Book of Abstracts

XXI International Workshop on Bunt and Smut Diseases

May 5-6, 2021
BOKU Campus TULLN
virtual event
bunt.boku.ac.at

Hosted by:
University of Natural Resources
and Life Sciences Vienna

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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

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Status of *Tilletia* spp. in Serbia - past, present, future

Radivoje Jevtić | Vesna Župunski | Mirjana Lalošević

Institute of Field and Vegetable Crops, Serbia

Presented by: Vesna Župunski, vesna.zupunski@ifvcns.ns.ac.rs

Bunt of wheat is a fungal disease that occurs worldwide. *Tilletia caries* is predominant in Serbia, but the presence of *T. foetida* and putative hybrid of *T. caries* and *T. foetida* was also reported. Quarantine species for Serbia include both *T. contraversa* Kühn and *T. indica* Mitra. In the past, common bunt had a severe impact on wheat seed production due to the economic embargo imposed on our country. Outbreaks of common bunt were usually caused by sowing undeclared seed, discontinuation of fungicide treatments, and prevalence of disease-conducive environment. Nowadays, common bunt is successfully controlled in conventional production, but the survey on the presence of *Tilletia* spp. shown that in 151 samples, 74 % were contaminated with < 0.1 teliospore per seed, while 4 of the 16 commercial seed samples were contaminated above the threshold level of 0.01% determined for mercantile wheat by the Ministry of Agriculture, Trade, Forestry and Water Management. These findings indicated the great potential for seed infection and contamination with *Tilletia* spp. in Serbia which could pose a serious trait for organic production. In addition to that, 3.3 % of samples were contaminated with teliospores with prominent gelatinous sheath and reticulation exceeding 1.5 µm. It was assumed that these teliospores belong to either *T. contraversa* or *T. bromi,* but because of morphological characteristics overlapping, their identification was not possible. The low number of teliospores per seed samples disenable molecular identification either, indicating that more attention should be paid to development of molecular techniques suitable for discrimination of *T. contraversa* from grass bunts in seed quality testing. It is usually reported that climate-change-driven environment affects agricultural production through impact on crop growth and plant response to combined abiotic and biotic stressors. However, changes in pathogen population could also affect wheat production. In Serbia, later-season infections with *Tilletia* spp. were determined assuming to be caused by a new race of *T. caries*. Great variability in susceptibility response of artificially infected non-resistant varieties in fungicide efficacy trials was determined, as well as non-stable resistance/susceptibility response of commercial varieties tested for resistance in field conditions in different localities within an interval of eight years. Only variety Lasta has shown a stable resistance response. This indicated that plant-environment interaction could influence a broad range of susceptibility reactions to *Tilletia* infection and that more investigation is needed to predict the risk of bunt occurrence in wheat production.

Common bunt: study of prevalent virulences in France and development of a resistance test for registration in the French Catalogue of common wheat varieties

Orgeur Geoffrey[1] | Cadot V.[1] |Baldwin T.[1] | Gombert J.[2] | Fontaine L.[3] | du Cheyron P.[4] | Bruyère J.[5] | Oste S.[5] | Champion J.[6] | Maigniel J.P.[7] | Mailliard A.[7] | Grimault V.[1]

[1] GEVES Beaucouzé, 25 rue Georges Morel, F-49071 Beaucouzé | [2] FNAMS, Impasse du Verger, 6-49800 Brain sur l'Authion | [3] ITAB, 9 rue André Brouard, CS 70510, F-49105 Angers Cedex 02 | [4] Arvalis Institut du Végétal, Route de Châteaufort, ZA des graviers, F-91190 Villiers le Bâcle | [5] FREDON Nord Pas-de-Calais, 265 rue Becquerel, BP 74, F-62750 Loos-en-Gohelle | [6] CA de la Drôme, Ferme expérimentale d'Etoile, 2485 route des Pécollets, F-26800 Etoile sur Rhône | [7] GEVES, Unité expérimentale de l'Anjouère, F-49370 La Pouëze

Presented by: Geoffrey ORGEUR, geoffrey.orgeur@geves.fr

Common bunt is a re-emerging seed-borne disease particularly affecting organic cereal farming. GEVES, throughout a new research project CASDAR named ABBLE, in collaboration with ITAB, FNAMS, CA26, FREDON and Arvalis, developed and adapted a pathotest in controlled conditions with early detection by PCR on plantlets. This early detection, allows the evaluation of pathogen transmission rates from seed to plant and the determination of threshold damage and was correlated with expression of the symptoms on ears in the field (3 years of tests). This pathotest was used for evaluation of varietal resistance.

The program ABBLE was created to study the variability in populations of common bunt in France and to identify the main virulences present in France. Evaluation of presence of *Tilletia* spp. in French territory proved that *Tilletia caries* was identified as the predominant species in 26 isolates collected with 100% occurrence, compared with *T. foetida*, at 15.4%. Twenty strains of *T. caries* were selected to identify their virulence, from their behavior on a differential host range, revealing a predominant virulence on Bt-7 resistance gene, with a frequency of 48%, followed by Bt-2 (24%) and Bt-15 (19%).

An early varietal resistance test was carried out by artificial contamination of seeds with a representative isolate and qPCR at the 2-3 leaf stage to quantify *Tilletia sp*. DNA in plantlets. This early test (7-8 weeks) was found to be well correlated with the adult field test (8 months), based on visual notation of the bunted ear rate (r_{Pearson}= 0.89) in 3 years of trials (2018, 2019 and 2020).

This resistance test using the predominant virulences is now taken into account for the registration in the French Catalogue of soft wheat varieties for organic farming. It is also used by breeders who want to study resistance of their varieties. It will allow the selection of resistant varieties which can replace chemical seed treatments or be combined with alternative treatments.

