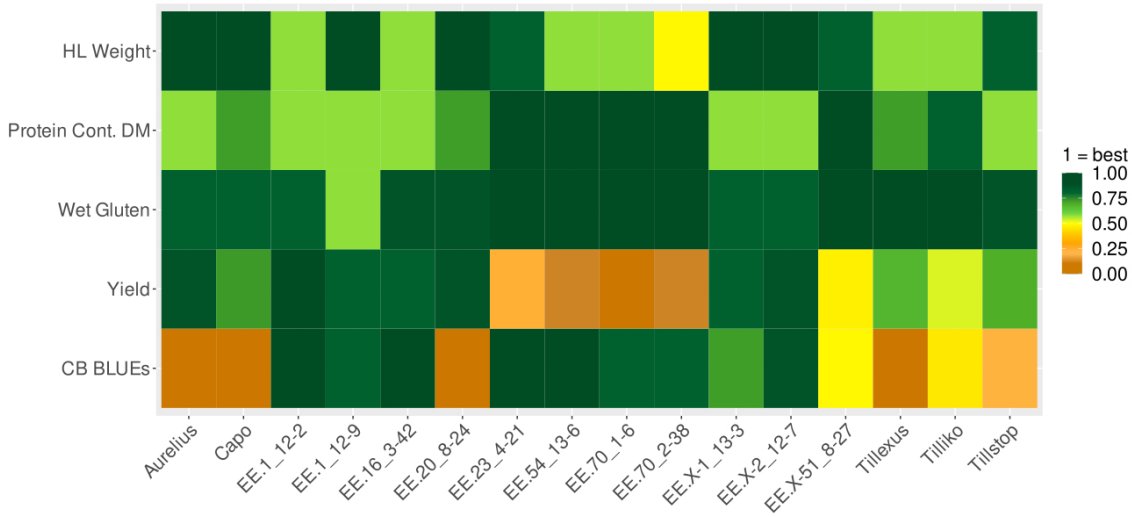

Figure 1: Heatmap showing scores for hectolitre weight (>76kg=0.5, >78=0.6, >79=0.8, >80=1), protein content in dry matter (>13%=0.6, >14=0.7, >14.5=0.85, >15=1), wet gluten content (>28%=0.6, >30=0.8, >33=0.9, >35=1), yield (>50dt/ha=0, >60=0.2, >70=0.5, >75=0.6, >80=0.8, >85=0.9, >88=1) and common bunt infections across two seasons (>70%=0, >40=0.5, >20=0.6, >4=0.8, 0=1) (y-axis, top row to bottom row) normalized to a range between 0 and 1. Scores are given for two bunt-susceptible standards (Aurelius and Capo), the six best-performing experimental lines in terms of yield and the five best-peforming lines in terms of protein content (genotype names starting with "EE") as well as three cultivars registered as bunt-tolerant in Austria (Tillexus, Tilliko and Tillstop). Data on all traits except common bunt is from replicated field trials conducted in Tulln in 2022. Data on common bunt incidence is shown as best linear unbiased estimates (BLUEs) across 2021 and 2022.

We therefore conclude that MAS is a suitable method to reduce time and resources for the development of bunt resistant and high-performing winter wheat lines. The experimental lines in our population were tested in generation BC_3F_{2n}. Using MAS, it is possible to reach this generation in 2.5 years, while selecting exclusively via phenotypes would take 5.5 years for the same outcome and require a lot of additional resources.

Keywords

winter wheat, common bunt, organic agriculture, resistance breeding, marker-assisted selection

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Loose smut resistant spring barley breeding for organic farming

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Loose smut is a seed-born fungal infection caused in barley by pathogen (*Ustilago nuda* (Jens.) Rostr.). Fungi colonizes florets and as a dormant mycelium can overwinter in mature seeds (Zang et al., 2015). Upon seed germination, the mycelium aggregates into the florets – all parts of the ear, except for the rachis, are replaced by spores which are produced by segmentation of the hyphae (Malik and Bats, 1960). The teliospores mature at about the same time when cereals head emerges and are easily spread by the wind to other developing crop seeds where they infect healthy embryos and remain dormant until the seed germinates (Eckstein et al., 2002; Wunderle et al., 2012). Infection effectively takes place immediately before pollination until 4-8 days after (Pedersen, 1960; Wunderle et al., 2012). Infection rates are increased in cool high-rainfall areas and the year following a wet spring (Asaad et al., 2013). Currently most grown cultivars are susceptible to loose smut and resistance to this disease was not a breeding goal for conventional farming. It gains importance with the increase of organic production and the need for breeding for organic farming. At least 15 resistance genes to controlling race specific resistance to *U. nuda* have been identified (Zang et al. 2015; Legkun 2016). Un8 confers resistance to all known isolates of *U. nuda* and is one of the most effective resistance genes (Eckstein et al., 2002; Zang et al., 2015; Legkun 2016).

We started to breed spring barley (*Hordeum vulgare* L.) for organic farming around 20 years ago and used some loose smut resistance sources recommended by K.-J. Mueller (2006). The most broadly used was *Un8* resistance from Canadian hulless barley variety CDC Freedom, as well as *Un15* resistance from Mik-1 (Russia), Un6 resistance from Steffi (Germany) and Keystone (USA), *Un3/6* resistance from Jet (Ethiopia) and unknown resistance from Pervonez (Ukraine).

Artificial inoculation was performed to screen breeding lines usually in F_5 or F_6 generation during anthesis time, as soon as anthers opened. At least three ears per sample were inoculated, pricing every flower with a droplet of syringe containing locally collected *Ustilago nuda* population spore suspension (Mueller, 2006).

Molecular marker was developed from the candidate *Un8* gene, and co-segregation tested with loose smut resistance in a recombinant inbred line (RIL) barley population. The new marker *Un8*-PvuII was used to genotype 98 lines of the previously phenotyped RIL population CDC Freedom/Samson and had 99% correspondence with resistance/susceptibility as determined by artificial inoculation (Sokolova et al., 2022).

One of our breeding directions is development of heterogeneous material. Selection of *Un8* resistant plants from two barley composite cross populations (CCP-5 and CCP-5HB) using molecular marker was done in order to obtain improved populations with resistance to loose smut. Agronomic performance of selected and unselected populations was compared during two seasons (in two and one location, respectively).

First Un8 resistant candidate variety from organic breeding program is currently under official testing. It is hulless barley with comparatively high beta-glucan content and large kernels and seems to have comparatively low susceptibility to covered smut (*Ustilago hordei*), which is usually a problem for *Un8* resistant hulless barley genotypes. A hulled barley breeding line with possible *Un15* and/or *Un3/6* resistances with stable grain yield, good nitrogen use efficiency and competitive ability against weeds is under consideration for registration. More than 10 other advanced lines (F_7-F_{10}) with possible presence of previously mentioned resistance genes are currently under evaluation.

The amount of *Un8* resistant individuals was higher in hulless than hulled CCP population (37 vs 19%). One-year agronomic testing in two testing sites showed on average 10% yield reduction for hulled *Un8* CCP in 1st year but 10% yield improvement in 2nd year, whereas hulless *Un8* CCP provided significant yield increase by 13% in one of two sites in 1st year and 23% improvement in the 2nd year in comparison to the unselected populations. Both *Un8* CCPs provided lower powdery mildew resistance.

Future perspectives include molecular markers for other resistance genes besides *Un8* in order to perform gene pyramiding, as well as combining resistance to loose and covered smuts, which would be especially necessary for hulless barley.

Keywords

Spring barley, loose smut, organic agriculture, resistance breeding, marker-assisted selection

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Identification of novel seed treatments and adapted agronomic practices to control common bunt in organic wheat production

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Common bunt caused by the fungus *Tilletia caries* (DC.) *Tul.* & *C. Tul.* and *T. laevis J.G. Kühn* can lead to severe yield and quality losses in wheat (Mourad et al. 2018). The disease is of particular importance in organic farming systems, because the use of synthetic fungicidal seed treatments effectively suppressing the pathogen is not allowed, while permitted treatments often solely provide limited control, especially under high disease pressure (Ehn et al., 2022).

Currently, there is only a low number of common bunt resistant wheat varieties available (Mourad et al., 2023). In addition, bunt pathogens are able to overcome plant resistance (Ehn et al., 2022). Thus, a promising control strategy in organic wheat growing could be to combine seed treatments with appropriate agronomic management practices.

The main aims of this study are therefore to identify seed treatments able to reduce the common bunt severity in wheat and compliant with organic production standards and to validate their efficacy in field trials in combination with adapted agronomic practices.

Ten different seed treatments with products already registered in European input lists for organic production or having the potential to become registered in these lists were tested in several greenhouse experiments. The chosen products contained different types of active ingredients, including bacterial and fungal microorganisms, plant extracts, micronutrients, and natural polysaccharides.

In each greenhouse experiment, a synthetic chemical seed treatment (Coral® Extra with difenoconazole and fludioxonil as active substances) was used as reference treatment and two control treatments were included, consisting of non-inoculated and non-treated plants (water control treatment) and of pathogen-inoculated and non-treated plants (pathogen control treatment).

Every treatment comprised five replicates (i.e. five pots with five wheat seeds sown in each pot). Summer wheat variety Diavel was used in each experiment and one experiment additionally included the summer wheat variety Fiorina. For all treatments (except the water control treatment), seeds were artificially inoculated with *T. caries* prior to sowing. The common bunt disease severity (expressed as percentage of seeds showing common bunt symptoms) was assessed visually at the time of grain harvest (i.e. at the end of the wheat ripening stage).

Results from the different greenhouse experiments showed that several of the tested products could significantly reduce the common bunt severity on wheat when applied as seed treatment prior to sowing. The most effective seed treatment consisted of a formulated product containing bacteria of the genus Pseudomonas (not yet commercially available) and was able to significantly reduce the common bunt severity on seeds by 100% compared to the pathogen control treatment in two independent experiments (at a significance level of 0.05). Its efficacy was identical to the synthetic chemical reference treatment.

Four other products were able to significantly reduce the common bunt severity on seeds in at least one greenhouse experiment.

To conclude, this study could highlight the potential of organic seed treatments to provide an additional tool to control common bunt in wheat production. For the most promising treatments, validation of the results from the greenhouse assays under field conditions is currently ongoing.

These field trials also include testing the effect of agronomic practices, including different cover crop types, on the common bunt severity and first results are expected by the end of 2023.

Keywords

Tilletia caries, common bunt, *Triticum aestivum*, seed treatment, organic farming

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Microbiome signature of endophytes in wheat seed response to wheat dwarf bunt caused by *Tilletia controversa Kühn*

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This is the first study on the microbiome signature of endophytes in wheat seed response to wheat dwarf bunt caused by *Tilletia controversa Kühn*. Some antagonistic microbes suppressed the germination of teliospores of the pathogen significantly, which will provide clues for future studies against wheat dwarf bunt. Collectively, this research first advances the understanding of the microbial assembly of wheat seed upon exposure to the fungal pathogen (*T. controversa*) infection.

Wheat dwarf bunt leads to the replacement of seeds with fungal galls containing millions of teliospores of the pathogen *Tilletia controversa Kühn*. As one of the most devastating internationally quarantined wheat diseases, wheat dwarf bunt spreads to cause distant outbreaks by seeds containing teliospores. In this study, based on a combination of amplicon sequencing and isolation approaches, we analyzed the seed microbiome signatures of endophytes between resistant and susceptible cultivars after infection with *T. controversa*. Among 310 bacterial species obtained only by amplicon sequencing and 51 species obtained only by isolation, we found 14 overlapping species by both methods; we detected 128 fungal species only by amplicon sequencing, 56 only by isolation, and 5 species by both methods. The results indicated that resistant uninfected cultivars hosted endophytic communities that were much more stable and beneficial to plant health than those in susceptible infected cultivars. The susceptible group showed higher diversity than the resistant group, the infected group showed more diversity than the uninfected group, and the microbial communities in seeds were related to infection or resistance to the pathogen. Some antagonistic microbes significantly suppressed the germination rate of the pathogen's teliospores, providing clues for future studies aimed at developing strategies against wheat dwarf bunt. Collectively, this research advances the understanding of the microbial assembly of wheat seeds upon exposure to fungal pathogen (*T. controversa*) infection.

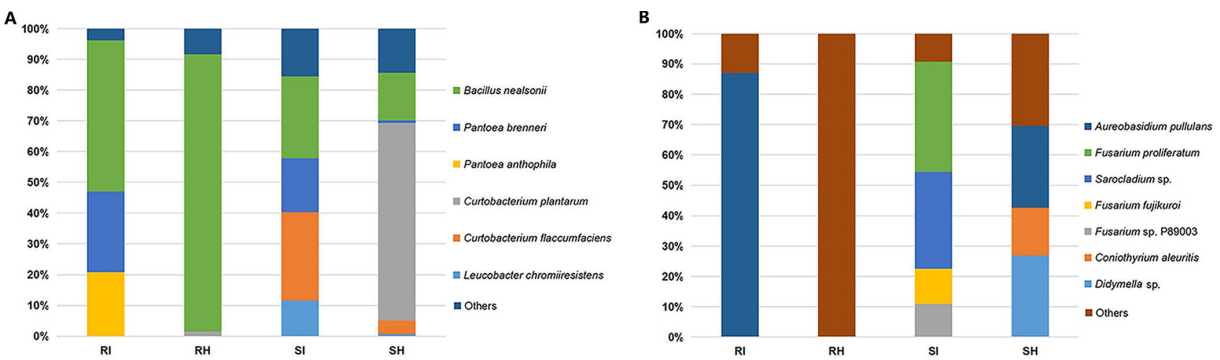

Figure 1: Total abundance of endophytic bacteria and fungi in different groups. (A) Total abundance of endophytic bacteria in different groups. (B) Total abundance of endophytic fungi in different groups. RI, *T. controversa*-infected resistant cultivar; RH, uninfected resistant cultivar; SI, *T. controversa*-infected susceptible cultivar; SH, uninfected susceptible cultivar

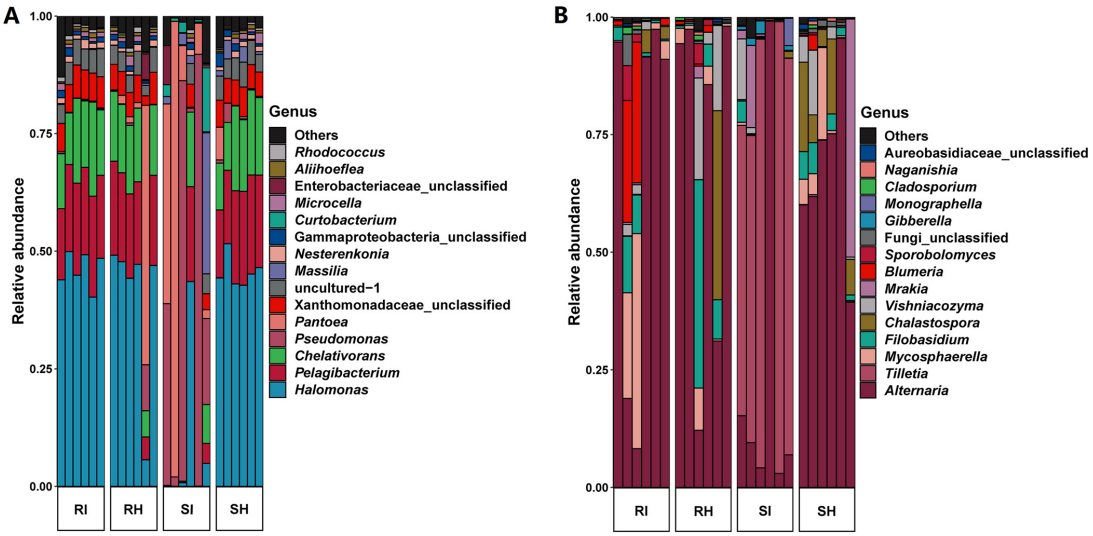

Figure 2: The relative abundances of bacteria and fungi at the genus level by amplicon sequencing. (A) The relative abundances of bacterial taxa in *T. controversa* infected and uninfected resistant and susceptible wheat samples. (B) The relative abundances of fungal taxa in *T. controversa*-infected and uninfected resistant and susceptible wheat samples. RI, *T. controversa*-infected resistant cultivar; RH, uninfected resistant cultivar; SI, *T. controversa*-infected susceptible cultivar; SH, uninfected susceptible cultivar.