The development of an early, rapid and reliable resistant test will provide breeding with a new tool for screening resistant varieties. These new tools, adaptable to other pathotest, are currently available to control wheat common bunt.

Development of a qualitative and quantitative qPCR assay to detect teliospores of *Tilletia controversa* in wheat seed samples

Monika K. Forster[1] | Somayyeh Sedaghatjoo[2] | Wolfgang Maier[2] | Berta Killermann[1] | Ludwig Niessen[3]

[1] Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding | [2] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics | [3] Technical University of Munich, TUM School of Life Sciences, Chair of Technical Microbiology

Presented by: Monika K. Forster, monikaforster@online.de

In wheat-growing areas worldwide and especially for countries trading seeds internationally, the reliable detection of wheat bunt species is essential. Regarding the best choice of variety and seed treatment as well as due to quarantine regulations the causal agent of dwarf bunt of wheat, *Tilletia controversa* Kühn, must be distinguished from other *Tilletia* species. Determination and quantification of the teliospores in seed lots is usually performed by combining the filtration method according to the International Seed Testing Association (ISTA) Working Sheet No 53 with a microscopic examination. However, distinguishing the species by means of their morphological characteristics is challenging and requires highly experienced staff. As an alternative to classical methods, molecular methods can help to facilitate reliable species determination.

We have developed a probe-based qPCR assay to detect and quantify teliospores of *T. controversa* in seed samples. The species-specific DNA region was obtained by comparison of 16 *Tilletia* genomes. The most promising primer pairs and probes were tested against 224 specimens of *Tilletia* spp. and further fungal pathogens infecting wheat, including 34 *T. controversa* specimens. Accuracy of the test was 98.21 % with 1.79 % false positive results. All *T. controversa* specimens were specifically detected. Taking advantage of cloning, the specific DNA fragment was multiplied to be applicable as an internal standard for quantitative real-time PCR (qPCR). Analyzing spore suspensions, the limit of detection (LOD) was 580 spores in total, which is equivalent to 1.36 spores per kernel (s/k). To simulate a naturally infested seed sample, defined amounts of teliospores of both *T. controversa* and *T. caries* were added to cleaned and sterilized wheat grains and correctly determined by the new qPCR assay. Usefulness of the assay was demonstrated by testing 64 naturally infested wheat seed samples with the official filtration method used in German seed testing laboratories and the new qPCR assay in parallel analyses. Teliospores of *T. controversa* were determined in 40 samples by filtration method. Three of them contained 0.4 s/k or less and were not detected by qPCR. A closer look at the official German threshold value of 20 s/k reveals that 95.31 % of the samples were consistently determined with both methods. In three cases, the quantified number of *T. controversa* teliospores analyzed by qPCR was above the threshold while filtration method obtained numbers below 20 s/k. Thus, the developed qPCR assay is a promising tool to be used in seed testing laboratories in future.

Inter- and intraspecies genomic comparison of *Tilletia caries*, *T. controversa*, and *T. laevis*

Somayyeh Sedaghatjoo[1] | Monika K. Forster[2] | Berta Killermann[2] | Wolfgang Maier[1]

[1] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany | [2] Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Vöttinger Straße 38, 85354 Freising, Germany

Presented by: Somayyeh Sedaghatjoo, ssedagh@gwdg.de

In central Europe, three *Tilletia* species are reported to infect wheat species (*Triticum* spp.). *Tilletia caries* and *T. laevis* cause common bunt and *T. controversa* is the causal agent of dwarf bunt of wheat. These historically notorious pathogens have been re-emerging in recent years especially in organic farming. The three species are closely related and can mainly be distinguished from one another by morphological characters of their teliospores. Little is known about their intra- and interspecific genomic variation including what genetically sets these three species apart.

We compared 16 whole-genome sequences of 5 *T. caries*, 7 *T. controversa* and 4 *T. laevis* isolates of which 11 were generated by us. In general, the three species were very similar in genome size and number of predicted genes. No sign of expansion or reduction of repetitive elements were found in any of the species. Additionally, most of the predicted secondary metabolite gene clusters, carbohydrate-active enzymes, secreted proteins, and half of the predicted effector-like proteins were conserved and shared across all 16 isolates. Only a few species-specific genes (<1%) were identified for each of the three species. However, the functions of these genes mostly remained unknown. In non-repetitive regions, the number of single nucleotide polymorphisms (SNPs) and small insertions or deletions (indels) were lowest within T. *laevis* isolates, with max. 0.52 SNPs/kb and 1.09 bp indels/kb and highest within *Tilletia controversa* isolates with max. 1.47 SNPs/kb and 2.48 indels bp/kb. We also observed extensive sequence conservation between the two species of *T. caries* and *T. laevis*, which was in a similar range as observed within each of the two species (0.51 SNPs/kb and 1.04 indels bp/kb on average) suggesting that *T. caries* and *T. laevis* have either just recently diverged or could even be conspecific. At the same time, both species showed equal genetic distances to *T. controversa.* These observations correlate with the fact that the causal agents of common bunt cause identical disease symptoms and have the same germination requirements as well as infection biology, which all differ from those of dwarf bunt.