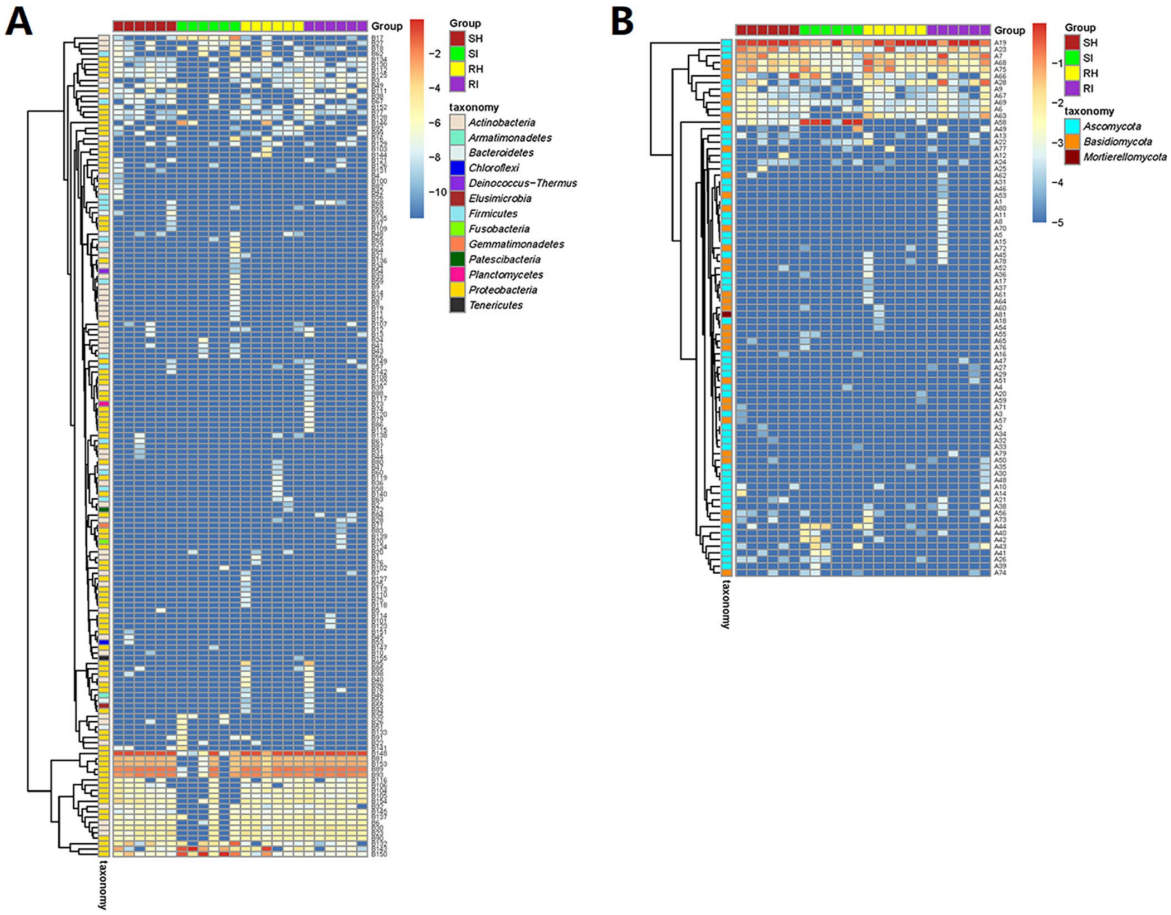

Figure 3: Heatmaps of enriched bacterial and fungal genera by amplicon sequencing. (A) Heatmap of bacterial genera in all groups. (B) Heatmap of fungal genera in all groups. The dendrogram shows the clustering tree. The box was colored based on the relative abundance data. The red color shows a higher abundance of genus, and the blue color shows a lower abundance. The number on the right corresponds to Table S7 in the supplemental material. RI, *T. controversa*-infected resistant cultivar; RH, uninfected

resistant cultivar; SI, *T. controversa*-infected susceptible cultivar; SH, uninfected susceptible cultivar.

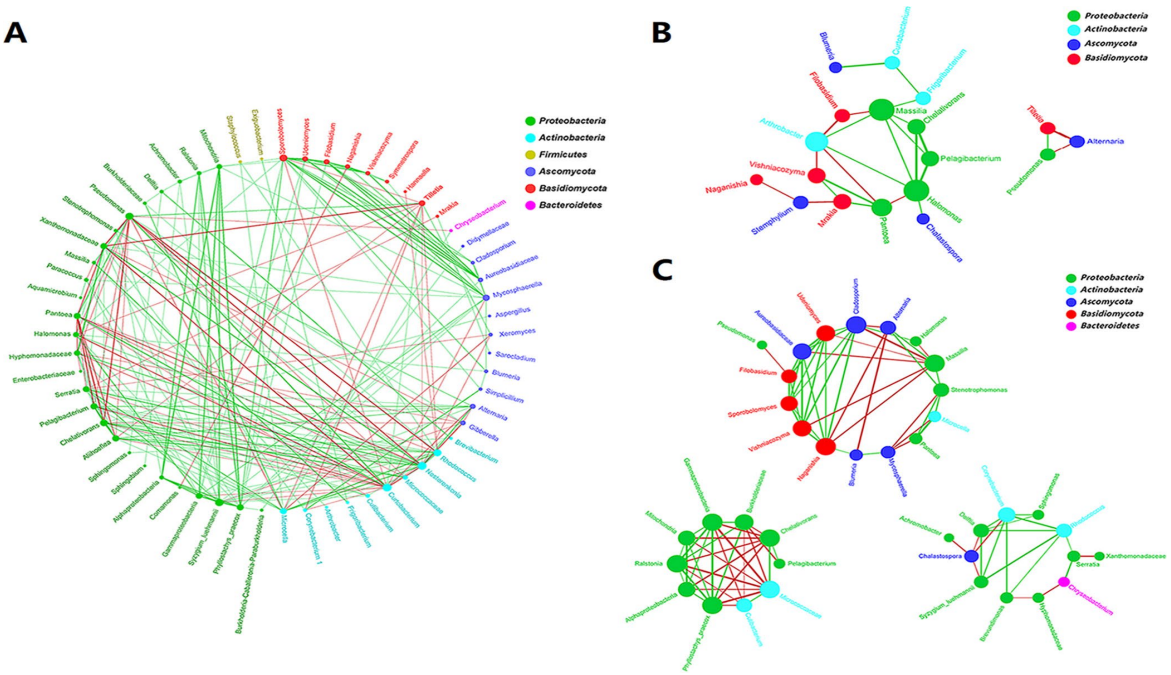

Figure 4: Visualization of microbial community co-occurrence network properties between uninfected and infected groups. (A) Potential keystone taxa based on bacterial and fungal network analysis of all groups. (B) Co-occurrence networks of diseased groups. (C) Co-occurrence networks of uninfected groups. Nodes represent ASVs and are colored by bacterial and fungal phyla.

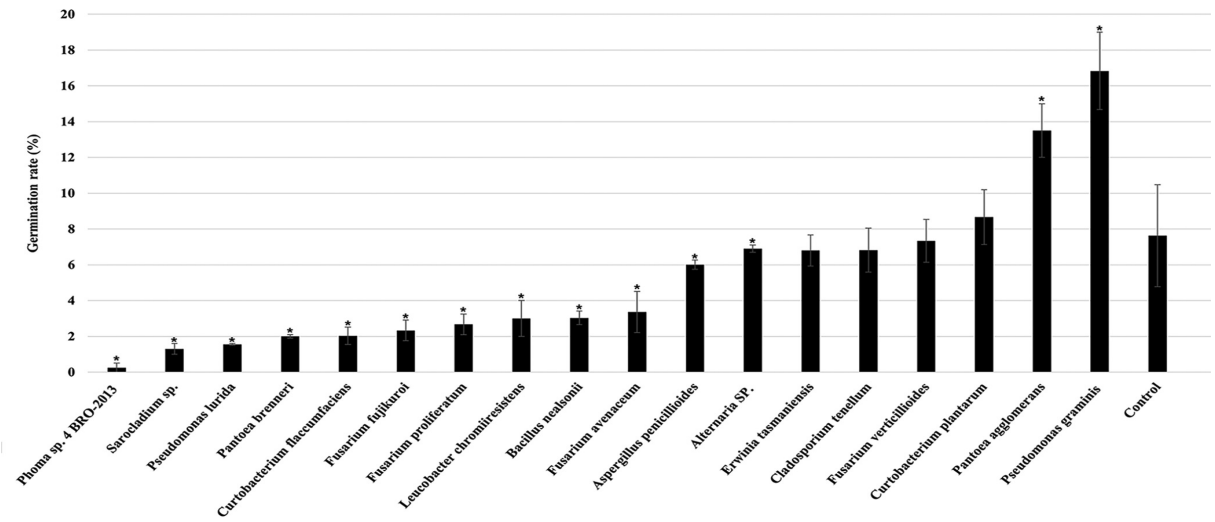

Figure 5: Effects of potential antagonist isolates on the germination of *T. controversa* teliospores. *, P , 0.05. RI, *T. controversa*-infected resistant cultivar; RH, uninfected resistant cultivar; SI, *T. controversa*-infected susceptible cultivar; SH, uninfected susceptible cultivar.