Transcriptome analysis of wheat spikes infected by *Tilletia controversa* Kühn and characterization of histological changes in resistant and susceptible varieties

Zhaoyu Ren | Tongshuo XU | Dandan Qin | Wanquan Chen | Li Gao

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China

Presented by: Li Gao, xiaogaosx@hotmail.com

Wheat dwarf bunt is caused by *Tilletia controversa* Kühn, which is one of the most destructive diseases of wheat worldwide. To explore the interaction of *T. controversa* and wheat, we analyzed the transcriptome profile of spikes of the susceptible wheat cultivar Dongxuan 3, which was subjected to a *T. controversa* infection and a mock infection. The results obtained from a differential expression analysis of *T. controversa*-infected plants compared with mock-infected ones showed that 10,867 out of 21,354 genes were upregulated, while 10,487 genes were downregulated, and these genes were enriched in 205 different pathways. Our findings demonstrated that the genes associated with defense against diseases, such as PR-related genes, WRKY transcription factors and mitogen-activated protein kinase genes, were more highly expressed in response to *T. controversa* infection. Additionally, a number of genes related to physiological attributes were expressed during infection. Three pathways were differentiated based on the characteristics of gene ontology classification. KEGG enrichment analysis showed that twenty genes were expressed differentially during the infection of wheat with *T. controversa*. Notable changes were observed in the transcriptomes of wheat plants after infection. The results of this study may help to elucidate the mechanism governing the interactions between this pathogen and wheat plants and may facilitate the development of new methods to increase the resistance level of wheat against *T. controversa*, including the overexpression of defense-related genes. Based on these results, we also found histological changes in the wheat roots, stems and leaves were much more severe in *T. controversa* infected susceptible plants than in infected resistant plants at the tillering stage (Z21). Specifically, we used scanning electron microscopy and transmission electron microscopy to characterize the histological changes at this stage in resistant and susceptible wheat cultivars infected by *T. controversa*. The root epidermal and vascular bundles were more severely damaged in the susceptible *T. controversa*-infected plants than in the resistant plants. The stem cell and longitudinal sections were much more extensively affected in susceptible plants than in resistant plants after pathogen infection. However, slightly deformed mesophyll cells were observed in the leaves of susceptible plants. With transmission electron microscopy, we found that the cortical bundle cells and the cell contents and nuclei in the roots were more severely affected in the susceptible plants than in the resistant plants; in the stems and leaves, the nuclei, chloroplasts, and mesophyll cells changed significantly in the susceptible plants after fungal infection.

Assessment of two quantitative trait loci for dwarf bunt resistance in winter wheat grown in Pacific Northwest of the USA

Jianli Chen[1] | Rawnaq Chowdhury[1] | David Hole[2] | Margaret Krause[2] | Tyler Gordon[3]

[1] Department of Plant Sciences, University of Idaho Aberdeen Research & Extension Center, Aberdeen, ID 83210 | [2] Department of Plants, Soils and Climate, Utah State University, 2325 Old Main Hill, Logan, UT 84322, USA | [3] USDA-ARS, Small Grains and Potato Germplasm Research Unit, 1691 S. 2700 W., Aberdeen, ID 83210, USA

Presented by: Dr. Jianli Chen, jchen@uidaho.edu

Dwarf bunt caused by *Tilletia controversa* is an endemic and recurring disease of winter wheat in regions with long periods of snow cover. In these regions, severe loss of grain yield and quality can occur in epidemic years. Breeding resistant cultivars is an effective approach to combat this disease and molecular marker-assisted breeding is an alternative to conventional breeding based solely on phenotypic selection of resistant progeny. In this study, markers tightly linked with two previously identified major QTL on chromosome arms 6DL and 7DS were used to screen winter wheat cultivars that were adapted to the Pacific Northwest. Additionally, Dwarf bunt resistant lines with both QTL were selected for future release and the marker haplotypes of the two QTL will be used as a tool for future selection of dwarf bunt resistance. Candidate genes for the 7DS QTL were tentatively identified and will be validated with CRISPR-CAS9 in the future study.

Mapping common bunt resistance loci in Canadian wheat cultivar AAC Tenacious

Raman Dhariwal | Therese Despins | Reem Aboukhaddour | Harpinder S. Randhawa

Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada

Presented by: Harpinder S. Randhawa, harpinder.randhawa@canada.ca

Common bunt or stinking smut, caused by fungal pathogens *Tilletia tritici* (syn. *T. caries*) and *T. laevis* (syn. *T. foetida*), is one of the devastating priority one wheat diseases for western Canadian breeding programs. It is a seedborne disease and significantly reduces grain quality and yield as the pathogen replaces the healthy wheat kernels with a black mass of spores, referred to as bunt balls. Current wheat cultivars lack an adequate level of resistance to common bunt which has caused several outbreaks in recent years throughout north America. In order to identify novel sources of common bunt resistance, a doubled haploid populations was developed utilizing common bunt susceptible female parent cv. AAC Innova and highly resistant male parent cv. AAC Tenacious. This population along with parents and checks were screened for disease severity in field in common bunt disease nursery in 2017 and under controlled conditions in greenhouse in 2018 in Lethbridge, Alberta, Canada. This population was genotyped using 90K Infinium iSelect SNP assay and a high density genetic linkage map was constructed. The linkage map along with phenotypic data was utilized for QTL analysis which identified two QTL for common bunt resistance on wheat chromosomes 3A and 5A. Together, these QTL explain up to ~ 18% phenotypic variation for common bunt. Interestingly, where 3A QTL was contributed by AAC Innova, 5A resistance QTL was contributed by AAC Tenacious. This population is again being grown in common bunt disease nursery in 2021 in Lethbridge to generate adequate disease severity data to be utilized for QTL mapping. The identification of these new sources of resistance will facilitate marker-assisted breeding for common bunt resistance.

Association mapping for common bunt resistance in wheat

Philipp Matthias Steffan[1] | Anders Borgen[2] | Anna Maria Torp[3] | Søren K. Rasmussen[3] | Gunter Backes[4]

[1] UCPH, current KWS | [2] Agrologica | [3] University of Copenhagen | [4] University of Kassel

Presented by: Søren K. Rasmussen, skr@plen.ku.dk

Common bunt is a seed borne disease of wheat whose importance is anticipated to increase with a growing organic seed market which, in addition to seed phytosanitary measures, relies on genetic resistances towards common bunt. Genome wide association studies have been proven a useful tool in the detection of genetic polymorphisms underlying phenotypic trait variation in wheat. We screened 248 wheat accessions for two years for their resistance reactions towards common bunt. The majority of lines exhibited high levels of susceptibility towards common bunt, but 25 accessions had less than 10 % infection. Using Diversity Array Technology (DArT) markers for genotyping and correcting for population stratification by using a compressed mixed linear model, we identified two significant marker trait associations for common bunt resistance, designated Q Cbt-cph-2 B and Q Cbt-cph-7 A, located on wheat chromosomes 2 B and 7 A, respectively. We show that genome wide association studies are applicable in the search for genetic polymorphisms for resistance towards rare plant diseases in the context of an under-representation of resistant lines.

Network-based GWAS of race-specific resistances to common bunt (*Tilletia caries*) in wheat

Jelena Baćanović-Šišić | Dominic Dennenmoser | Gunter Backes

University of Kassel, Section of Organic Plant Breeding and Agrobiodiversity, Germany

Presented by: Jelena Baćanović-Šišić, bacanovic@uni-kassel.de

With the goal of developing molecular markers for the range of resistance genes (Bts) against common bunt (*Tilletia caries)* in wheat*,* in total 445 wheat varieties and breeding lines were phenotyped in field trials in 2018 and 2019, and resistance response to inoculation with 7 to 11 different common bunt races was recorded. Out of them 274 selected lines have been genotyped with 25K chip at the TraitGenetics GmbH and obtained data were used in the genome-wide association study (GWAS).

The network-based GWAS has been selected as the most appropriate statistical methods for association because it can handle ordinal, non-Gaussian continuous data, and mixed discrete-and-continuous data. It also adjusts for the effect of all other SNPs and phenotypes while measuring the pairwise associations between them, and therefore accounting for population structure by definition. The resulting genotype-phenotype network is a complex network made up of interactions among: (i) genetic markers, (ii) phenotypes, and (iii) between genetic markers and phenotypes. Maximum value of the standardized and log+1 transformed data of both years have been used in the GWAS. Lines represented in just one experimental year as well as those Vr that are tested on small number of lines were excluded from the calculation, leaving only five Vr (Vr13, Vr10, Vr5, Vr2 and Vr3) to be included in the data analysis.

GWAS revealed in total 120 SNP markers associated ($r^2 \leq 0.2$) with the response of the wheat to the infection with the common bunt. There were 43 SNPs for the Vr13, 31 for the Vr5, 29 for the Vr10, 14 for the Vr2 and 3 for the Vr3 (Figure 1). Associated markers are distributed over whole wheat chromosome, with the highest number of markers located on 1A (14 SNPs), 7A (12) and 2B (11) (Table 1).

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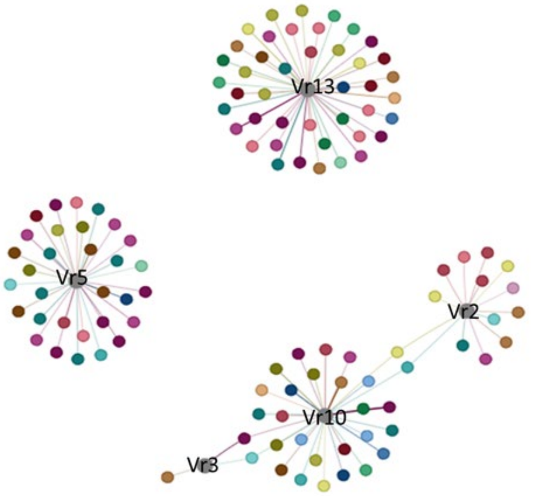

Figure 1. Strain-marker interactions of 274 genotyped lines of wheat. Each edge represents the connection between two nodes, where each node is a SNP marker (colored, where each color represent a chromosome on which marker is located) or a virulence race (grey).

Table 1. Distribution of associated SNPs with different virulent races over wheat chromosome.

	Vr10	Vr13	Vr2	Vr3	Vr5	Total
1A	3	5		1	5	14
1B	2				1	3
1D	2	2	3			7
2A	1	1			4	6
2B	1	4	1		5	11
2D	1		1	1	1	4
3A	1	3				4
3B	1	3	2	1		7
3D			1			1
4A	2	1			1	4
4B	1	3				4
4D	1	1				2
5A	1	3			1	5
5B	1	1				2
5D		1			1	2
6A	3				2	5
6B	2	1	3		1	7
6D	3					3
7A	2	3	1		6	12
7B	1	5	1		1	8
7D		6	1		2	9

Is *Bt8* located at Chromosome 6D and closely linked to *Bt10*?

Dennis Kjær Christensen | Anders Borgen

Agrologica, Houvej 55, DK-9550 Mariager, Denmark

Presented by: Dennis Kjær Christensen, dennis@fastcode.dk

The biparental populations with the parents PI178383/Midas and PI178383/Rainer described in (Hagenguth, 2016) segregated, as there were two common bunt resistance genes in PI178383. As PI178383 is known to carry Bt8, Bt9, Bt10 and a minor factor, it is possible to speculate that two of the genes must be tightly linked. Bt9 and Bt10 are known to be located at chromosome 6D, but are not tightly linked. This leads to the hypothesis that Bt8 is closely linked to Bt10 or Bt9.
Comparing and filtering genotyping data for 6D from the TG15K array for PI178383, Hansel, Bt8 differential M82-2161, Bt9 differential M90-387, Bt10 differential M82-2102, Stava, Starke II Bt9 NIL, Starke Bt10 NIL revealed that Bt8 and Bt10 may be located closely together at the short end of 6D in the interval 1,773,421 – 6,342,831 bp.
Crosses with Pi178383 has led to several bunt resistant varieties, including the Swedish variety Stava. Stava miss the marker for Bt10 (J. G. Menzies, 2006) and Stava-crosses never gives lines that are infected with races virulent to Bt10. Stava has markers in the interval developed by Steffan et al 2017 indicating Bt9. Dwarf bunt at Gotland, Sweden, is virulent to Bt8 but not to Bt9 (Mascher et al 2016). Crosses with Stava is resistant to dwarf bunt at Gotland, and crossing with Stava does give bunt resistant lines of which some are resistant to dwarf bunt at Gotland (including Hellfrida), and some are not (including Magnifik). It is therefore most likely that Stava have both Bt8 and Bt9, whereas Magnifik has only Bt8.
A new race of *Tilletia laevis* has developed in Sweden virulent to Stava, and therefore must likely virulent to Bt8 and Bt9. This development jeopardize the strategy for Swedish bunt resistance breeding, but will in future make it easier to identify resistance genes by reactions to local virulent races.