Keywords

Tilletia controversa Kühn, wheat dwarf bunt, amplicon sequencing, isolation, endophytes

Acknowledgments

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Genetic mapping of common bunt resistance gene *Bt1*

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Abstract

Common bunt is a soil and seed borne disease of wheat causing kernels to be replaced by sori balls of fungal spores. Common bunt is controlled in conventional agriculture by seed treatment but causes many problems in organic agriculture where seed cleaning, seed testing and plant resistance are the main tools available.

The *Bt1* gene was discovered by Fred N. Briggs in 1926 in the variety Martin (Briggs 1926). Briggs and Holton (1950) found that Martin has two resistance factors, M1 and M2. M1 was later renamed to *Bt1*, and M2 to *Bt7* (Metzger, 1970). The two Martin factors were located using nullisomic and monosomic lines to chromosome 13 = 2B (*Bt1*) and chromosome 16 = 2D (*Bt7*) (Sears, Schallearn, & Briggs, 1960). The Martin factor M1 was later found in Hussar, Odessa, White Odessa, Banner Berkeley, Regal, Sherman and Albit (Hybrid 128 x White Odessa) (E. N. Bressman 1932, Fred N. Briggs 1935, Fred N. Briggs 1929, E. N. Bressman 1931)

PI 554101/ Selection 2092 is used worldwide as the common bunt differential line for *Bt1* (Goates 2012).

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. A NIL with *Bt1*, NGB-11503, exist. Albit is the *Bt1* donor.

The mapping population consist of 1192 wheat varieties and breeding lines that were phenotyped and genotyped in different trials in the LIVESEED, BOOST and DIVERSILIENCE projects. Each line was postulated to have or not have *Bt1* based on phenotype data and information about their pedigree. 62 lines were postulated to have *Bt1* alone or in combination with other genes.

A GWAS with gene postulates as input produced signals at 2B and 2D and the detailed analysis revealed that markers at 2D in reality are positioned at 2B and part of the same signal. The marker at 3A, Kukri_c18420_705, is known to be associated with spike fertility and is probably due to unaccounted for population structure.

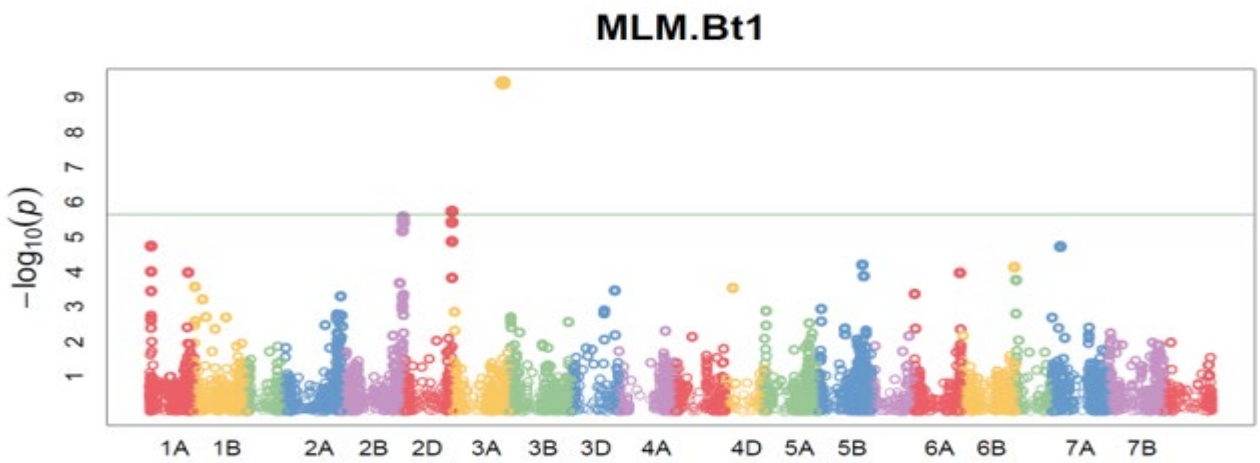

Figure 1: GWAS Manhattan plot made with the MLM method

Investigation of recombination events in the Starke II NIL / Starke II / Albit triplet together with a comparison of haplotypes for all *Bt1* postulated lines in our mapping population across an interval extending the GWAS interval at 2B allowed identification of the 11,043,031bp interval 799,983,180 – 811,026,211bp.

Markers were a match in 91% of lines postulated to have *Bt1*.

The false positive rate was 24%. For eight of these lines phenotyping cannot rule out that that they actually have *Bt1*, because resistance from other genes mask the effect of *Bt1*. Notable false positives was the *Bt2* differential line Selection 2075 / PI 554103 (Elgin x Selection 1403 / PI 554102). The *Bt11* differential line M82-2123 / PI 554119 also is a false positive as is the *Bt12* differential P78-24 / PI 554106 (1696 / PI 119333 x Elgin). Butaro is a parent of many lines in the population and is responsible for 26 false positives in them.

Table 1: Markers, Markers highlighted in brown define the interval. Markers highlighted in green can be used for MAS. Markers in orange are significant in the GWAS.

AX-158609666	A
Excalibur_c48404_59	C
wsnp_Ex_c15646_23969140	A
BS00065302_51	G
AX-94890379	G
BS00083998_51	G
Ra_c105904_187	C
Ra_c105904_1191	G
AX-158610188	A
AX-94808568	G
AX-158562114	C
Kukri_c49784_86	A
Excalibur_rep_c106698_235	A
BS00065264_51	G
Excalibur_c25043_357	A
Kukri_c900_1334	T
AX-95017900	G

Keywords

Wheat, gene mapping, common bunt, organic agriculture, resistance breeding, marker-assisted selection

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Sears, E. R., Schallearn, C., & Brigg, D. F. Identification of the Chromosome Carrying the Martin Gene for Resistance of Wheat to Bunt. Canadian Journal of Genetics and Cytology, September 1960, https://doi.org/10.1139/g60-026.

Genetic mapping of common bunt resistance gene *Bt7*

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The *Bt7* gene was discovered by Briggs and Holton (1950) as one of two resistance factors, M1 and M2, in the variety Martin. M1 was later renamed to *Bt1*, and M2 to *Bt7* (Metzger, 1970). The two Martin factors were mapped using nullisomic and monosomic lines to chromosome 13 = 2B and chromosome 16 = 2D (Sears et al. 1960). PI554100/ Selection 50077 is a selection from a cross of Martin with Elgin, and is now used worldwide as the common bunt differential lines for *Bt7* (Goates 2012). GWAS was conducted by using the MLM algorithm implemented in GAPIT R package (3.3), with kinship correction and population structure controlled by principal component analysis. The number of principal components was determined by the forward model selection implemented in GAPIT, using the Bayesian information criterion (BIC) to determine the optimal number of PCs to include. Physical positions for markers were supplied by Trait Genetics and also obtained by a BLAST against RefSeq 2.1.

We calculated linkage disequilibrium (r^2) in a region of approximately 20 Mbp, according to the physical map provided by Trait Genetics, around significant markers using the R package "gaston" (Gaston). To identify linkage groups within these markers, we clustered the resulting LD matrix hierarchically as implemented in R package "stats" (stats) treating the LD values as a distance matrix (dist = 1 - LD) and using method "ward.D2".

The mapping population consists of 1192 lines of which 301 was postulated to have *Bt7*, by pedigree analysis and multi race phenotyping (Borgen *et al* 2018), either alone or in combination with other genes. Gene postulates were used as binary phenotypic input to the GWAS.

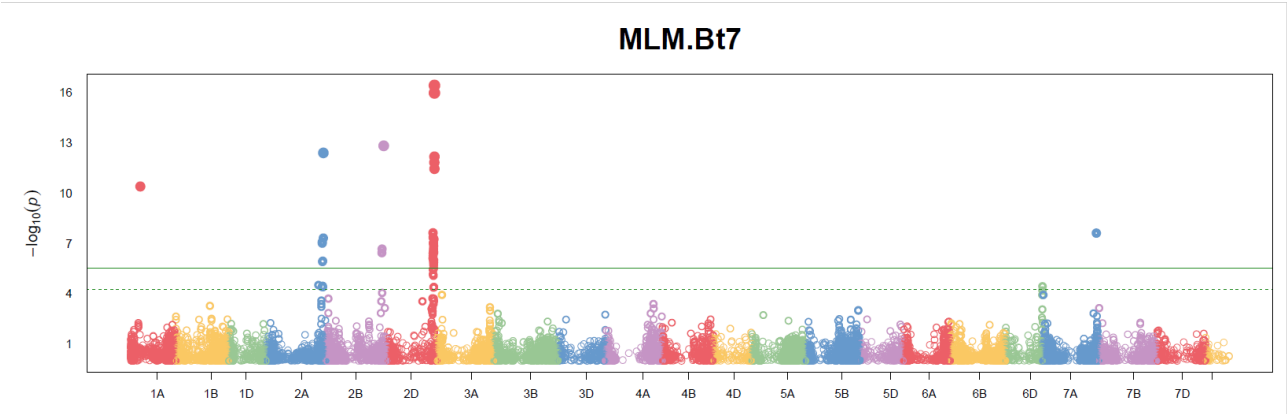

Figure 1: GWAS Manhattan plot made with the MLM method

The GWAS gave three distinct signals on the three homeologous chromosomes 2A/2B/2D. We investigated LD patterns in the regions around significant marker positions and aligned markers to the most recent IWGSC RefSeq v2.1 assembly. LD analysis showed that significant markers were in strong LD, indicating a single locus on one chromosome as the location of the resistance.

Three of the markers had unique alignments in the unfiltered physical map and all of them were at chromosome 2D on TG maps and BLAST results, suggesting that the signal is at chromosome 2D.

Table 1: Markers. The three markers in brown can be used to track the presence of *Bt7* and the two markers in green define the interval.

BS00110411_51	C
RAC875_c30919_311	G
RAC875_rep_c114621_200	C
wsnp_Ex_c42970_49408712	A
wsnp_RFL_Contig4402_5154408	A

Using only markers from the 2D signal, the full genotype – phenotype relation was explained. In summary all analyses suggested that only one signal exists and it is located at chromosome 2D. This is in agreement with the result from the nullisomic and monosomic analysis by Sears *et al* (1960).

GWAS resulted in a 7.33 Mbp interval 616,243,864 – 623,541,433 bp for the position of *Bt7*. Markers BS00110411_51 and wsnp_RFL_Contig4402_5154408 are flanking the interval and the three markers, RAC875_c30919_311, RAC875_rep_c114621_200 and wsnp_Ex_c42970_49408712 identify the *Bt7* haploblock.
The markers matched in 87 % of lines postulated to have *Bt7*, but at the same time they matched in 28% of lines not postulated to have *Bt7*. In many cases it is not possible determine whether these lines represent false positives because the presence or not of *Bt7* is masked by other genes, such as *Bt5*, *Bt9* or *BtZ*.

Keywords

Wheat, gene mapping, common bunt, organic agriculture, resistance breeding, marker-assisted selection

Acknowledgement

Phenotyping was done with support from the projects LIVESEED (H2020), BOOST (Organic RDD), DIVERSILIENCE (CoreOrganic Co-fund). Genotyping was supported by LIVESEED, Fonden for Økologisk Landbrug, Promilleafgiftfonden, and the European Consortium for Bunt Research.

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Trait Genetics. SGS INSTITUT FRESENIUS GmbH - TraitGenetics Section. https://sgs-institut-fresenius.de/en/gesundheit-und-ernaehrung/traitgenetics

Genetic mapping of common bunt resistance gene *Bt9*

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R. J. Metzger found a resistance gene in the line C.I. 7090 / PI 57143 that was different from *B1-Bt8* and hence named *Bt9*. C.I. 7090 / PI 57143 also contains *Bt7*. The *Bt9* differential line is R63-6968 / PI 554099 which is a selection from the cross Elgin / PI 178383.

Bt9 was first mapped to the distal end of 6DL in a biparental population of 91 double haploid (DH) lines. The parents were PI 554099 (National Small Grains Collection, Aberdeen, Idaho, USA), carrying resistance gene *Bt9*, and common bunt susceptible-wheat cv. Cortez (Wiersum Plant Breeding, Winschoten, The Netherlands). (Steffan et al. 2017).

Bt9 was later mapped to the interval 469,830,275 – 471,017,889 bp (IWGSC RefSeq v1.0 positions) (491,342,078 – 492,585,860 in RefSeq 2.1 positions) in a biparental doubled haploid population with parents IDO835 (*Bt9* donor) and Moreland by QTL mapping.

NordGen has a 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. One of the NILs possesses *Bt9* (NGB-11505). The source of *Bt9* in the Starke II *Bt9* NIL is MK 2-6244 / NGB 21193. Comments in Nordgen state that it is Selection M73-2260 from PI 264255, but this is a Durum wheat named Akbasak.

Our mapping population have 1192 lines and 92 of them was postulated to have *Bt9*. Gene postulation was made difficult by the fact that many lines could have *Bt8* + *Bt9* or *Bt9* + *Bt11* and the presence of *Bt8* or *Bt11* will mask the presence of *Bt9*. A GWAS using the MLM method implemented in the R-package GAPIT was run with gene postulation as input.

The GWAS signal consists of four tightly linked markers in the interval 490,706,843 – 490,708,662 bp (RefSeq 2.1 positions).

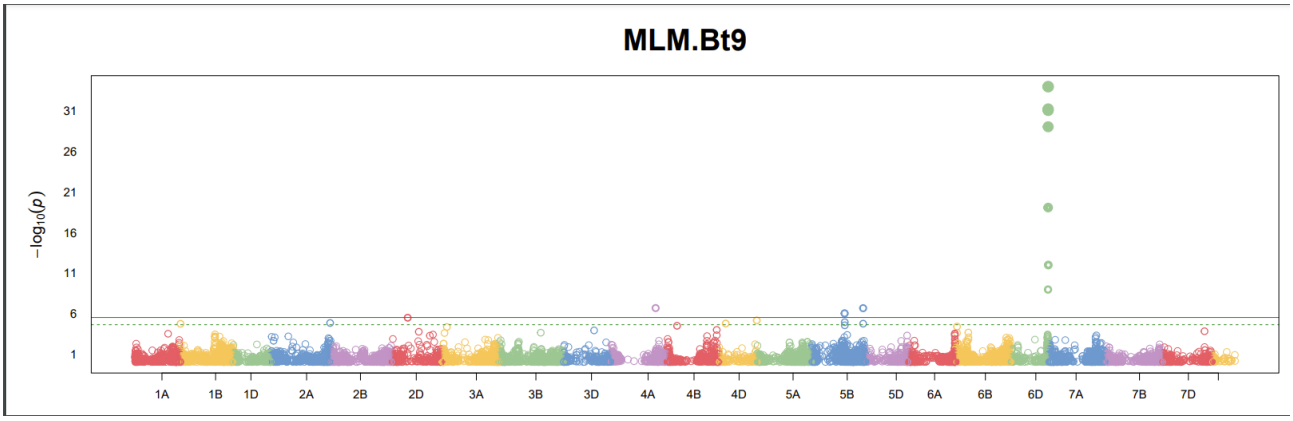

Figure 1: GWAS Manhattan plot made with the MLM method

An interval extending from approximately 10 Mbp below GWAS signal and to the end of the chromosome was used to search for recombination events in lines where one parent had *Bt9*, including the Starke II NIL. This lead to the discovery of the 1,005,254 bp *Bt9* candidate interval 490,336,412 – 491,341,666 bp.

Markers correctly identifies 84% of lines postulated to have *Bt9* alone or in combination with other genes. For lines postulated to have *Bt9* alone the hit rate is 98%. The false positive rate is 5%.

Some notable lines supposed to have *Bt9* that does not match the markers are the *Bt11* source Dimenit / PI166910, Malkesi / PI 178201 and Ark / CItr 15286.

Crosses for fine mapping should be designed to have maximum marker contrast inside the candidate interval, and multiple lines with 100% contrast are available, such as Ikarus (*Bt5*), Hypnos (*Bt5*) and T325-7717 (*Bt0*).

Table 1: Significant markers in GWAS.

wsnp_JG_c5646_2148296	C
Kukri_rep_c107605_164	T
wsnp_CAP8_rep_c4586_2232878	C
wsnp_CAP7_c1735_859875	G

Table 2: Markers for MAS. The six markers in green/orange can be used to track the presence of *Bt9*.

BS00022206_51	A
wsnp_JG_c5646_2148382	C
wsnp_JG_c5646_2148296	C
Kukri_rep_c107605_164	T
wsnp_CAP8_rep_c4586_2232878	C
wsnp_CAP7_c1735_859875	G
AX-94589700	A

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Genetic mapping of common bunt resistance gene *Bt10*

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

	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

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.

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 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.

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 6: Markers usable for *Bt10* MAS. The nine markers in green can be used to track the presence of *Bt10*. The two in brown define the interval.

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 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.