BOKU and NordGen kindly provided genotyping data.

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Reaction of Czech registered varieties and sources of resistance to common bunt and dwarf bunt

Veronika Dumalasová

Crop Research Institute, Praha 6 – Ruzyně, Czech Republic

Presented by: Veronika Dumalasová, dumalasova@vurv.cz

In the Czech Republic, common bunt [caused by *Tilletia caries* (DC.) Tul. & Tul. (syn. *T. tritici* (Bjerk.) G. Winter in Rabenh.) and *T. foetida* (Wallr.) Liro (syn. *T.laevis* Kühn in Rabenh.)] and dwarf bunt (caused by *Tilletia controversa* J.G. Kühn) occur on cereals. Prevention is the strongest weapon against common bunt and dwarf bunt. The most common preventive procedure for protection against common bunt and dwarf bunt involves seed treatment and using healthy certified seed.

Measures to reduce fungi in organic farming include seed analyses, seed cleaning, seed treatment registered for organic farming and use of varieties with low susceptibility to bunt. Breeding for bunt resistance offers efficient and sustainable plant protection strategy.

Reaction of Czech registered varieties and sources of resistance to common bunt and dwarf bunt has been tested in 2019-2020 in field experiments with an artificial infection. Disease reaction of common bunt spore mixture used for artificial inoculation was tested on a standard differential set of cultivars and lines carrying various genes of resistance (*Bt0-Bt13* and *BtP*). The average levels of infection were 52,0% (*Bt0*), 39,8% (*Bt1*), 14,9% (*Bt2*), 18,7% (*Bt3*), 50,0% (*Bt4*), 33,1% (*Bt5*), 7,4% (*Bt6*), 24,7% (*Bt7*), 0,0% (*Bt8*), 0,3% (*Bt9*), 0,0% (*Bt10*), 0,0% (*Bt11*), 1,0% (*Bt12*), 0,0% *Bt13*), (0,0% *Btp*). The varieties Genius, WPB Calgary, Rivero, Nordika, Bonanza and Safari registered in the Czech Republic were less sensitive to common bunt, with less than 10% infestation. The varieties Genius and Bonanza also had lower dwarf bunt infestation, not exceeding 1% (the infection pressure was lower in the dwarf bunt trials). Common bunt was not observed on varieties and breeding lines Aristaro, Blizzard, Bonneville, Deloris, Globus, Philaro, Quebon, Stava, SW Magnifik, Tilliko, Tillstop, UI SRG, Weston and V1-113-19 (breeding line with long glumes). Varieties Philaro, Stava, SW Magnifik and Weston were also free of dwarf bunt.

So far, we have been able to infect varieties of winter wheat, spring wheat, spelt wheat, durum wheat and several varieties of triticale. Barley, rye and oat infection has not yet been successful. Spelt wheat variety Sofia 1 was free of common bunt in five years of testing in CRI (2015-2019), both in hulled and dehulled variant. It was also free of dwarf bunt in 2016-2020 and may therefore offer a suitable genetic material for resistance breeding both to common bunt and dwarf bunt.

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Evaluation of Genomic Selection methods for dwarf bunt resistance in wheat

Margaret Krause[1] | Tyler Gordon[2] | David Hole[1] | Jianli Chen[3]

[1] Utah State University | [2] USDA ARS | [3] University of Idaho

Presented by: Margaret Krause, margaret.krause@usu.edu

By utilizing minor genes for resistance to dwarf bunt (DB), caused by *Tilletia controversa* J.G. Kühn, breeders can develop wheat cultivars with quantitative resistance that complement the larger-effect *Bt* resistance genes. Marker-assisted selection (MAS) strategies become less effective when breeding for quantitative resistance conferred by many genes of small effect. Genomic selection (GS) could therefore be useful to accelerate breeding for quantitative resistance to DB. We used a set of 292 bread wheat accessions from the National Small Grains Collection to compare MAS and GS breeding strategies for increasing DB resistance. Three modeling approaches were compared: multiple linear regression (MLR) using five trait-associated markers previously identified in a genome-wide association study of the panel, GS using genome-wide markers, and a GS model incorporating the same markers used in MLR as fixed effects (GS+FIX). Prediction accuracies of the GS model (0.51-0.90) were considerably higher than for the MLR (0.19-0.74), while GS+FIX showed similar accuracies (0.49-89) compared to GS. These results provide preliminary evidence that GS could be effective for improving DB resistance. Further studies to confirm these results in breeding populations and empirically test GS approaches for DB resistance breeding are warranted.

Summary on current research activities on resistance to bunt in winter wheat at the University of Natural Resources and Life Sciences Vienna

Madgalena Ehn | Barbara Steiner | Sebastian Michel | Iris Fallbacher | Anica Leonhard | Elisabeth Ritzer | Heinrich Grausgruber | Maria Buerstmayr | Hermann Buerstmayr

University of Natural Resources and Life Sciences Vienna, Institute of Biotechnology in Plant Production, Konrad Lorenz Str. 20, 3430 Tulln, Austria & Institute of Plant Breeding, Konrad Lorenz Str. 24, 3430 Tulln, Austria

Presented by: Magdalena Ehn, magdalena.ehn@boku.ac.at

We currently work on several research projects in connection to bunt resistance.

1) **Introgression project:** We develop a series of novel experimental lines harboring bunt resistance QTL from Bonneville, Blizzard or PI119333 in BC_2 or BC_3 genetic background composed of various regionally adapted winter wheat cultivars (and breeding lines). The project uses a combination of two selection techniques: on the one hand marker assisted foreground selection employing KASP markers linked to QTL on chromosomes 1A or 1B (from Blizzard or Bonneville, doi: 10.1007/s00122-020-03708-8) or *Bt12* on 7D (from PI119333, doi: 10.1007/s10681-020-02614-w) and on the other hand genomic assisted background selection for agronomic traits.

2) **Mapping *Bt11*:** segregating populations have been phenotyped during two seasons, and are now being genotyped using a 25k SNP platform for mapping *Bt11*.

3) **Association panel:** The same set of accessions that has been genotyped with a 90k SNP-chip and evaluated for dwarf bunt resistance by colleagues in Utah and Idaho (Gordon et al. https://doi.org/10.1007/s00122-020-03532-0) is being tested for common bunt resistance using a local bunt inoculum in Austria and will be subjected to association analysis.

4) We currently phenotype a ***Triticum tauschii* panel,** obtained from John Innes Centre Norwich, for common bunt response and aim to employ the data for association mapping of D-genome QTL.

5) Development of **TILLING** populations for the bunt resistance genes *Bt9*, *Bt12*, and the QTL on 1A and 1B. Lines possessing single *Bt* genes or QTL have been mutagenized using EMS and are currently being propagated for future phenotyping.

6) **Virulence pattern** of common bunt isolates from Austria. Based on the observation that a few previously resistant lines developed bunt symptoms in some fields we obtained a series of 8 different isolates – 6 from Michael Oberforster, AGES - and test these on a series of 40 lines with different resistance makeup, including the differential set in an inoculated field trial. This study is ongoing and should shed some slight on the mosaic of virulence in the bunt pathogen in Austria.

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Starke-II NIL based common bunt resistance gene mapping

Dennis Kjær Christensen | Anders Borgen

Agrologica, Houvej 55, DK-9550 Mariager, Denmark

Presented by: Anders Borgen, borgen@agrologica.dk

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. The NILs possesses *Bt1* (NGB-11503), *Bt5* (NGB-16106), *Bt6* (NGB-11504), *Bt9* (NGB-11505), *Bt10* (NGB-11506) and an unknown gene (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).

NILs and Starke II had all but 23-202 markers in 1-4 linkage groups in common. Linkage groups for each NIL was extracted and filtered against differential lines containing the Bt gene in question. Chromosomal locations of remaining markers were compared to suggested locations from the literature, enabling separating the major Bt gene from additional genes or QTLs.

- *Bt1* was mapped to chromosome 2B in the interval 789,867,236-801,253,554 bp.
- *Bt5* was mapped to chromosome 1B in the interval 285,345,287-285,608,205 bp. Corresponding markers in the bunt resistant varieties Globus and Tommi widely used in European bunt resistance breeding, confirms the phenotypic data indicating that these lines carry *Bt5* resistance (Borgen et al. 2018B).
- *Bt9* was remapped to chromosome 6D in the interval 469,248,476 bp – 469,919,743 bp (Steffan et al. 2017; Wang et al. 2019).
- The *Bt6* NIL (NGB-11504) seemed to be identical to the Bt9 NIL (NGB-11505) indicating miss information about this line, and no conclusion is therefore drawn for *Bt6*.
- *Bt10* was remapped to chromosome 6D in the interval 1,773,421 bp – 11,407,937 bp (Menzies et al. 2006).
- Unknown resistance in Starke NIL NGB-16160 is expected to be *Bt12* and was remapped to chromosome 7D in the interval 7,073,045 bp – 10,835,093 bp (Muellner et al., 2020).

All markers/intervals were analysed against the remaining 266 LIVESEED lines revealing that they were present in lines with and without the Bt gene. Whether the marker matches in other lines are linked to resistance or not is not known. An example is the presence of the *Bt12* markers in Thule III having *Bt13* (Goates 2012).

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Bunt resistant varieties, breeding and variety development at Saatzucht-Donau, Austria

Christian Gladysz[1] | Almuth E. Müllner[2] | Franziska Löschenberger[2]

[1]SAATBAU LINZ eGen, Schirmerstraße 19, 4060 Leonding, Austria | [2]Saatzucht Donau GesmbH & Co KG, Saatzuchtstrasse 11, 2301 Probstdorf, Austria.

Presented by: Christian Gladysz, Christian.Gladysz@saatbau.com

More than 25% of the agricultural acreage and more than 20% of the arable land in Austria is under organic crop management in 2021. In parallel with the rising acreage, a continuous growth of seed borne diseases has been observed in cereals over the past decade. Hereunder the most important diseases are common bunt (*Tilletia caries*) and loose smut (*Ustilago nuda*) on wheat and barley, respectively.

In general, we have two options to compensate for the ban of fungicide treatment in organic farming: resistance breeding and (physical) sterilization of seed.

Right now, no resistance sources are in use in barley breeding against loose smut. Due to its localization inside the kernel, lose smut also detracts from physical seed treatments successfully. The only way to control loose smut in organic farming is the use of certified seed and a restrictive seed hygiene regime during seed multiplication.