Acknowledgements

Phenotyping was done with support from the projects LIVESEED (H2020), BOOST (Organic RDD), DIVERSILIENCE (CoreOrganic Co-fund). Genotyping was supported by LIVESEED, Fonden for Økologisk Landbrug, Promilleafgiftfonden, and the European Consortium for Bunt Research.

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Preliminary genetic mapping of common bunt resistance gene *Bt13*

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Two kinds of plant resistance against common bunt exists. Race specific qualitative resistance following the gene for gene principle by Floor and non-race specific quantitative resistance. Until now seventeen genes named *Bt1-Bt15*, *Btp* and *BtZ* are known to confer race specific resistance and they make up the set of differential lines used to differentiate virulence races. The differential lines carrying *Bt14* and *Bt15* are the Durum varieties Doubbi and Carleton, while the remaining lines are *Triticum aestivum*.

The *Bt13* gene was identified and added to the differential line reference set by Blair Goates (Goates 2012). Thule III (PI 181463), not to be confused with the Swedish cultivar Thule III (NGB 6714) (Borgen 2014), is used as the differential line for identifying the *Bt13* resistance gene (Goates, 2012).

1192 wheat lines were phenotyped using a design described in Borgen et al (2018) and genotyped using TG25k SNP markers in different trials in the LIVESEED, BOOST and DIVERSILIENCE projects. Each line was postulated to have or not have *Bt13* based on phenotypic data and information about the pedigree. Our mapping population has strong population structure regarding *Bt13* because Thule III is a direct parent of most of the 64 lines postulated to have *Bt13*. For this reason there are a many sporadic significant markers scattered across chromosomes.

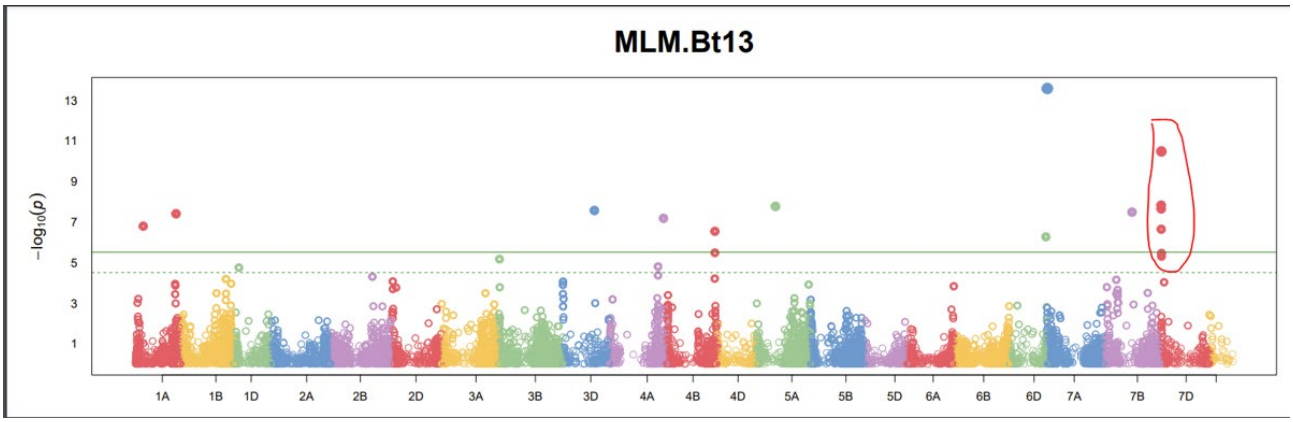

Figure 1: GWAS Manhattan plot made with the MLM method

At chromosome 7D in the interval 6,820,874 – 11,141,495 bp we have what appears to be the correct signal. The marker at 7A, which is the most significant of all, was investigated further and was found to placed at 7D by linkage analysis in the BOKU *Bt12* mapping population. Furthermore, a BLAST against RefSeq 2.1 had 7D 8,602,319 bp as a possible (and very likely) location. The likelihood that the 7D signal is the correct one is strengthened by this placement of the most significant marker in the middle of it.

The *Bt13* signal is identical to the *Bt12* interval mapped by Muellner et al. (2020). For this reason, the *Bt13* GWAS markers also matches in most lines containing *Bt12*. This 7D haploblock can be considered a signature shared between Bt12 (including Snow Mold Tolerant Selection 1 / CItr 14106 and Snow Mold Tolerant Selection 2 / CItr 4107) and *Bt13* containing lines and also with Erythrospermum 5221 / PI 572845, TU86-42-01-6 / PI 60848 and PI560603-sel-wclrs / PI 636148 having unknown resistance.

A detailed analysis of cross-over events in lines having Thule III in the pedigree in an extended interval around the GWAS signal was done to get a candidate interval. The two Bt13 postulated lines SegThul LS180 and SegThul LS169 had recombination in the GWAS interval, between markers at 9,201,720 and 9,642,370 bp. The three lines SegThul-veksel LS158 (No *Bt13*), SegThul LS168 (*Bt13*) and SegThul LS173 (*Bt13*) had recombination in the GWAS interval, between markers at 9,201,720 and 9,642,370 bp and also between 5,005,433 and 5,357,634 bp. From these five lines we get the final candidate interval 5,005,433 - 9,642,370 bp.

The MAS markers match in 95% of *Bt13* postulated lines and the false positive rate is 7%. The low false positive rate is partly explained by the presence of very few *Bt12* containing lines in the mapping population.

Table 1: Significant markers at 7D

TA001746-1415	G
AX-158595238	T
Kukri_c80931_147	A
IAAV9104	C
AX-111707392	T
RFL_Contig1323_544	G

Table 2: Markers. Markers in blue define the interval. Markers in green can be used for MAS. Markers in red letters are significant in the GWAS.

AX-158555104	C
AX-94804328	C
Kukri_c37227_579	A
AX-95237430	G
Ra_c30952_531	T
AX-158544378	T
AX-94708419	G
TA001746-1415	G
AX-158595238	T
Kukri_c80931_147	A
IAAV9104	C

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Keywords

Wheat, gene mapping, common bunt, organic agriculture, resistance breeding, marker-assisted selection

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Genetic mapping of common bunt resistance gene *BtZ*

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Once among the most devastating wheat diseases, common bunt caused by *Tilletia tritici* and *T. laevis* was successfully eliminated as a problem by the invention of seed dressings with hexachlorobenzenes (HCBs) in the 1950s. During the past decades, a continuously increasing area of agricultural land has been converted to organic management, refraining from the use of chemical pesticide applications, including seed treatments. Therefore, common bunt as a primarily seed-borne disease is experiencing a limited come-back since no alternative and equally effective treatments to seed dressings are available. The most sustainable and efficient way to avoid yield and quality losses due to bunt infections is the use of resistant cultivars. Seventeen different resistance genes have been characterized so far, and fifteen of them have been mapped and are available for applied breeding.

BtZ is introgressed into *Triticum aestivum* from *Thinopyrum intermedium* via the line Hybrid 599 / W0480. The cultivar Zarya has Hybrid 599 in its pedigree and is the main source of *BtZ* in European breeding material (Sandukhadze et al 2021).

No differential line has been agreed upon and *BtZ* is not part of the standard differential set. Hybrid 599 and Zarya are obvious candidates.

Using 152 breeding lines, mainly from Cultivari, Germany, of which 103 where postulated to carry *BtZ* based on parental information and phenotypic results, for a GWAS gave a signal at 6D in the interval 3,118,642 – 4,572,453bp.

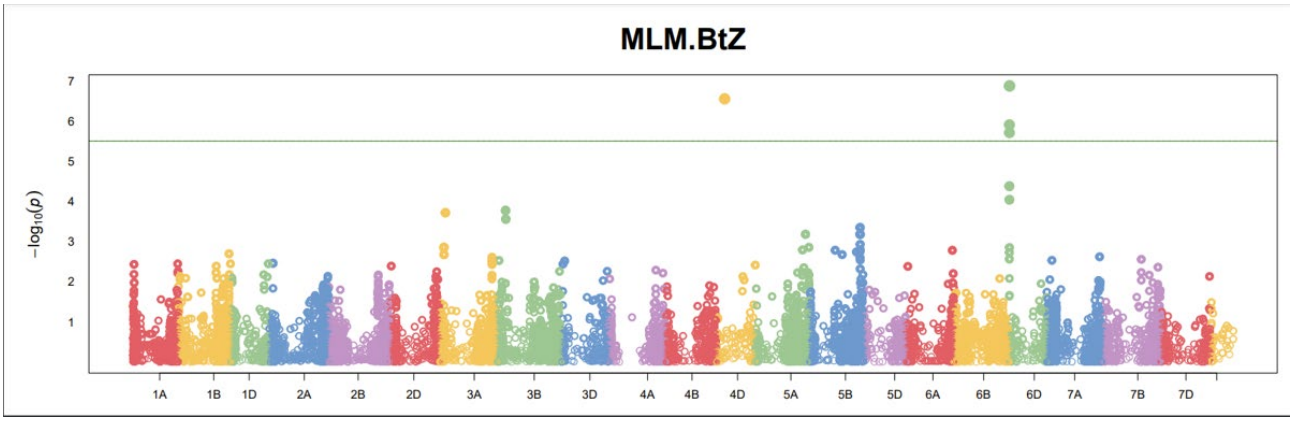

Figure 1: GWAS Manhattan plot made with the MLM method.

The single marker at 4D was later found to be located at 6D and linked to the other markers.

Our mapping population consists of 1192 lines and among them is a small RIL population with 14 lines from the cross of Inna (*BtZ*) and Starke II (*Bt0*). As the mapped interval is close to the telomere at 6DS, plenty recombination is happening and it was possible to narrow down the interval to 3,444,603 – 4,572,453 bp (wsnp_CAP12_c720_382116 - Kukri_c73802_205).

Markers from the GWAS were a match in 70% of the 122 lines postulated to have *BtZ* alone or in combination with other genes. There are multiple possible reasons for this low match rate. Many lines have other resistance genes and phenotyping cannot clearly detect the presence of *BtZ*. It also appears that a lot of recombination is happening and this breaks linkage between the markers and the gene. For lines having *BtZ* alone, the marker match rate is 86% indicating that gene postulations errors in lines with multiple genes are the main source of error. The false positive rate was 4%.

Table 1: Significant markers from GWAS. These four markers from the GWAS can be used to track the presence of BtZ in breeding material.

RAC875_rep_c118305_446	C
Excalibur_c7731_2743	G
AX-158531240	T
Kukri_c73802_205	A

Table 2: Markers for MAS. The two markers in brown define the interval and the one in green could be used for marker-assisted selection, but the markers from the GWAS are more effective.

wsnp_CAP12_c720_382116	G
BS00065960_51	C
Kukri_c73802_205	A

Bt10 has been mapped to an interval overlapping intervals for *BtZ* (Christensen and Borgen 2023). Alsp the phenotypic results are hard or even impossible to separate (Borgen et al 2023). Further investigation is needed to clarify this issue.

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Phenotyping was done with support from the projects LIVESEED (H2020), BOOST (Organic RDD), DIVERSILIENCE (CoreOrganic Co-fund). Genotyping was supported by LIVESEED, Fonden for Økologisk Landbrug, Promilleafgiftfonden, and the European Consortium for Bunt Research.

Keywords

Wheat, gene mapping, common bunt, organic agriculture, resistance breeding, marker-assisted selection

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Genome-wide association mapping identifies common bunt resistance loci in a wheat diversity panel

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Common bunt caused by *Tilletia caries* and *T. laevis* was successfully controlled by seed dressings with systemic fungicides for decades but has become a renewed threat to wheat yield and quality in organic agriculture where such treatments are forbidden. As the most efficient way to address this problem is the use of resistant cultivars, this study aims to broaden the spectrum of resistance sources available for breeders by identifying resistance loci against common bunt in bread wheat accessions of the USDA National Small Grains Collection.

A panel of 238 bread wheat accessions that has already been tested for dwarf bunt (DB) resistance by Gordon et al. (2020) was evaluated for reaction to CB in three subsequent years (2019-2021) at the experimental station of IFA Tulln, Austria. Sowing took place in autumn and all seed samples were artificially inoculated with a suspension of CB teliospores in a solution of methylcellulose in water. CB incidence was scored at the time of ripening in June and July by cutting open 75 randomly chosen spikes in each plot and calculating the percentage of diseased spikes. Genotypic data comprising 18953 SNP markers was used for genome-wide association mapping. Best Linear Unbiased Estimates (BLUEs) were determined for each genotype and trait, respectively, in individual years and across years using linear mixed models. To identify marker-trait associations (MTAs), compressed mixed linear models (CMLM, Zhang et al. (2010)) with compression through partitioning around medoids clustering (Kaufman and Rousseeuw, 1990) of the SNP marker data were fitted. For each data set (2019-2021 and across years) the optimum compression level was determined, allele calls were averaged across all genotypes assigned to a single cluster and the additive relationship matrix K was calculated based on this averaged, clustered marker data for all 238 accessions. To determine the influence of the ratio of susceptible vs. resistant genotypes in the population on GWAS-results, a leave-one-out cross-validation was conducted, excluding one of the six subpopulations identified in the panel by Gordon et al. (2020) (Fig. 1a) at a time from the analysis.

More than two thirds (66.8 %) of all lines were resistant with ≤ 10 % CB-NI. Across-year BLUEs for DB-NI in Logan, U.S., (Gordon et al., 2020) and CB-NI in Tulln, Austria, were positively correlated with $r = 0.37$ ($p \leq 0.0001$). Twenty accessions showed ≤ 1 % incidence across years for both bunt diseases. The bunt differential set comprising 20 genotypes was included in the test panel and DB-NI was higher than CB-NI for most of these known resistance sources except for the differential lines for *Bt8*, *Bt14*, *Bt15*, *BtP* and the unknown resistance in PI 173438. Broad-sense heritability of CB-NI across trials was 0.96. Accessions from Iran, Serbia and Turkey showed the highest proportions of susceptible lines whereas lines from the U.S. were for the most part highly resistant. The six previously identified subpopulations reacted differently to CB compared to DB (Fig. 1b). Four significant MTAs (CB-1A, CB-2B, CB-7A1 and CB-7A2) were found for CB- NI in at least two out of four data sets (2019-2021 and BLUEs across years). Allele frequencies ranged from 91.2 % to 94.1 %. Differences in average CB-NI levels between accessions carrying the resistant vs. the susceptible allele ranged from 29.4 % (CB-7A1) to 52.1 % (CB-2B).

A very high ratio (42 %) of all tested genotypes were highly resistant with ≤ 1 % CB-NI, leading to low variation in CB-NI and a rare nature of susceptible alleles. Therefore, a compressed kinship matrix was used to reduce matrix complexity and avoid overfitting. Of the four significant MTAs found across data

sets, CB-1A and CB-7A2 overlap with or are in proximity of regions previously reported to be associated with bunt resistance (Muellner et al (2020), Wang et al (2019)). Marker CB-2B has not been reported in any other publication, but wheat chromosome 2B is hypothesized to harbor bunt resistance gene *Bt1*. The robustness of our results is supported by the leave- one-out cross-validation method, showing that the unbalanced nature of the data set was handled well by the CMLM. The 20 accessions identified as being highly resistant to both diseases may provide valuable new genetic variation for research and breeding programs. More results of this work are available in Ehn et al. (2022).

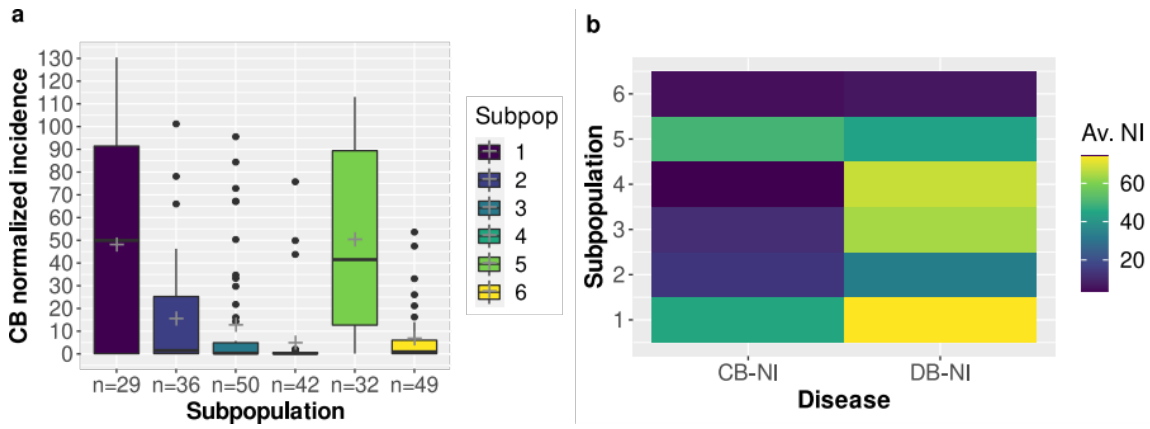

Figure 2: a Best linear unbiased estimates (BLUEs) across three years for CB-NI in percentages for genotypes assigned to different subpopulations. Number of genotypes per subpopulation is shown on the x-axis, crosses mark average CB-NI. **b** Heatmap comparing subpopulation averages of BLUEs across years for normalized incidence (NI) of dwarf bunt (DB-NI, Gordon et al. 2020) and CB-NI