For common bunt, in contrast, a limited number of resistance sources are available and currently in use in wheat breeding programs. In our organic wheat breeding program, cultivars Tillexus and Tillstop were selected from crosses with Weston (donor of *Bt10*) and local high quality wheat varieties and carry the race specific resistance gene *Bt10.* While highly effective in AGES race mixture tests, *Bt10* provides only partial resistance when tested with individual inoculum samples collected from different locations in Austria by AGES. The race spectrum can override the protective effect of single race specific resistance genes (as *Bt10* in Tillexus/Tillstop, *BtZ* in Tilliko). Due to this scattered resistance/susceptibility picture, none of the two registered varieties can be entirely recommended to Austrian organic farmers to protect their wheat from common bunt. Consequently, Saatzucht-Donau is not marketing these cultivars to Austrian farmers. In contrast to Tillexus and Tillstop, the SZD winter wheat variety Tillsano, listed in the Austrian catalogue since 2020, was identified as bunt resistant “by chance” and its – to date not fully clarified resistance – is up to now highly effective in AGES race mixture as well as individual inoculum tests. Current breeding efforts of improved bunt resistant varieties for organic farming at SZD make use of race specific as well as non-race-specific resistance - and is particularly interested in combining these resistance types in order to provide highly and durably resistant varieties to organic farmers in near future.

In conclusion, breeding for common bunt resistance is important and necessary to compensate for the not available fungicide option in organic farming. Providing Austrian farmers with varieties which combine effective resistance, high end use quality and good agronomic characteristics has already been achieved and will continue with further registrations in near future. In contrast to bunt, the problems with loose smut remain genetically unsolved till now.

Studies on variability and host resistance for Karnal bunt of wheat in India

M. S. Saharan | Malkhan S. Gurjar | Niharika Mallick | Niranjana M. | Vinod | Rashmi Aggarwal

Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

Presented by: M. S. Saharan, mssaharan7@yahoo.co.in

India harvested record wheat production of 107.20 mt from an area of 30.50 mha during 2019-20. Two diseases of wheat *viz*., Karnal bunt and head scab or *Fusarium* head blight are likely to be affected by global climate change in near future. Karnal bunt (KB) of wheat caused by *Neovossia indica (Tilletia indica)* was first reported from India by Mitra in 1931. Since then, it has been reported in other countries of South Asia (Afghanistan, Pakistan and Nepal) and from other countries of the world including Iran, Iraq, South Africa, Mexico and USA. In India, KB has been mostly severe in the north western plains zone (NWPZ) which provides wheat to the national buffer stock. The disease has not been recorded in Maharashtra, Gujarat, Orissa, Assam, Meghalaya, Karnataka, Andhra Pradesh, Tamil Nadu and Kerala. During 2013-14, 32.20 % samples were found infected with KB but during 2014-15, analysis of 12295 samples revealed KB incidence in 42.67 % samples from NWPZ. But during 2015-16 crop season, disease incidence was less as out of 8732 wheat grain samples, 25.22 % were found infected.

Pathogenic variation among *Tilletia indica* isolates collected during 2011-2015 from different Indian states have been studied by inoculating on a set of host differentials and virulent isolates causing KB have been identified. These isolates have also been characterized through molecular markers. Monosporidial lines of *T. indica* have been developed and compatible monosporidial pairs were identified by inoculating on susceptible wheat variety. Evaluation done under artificially inoculated conditions of a large number of germplasms comprising of advanced lines, popular cultivars, synthetic hexaploids, indigenous and exotic germplasm, CIMMYT Karnal bunt nursery and introgressed lines developed by using Chinese Spring and *Triticum militinae* have led to the identification of many KB resistant sources. The expected onslaught of climate change is a worrisome aspect and thus systematic efforts are required to tackle Karnal bunt of wheat which is very important from trade point of view.

Bunt and smut diseases in cereal seed production

David Blöch

Raiffeisen Ware Austria AG, Raiffeisenstraße 1, 2100 Korneuburg, Austria

Presented by: David Blöch, David.Bloech@rwa.at

Sustainable agriculture needs healthy seeds. In order to ensure seed health, there are standardized methods and criteria to monitor seed quality. Officially certified seed has been revised during field- and laboratory inspections that guarantee high quality and healthy seed. In this respect, the Federal Office for Food Safety is the responsible authority in Austria and seed companies can be authorized to carry out certain tasks on its behalf.

In Austria, the most important bunt and smut diseases are *Tilletia caries* and *Ustilago nuda,* others than those two are rare. Generally, the majority of current varieties are medium to highly susceptible to smut or bunt, yet, in recent years there was a slight increase of seed infections with these diseases. In other countries like Slovakia, Croatia and Serbia, however, there are no such problems. Basically, seed treatment is feasible in both conventional and organic farming. Despite the expanding acreage under organic production, bunt and smut is not an 'organic topic' only, but also important in conventional farming. While reasons for bunt or smut infected fields may be associated with poor seed multiplication, weather conditions, farming without good practice and sometimes variety susceptibility, the main problem is the use of farm-saved seed. Only certified seed guarantees clean seed that is free from seed-borne diseases. Also highly tolerant varieties cannot sanitize or compensate farming without good practice. WEINHAPPEL and RIEPL (2013) compared farm saved seed with certified seed and proved a higher percentage of infections with bunt in farm-saved seed. The reason for that is that seed-borne infections and contamination of soils with spores of such fungi remain in the soil for a long time.

Untreated seeds are revised within the quality inspection of the cereal seed production and only those exceeding a minimum quality standard value are placed on the market - for example 300 spores per grain of *Tilletia caries* are the upper limit for winter wheat seed and values between 10 and 300 spores per grain must be treated with an effective and registered fungicide by professional machinery. Notably, in recent years, there was an increase of seed lots that showed bunt contaminations of more than 10 spores per seed and therefore had to be treated.

In conclusion certified seed secures seed health and underlines that a good seed certification process is necessary to control seed-borne diseases. Efficient control of smut and bunt diseases also requires good agronomic practice and wide crop rotations.