Keywords

Tilletia caries, *Triticum aestivum*, genome-wide association mapping, resistance breeding, diversity panel

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Virulence patterns in a winter wheat panel tested with eight Austrian common bunt populations

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Common bunt (CB) caused by *Tilletia caries* can cause up to 50 % yield loss. Therefore, it is amongst the most destructive diseases in wheat (*Triticum aestivum*). Common bunt (CB) is a seedborne and, as recent studies have shown, as well a soilborne disease. Only one spore is sufficient to infect the seedling. If infection is successful, so-called bunt balls consisting of fungal teliospores are formed instead of regular grains. Because of the trimethylamine content of the teliospores, they spread an obnoxious fishy smell and the yield is consequently unusable. For several decades the disease has been controlled via chemical treatments. However, in low-input and organic agricultural systems, seed dressings with fungicides are not an option. Additionally, more and more people are concerned that the already limited selection of available chemical treatments will be further restricted by new EU regulations. These regulations could also let plant protection companies refrain from developing new products for the EU. Therefore, resistance breeding offers a sustainable solution to control the disease. The aim of this thesis is to monitor the virulence spectrum of the common bunt pathogen in Austria and to find durably common bunt resistant genotypes and crossing partners suitable for low-input and organic agricultural systems. For this purpose, field trials comprising 38 different winter wheat genotypes, including the common bunt differential set and putatively highly resistant breeding lines were conducted at the BOKU experimental station in Tulln. Bunt infections were provoked through seed inoculation with eight different Austrian common bunt populations prior to sowing. Each field plot was examined by visual assessment of the percentage of bunt infected spikes in June and July in the years 2021 and 2022. Thereby the following research questions were addressed: How similar or deviant are the virulence spectra of bunt pathogens collected in eight locations in Austria 2022 compared to 2021? Which bunt resistance donors, cultivars or experimental lines display stable resistance against all or most of the eight available bunt populations and can be recommended as crossing partner for resistance breeding?

The optimal growing conditions for CB in autumn 2021 led to an increase of the infection rates by about 50 %. Virulence patterns of bunt populations showed that the most aggressive sample in 2021 originated from Loosdorf whereas in the following year it came from Thening. The CB population “IFA Housekeeping” was the least aggressive sample in both years. Strong quantitative variation between bunt populations were observed for all four cultivars registered as bunt-tolerant or bunt-resistant (Tillstop, Tillsano, Tilliko and Tillexus) as well as for the differential lines for *Bt2*, *Bt3*, *Bt10*, *Bt13* and *BtP*. The largest part of the total variation observed for CB incidence across years was explained by the genotypic variance component. Genotype-environment interactions also had a significant effect in ANOVA analysis, whereas the amount of variation explained by the genotype-isolate interaction was significant but rather small.

Although a few genotype resistances from 2021 were cracked in 2022 there are still some genotypes that can be recommended as potential crossing partners for future breeding programs: P101.111.1 (IFA breeding line), PI 178383, P106.69.5 (IFA breeding line), Bonneville, PI 362695, 702-1102C, PI 560795-2_(9561.14), P106.51.2 (IFA breeding line), S7.4.1 (IFA breeding line), PI 166910. In general, all differential lines except for *Bt11* and *Bt12* can be regarded as (mostly) susceptible.

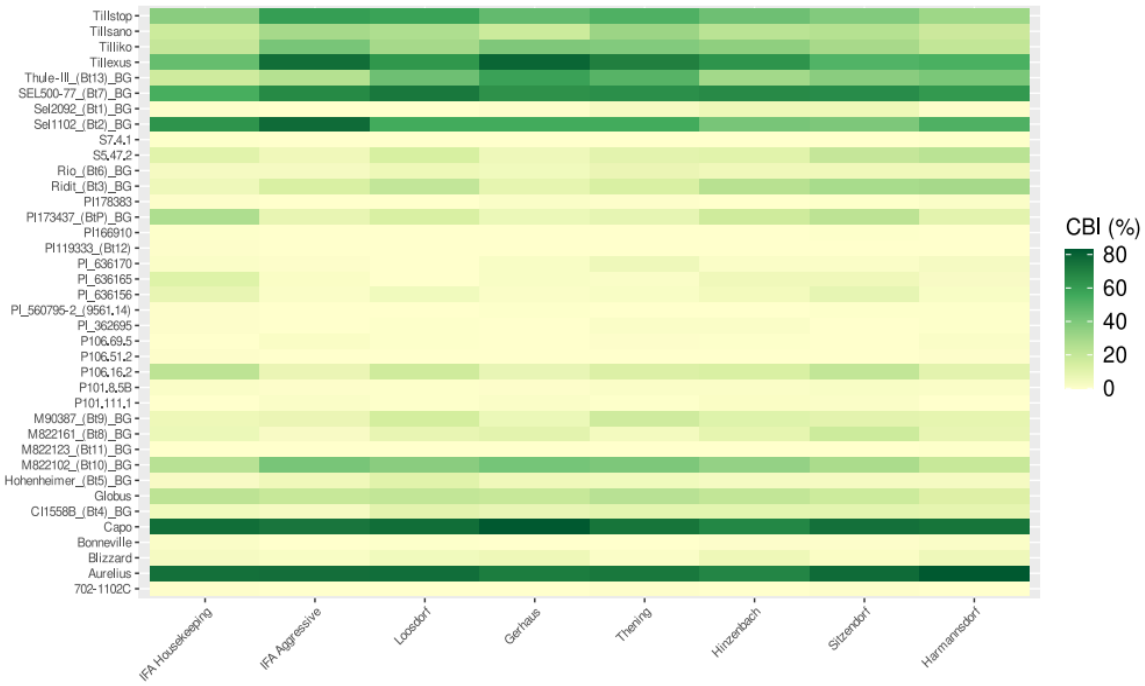

Figure 3: Heatmap of common bunt incidence (CBI) levels across two years (2021 and 2022) for a set of 38 test genotypes (y-axis) inlcuding the bunt differential set (all genotypes with *Bt*-designation indicated in brackets), resistance donors and breeding lines as well as cultivars adapted to mid-european growing conditions. Eight different Austrian common bunt populations were tested in both years, their names correspond to their geographical origin and are indicated on the x-axis.

Keywords

common bunt, virulence pattern, *Triticum aestivum*, resistance breeding, differential set

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HealthyMinorCereals spelt diversity panel reaction to rusts, powdery mildew, leaf blotch and common bunt

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Nowadays only three major species make up the majority of cereal food and feed. The minor species spelt wheat (*Triticum spelta L.*) was investigated for disease resistance traits as a first step of utilising ex-situ germplasm collections of spelt wheat in today's agriculture.

The risk of fungal diseases in spelt is non-negligible. Stem rust in particular can have a devastating impact, common bunt spores may contaminate the spelt seed lots.

The HealthyMinorCereals spelt diversity panel, including 80 genotypes of winter spelt, was tested for resistance to common bunt, leaf blotch, powdery mildew, leaf rust, stem rust and yellow rust. The reaction to diseases was investigated in field trials carried out at multiple European locations between 2013 and 2019. Resistance was assessed after artificial inoculation or natural infestation by visual scoring of symptoms. Presence of rust resistance genes was postulated using molecular markers.

Disease resistance genes are present in spelt and also new unknown and perspective resistance factors may be among them. Some genotypes showed a very low disease infestation in all environments tested, ´Sofia 1´ and ´Albin´ by common bunt, ´Sofia 1´, ´Riniken Weißkorn´, ´Zürcher Oberländer Rotkorn´ and ´Toess 5B´ by leaf blotch, ´Sofia 1´ by leaf rust and stem rust, ´Speltvete från Gotland´ by yellow rust. Multiple resistance to common bunt, leaf blotch, leaf rust, stem rust and powdery mildew has been found in ´Sofia 1´.It was found that presence of hulls plays a role as a passive resistance factor against common bunt. The influence of breeding period was examined and a differences between modern and old varieties have not been proven.

Data on the resistance of spelt varieties are beneficial for cultivation in organic farming conditions as well as for plant breeding against common bunt, leaf blotch, powdery mildew, leaf rust, stem rust and yellow rust. The results obtained can be used for the selection of suitable parental material for breeding spelt with improved disease resistance.

Keywords

spelt wheat, common bunt, leaf rust, stem rust, yellow rust, leaf blotch, powdery mildew

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