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Bunt management in cereal seed networks: the case of Rete Semi Rurali's Community Seed Bank in Italy

Bettina Bussi | Matteo Petitti | Giuseppe de Santis | Riccardo Franciolini | Riccardo Bocci

Rete Semi Rurali

Presented by: Matteo Petitti, Bettina Bussi, matteo.petitti@semirurali.net bettinabussi@semirurali.net

Rete Semi Rurali (RSR) is an Italian umbrella organisation and seed network promoting conservation and use of agricultural biodiversity in farmers' fields. It operates a Community Seed Bank (CSB), through which it collects, regenerates and distributes local varieties, old varieties and populations to farmers and growers throughout Italy.

Seed health is a prerequisite for the independence of farmers and to guarantee access to alternative seed sources, including farm saved seeds for own use or exchange within a network. Moreover, it represents a key element in building up trust-based relationships within seed networks and between a CSB and farmers.

For cereals such as wheat, in absence of accurate laboratory tests, it is crucial to proceed with seed treatment in order to avert the risk of important seed borne diseases such as Bunt (Tilletia caries). The bunt management protocol implemented by RSR for wheat and other susceptible cereals follows two main scenarios depending on detection of the disease.

If the disease is not confirmed, the strategy of risk prevention envisages: rotations between the crops (avoiding the succession of winter cereals), the preventative use of copper for seed dressing and disease checks at critical stages of the growing cycle and at harvest.

If evidence of bunt infection has been found or is suspected, the protocol is further extended to: mowing of the wild cereals surrounding the plot, washing of the seed and the farm tools with a sodium hypochlorite solution (2%) in order to inactivate the spores.

For wheat seed dressing of small lots against bunt in Italian conditions, the use of copper oxychloride is recommended. The application rate should not exceed 75 grams of metallic copper per 100 kg of seed treated. To facilitate dispersion it is advised to mix the seed with sunflower oil, in the proportion of 2 litres per 100 kg of seed. Once the oil is mixed, the copper powder is added: this operation guarantees contact with the dressing even in case of rain. Prolonged contact with copper oxychloride may reduce the germination rate of wheat seeds, therefore, the treatment should be done immediately prior to sowing.

When cereal seed accessions are distributed through the CSB, RSR provides farmers with instructions on how to treat cereal seed against bunt and additional information on how to detect the disease and best management practices.

Control of common bunt in organic wheat cultivation

Lars Wiik

Hushållningssällskapet Skåne, Sweden

Presented by: Lars Wiik, lars.wiik@hushallningssallskapet.se

In wheat, appropriate seed treatment fungicides have very good effect against seed-borne common bunt, caused by *Tilletia caries/tritici* or *T. laevis*. In organic farming, other methods than chemical pesticides must be used. In a project in Sweden some ecologically acceptable methods of treatment and sanitation against common bunt were tested. Brushing the seeds, seed treatment with mustard flour, herbal extracts and other substances were tested. In a total of seventeen field trials performed in different parts of Sweden and at one location in Denmark 2017–2020, many of these countermeasures proved to have a good effect and some very good effect. Unfortunately, some of the treatments significantly reduced the number of plants, but despite this, there were treatments well worth using at farm level.

Common bunt management on organic wheat: multi-factorial, knowledge-based

Stephanie Klaedtke[1] | Anders Borgen[2] | Miriam Lechner[3] | Angela Thueringer[3] | Judit Fehér[4] | Victor Petcu[5] | Jean-Pierre Bouchet[6] | Frédéric Rey[1]

[1] ITAB (FR) | [2] Agrologica (DK) | [3] AGES (AT) | [4] ÖMKI (HU) | [5] NARDI-Fundulea | [6] Ubios (FR)

Presented by: Stephanie Klaedtke, stephanie.klaedtke@itab.asso.fr

Techniques that allow the management of common bunt in organic farming - including sound crop management, observation, seed analyses and seed treatments – are well identified. When these are not put into practice, occurrences of common bunt still regularly devastate organic wheat crops. The research and development presented here follows two objectives: Firstly, collecting techniques available for bunt management and developing appropriate dissemination formats. Secondly, exploring new approaches, ranging from novel seed treatments to more holistic approaches to plant health.

An inquiry was performed over 4 years in the LIVESEED project, putting emphasis on the exchange of knowledge between European countries and across disciplinary boundaries. Meetings and workshops among researchers and practitioners allowed both for the exchange of knowledge on existing techniques for bunt management and for the emergence of unanswered questions. Field and laboratory trials were conducted to test and fine-tune seed treatments. Empirical experience with common bunt was explored through qualitative interviews. Particular attention was placed on farmers' varieties, which pose specific constraints.

As first outcome, several formats were developed for disseminating the knowledge on the combination of multiple practices that reduce the risk of common bunt, including workshops, websites, videos and Practice Abstracts. Specific knowledge gaps or frequent practical shortcomings were highlighted. We infer that reliable bunt management in organic farming requires specific knowledge on the disease cycle of the fungus, as well as practical and observation skills on behalf of practitioners.

As a second outcome, information on official thresholds for bunt spores in certified wheat seed in EU member states was retrieved, allowing for a comparison of national regulations, serving as basis for discussions on transparent rules for bunt management in organic wheat seed.

Thirdly, seed treatment examinations produced operational knowledge to optimize their application (storage of CERALL, use of vinegar).

Unanswered questions for future research include: To what extent is bunt present in organic cropping systems without causing symptoms? What role do soil microbiota play in suppressing bunt? What types of plant defense mechanisms come into play? Is genetic diversity reduced when breeding for resistant wheat cultivars? Are there allelopathic effects of previous or mixed crops in diversified crop rotation? Which farm-produced seed treatments may be efficient?