European Common Bunt Consortium –
News Letter No. 1 November 2022

Ed.: Dennis and Anders

A consortium on breeding for resistance to common bunt and related topics are forming, and this is the first newsletter issued from it.

We are planning an online meeting in December 2022 giving a status and to discuss topics of relevance to the ongoing bunt research. If you have not already done so, please fill out the Doodle to settle a date:
https://doodle.com/meeting/participate/id/bD1xxJYd
To prepare the meeting and to ease the conversation, we like to give you a written update.

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XXII International Workshop on Bunt and Smut Diseases in June 2022

For the past +40 years, international biannual workshops have been organised in Europe, America and a few times in Asia. From these events, an international network of bunt researchers has developed.
The next biannual workshop is planned for June 2023 in Tulln, Austria.

Hermann Bürstmayr <hermann.buerstmayr@boku.ac.at> from BOKU is chairman of the organising committee. A formal invitation will be distributed shortly. A homepage will soon be set up, most likely by updating the homepage from the former online workshop 2021 at https://bunt.boku.ac.at/

Status of the European breeding consortium for bunt resistance

Background

In July 2022, a minor workshop was organised by Agrologica, Denmark focusing on the ongoing research about development of genetic markers for bunt resistance at Agrologica. A small network is already established by researchers and breeders involved in the former LIVESEED project, ECOBREED project and DIVERSILIENCE project, but several new actors planning to start up new projects attended the workshop. Agrologica has phenotypic data from a field trial in 2022 that much exceeded the budget for genotyping in the DIVERSILIENCE project. The participants of the workshop therefore agreed to support the genotyping to develop a larger database for development of genetic markers, and to get first hand access to the collected data. In this way, the ongoing projects will be able to improve the ongoing research, and the new projects will be able to start on a higher level rather than starting up the projects from scratch.

So far, the cooperation between the ongoing projects and the new research and breeding projects has been informal, even though 9 different partners has agreed to support the genotyping with each 1500€. Once money is involved, a discussion on value for money and a formal agreements is a relevant topic to discuss. The informal agreement is that Agrologica will make available the phenotypic and genotypic data from this years trial, and also try to make the data from LIVESEED and ECOBREED available for the entire consortium. We will try to establish a common database accessible to all consortium members, and all consortium members are encouraged to upload additional phenotypic and genotypic data of relevance to bunt research. Based on the common dataset, the consortium members will assist each other with the analysis and development of markers that can be used in MAS and genomic selection. Until a formal platform is set up, the data will be shared by email correspondence.

Consortium partners

The consortium at present include:

1. **Agrologica**
    - Anders Borgen <borgen@agrologica.dk>
    - Dennis Kjær Christensen <dennis@fastcode.dk>
2. **BOKU**
    - Hermann Bürstmayr <hermann.buerstmayr@boku.ac.at>
    - Magdalena Ehn <magdalena.ehn@boku.ac.at>
    - Hermann Gregor Dallinger <hermann.dallinger@boku.ac.at>
3. **Breun**
    - Anja Hanemann <hanemann@breun.de>
4. **Saatzucht Donau**
    - Almuth Müllner <almuth.muellner@saatzucht-donau.at>
    - Franziska Löschenberger <franziska.loeschenberger@saatzucht-donau.at>
    - Herbert Bistrich <herbert.bistrich@saatzucht-donau.at>
    - Judit Koller <judit.koller@saatzucht-donau.at>
    - Monika Opalo <monika.opalo@saatzucht-donau.at>
5. **Dottenfelder Hof**
    - Carl Vollenweider <carl.vollenweider@dottenfelderhof.de>
6. **Cultivari**
    - Karl-Josef Müller <k-j.mueller@cultivari.de>
7. **GZPK**
    - Herbert Völkle <h.voelkle@gzpk.ch>
8. **Julius Kühn Institut**
    - Andreas Stahl <andreas.stahl@julius-kuehn.de>
    - Wolfgang Maier <wolfgang.maier@julius-kuehn.de>
9. **KWS**
    - Neele Wendler (KWS) <neele.wendler@kws.com>
    - Jenny Matthiesen <jenny.matthiesen@kws.com>
    - Monika Spiller (KWL DENO) <monika.spiller@kws.com>
    - Felix Bemm (KWS-KGaA DEEI) <felix.bemm@kws.com>
10. **Lantmännen**
    - Tina Henriksson <tina.henriksson@lantmannen.com>
11. **LfL**
    - Lorenz Hartl (LfL) <Lorenz.Hartl@lfl.bayern.de>
12. **NARDI**
    - Matilda Ciuca <mcincda@gmail.com>
13. **NIAB**
    - Kostya Kanyuka <Kostya.Kanyuka@niab.com>
    - Nick Fradgley <Nick.Fradgley@niab.com>
    - Phil Howell <phil.howell@niab.com>
14. **Nordic Seed**
    - Pernille Merete Sarup <pesa@nordicseed.com>
    - Ahmed Jahoor <ahja@nordicseed.com>
    - Jihad Orabi <jior@nordicseed.com>
15. **SLU**
    - Anna Berlin <Anna.Berlin@slu.se>

    - Fluturë Novakazi <fluture.novakazi@slu.se>
    - Mahbubjon Rahmatov <Makhbubdzhon.Rahmatov@slu.se>
    - Marwan Alamrani <marwan.alamrani@slu.se>
    - Therese Bengtsson <Therese.Bengtsson@slu.se>
16. **Wheat Gateway**
    - Andy Forbes <atty@area3.net>
17. **VÚRV**
    - Veronika Dumalasova <dumalasova@vurv.cz>

Ongoing research projects by consortium partners

Agrologica and NARDI

Agrologica is involved in BOOST project and the DIVERSILIENCE project, where also NARDI is a partner. NARDI is focussing on development of new sources of resistance transferred from synthetic hexaploid material, whereas Agrologica is focussing on identification of the known Bt-genes and development of new virulence races to be used in phenotyping trials.

BOKU

BOKU, Austria is involved in the ECOBREED project, where also Sz Donau and VURV, Czech Republic are partners. At present, BOKU is mainly working on Bt11 based on segregating bi-parental populations. BOKU has previously worked also on Bt12 and Blizzard/Bonneville resistance.

SLU and Lantmännen

SLU is mainly working on virulence in common bunt and dwarf bunt, and with a special focus on new races with virulence to Bt8 and Bt9.

KWS, JKI, LfB, Dottenfelder Hof and Breun

The partners have applied for a new project, but I'm not aware of the status of the application.

Progress from July-November 2022

Some partners have expressed a wish for a formal agreement for the consortium. Monika Spiller from KWS is working on it. However, those who has agreed to support the genotyping financially may expect an invoice from Agrologica within a few days.

It is still a bit unclear if the common database and communication platform should be open also for other researchers interested in bunt research.

The field trial presented at the workshop in Denmark 2022 has been further analysed during August-November and the gene postulations has been updated based on phenotypic data and parental information. Based on the updated gene postulations, 850 lines and varieties have been selected and sent to Nordic Seed for DNA extraction and once done, the DNA will be forwarded to TraitGenetics for genotyping on the 25k chip. We expect the result of this genotyping will be available in January 2023. Most of the 850 lines are from the phenotyping 2022, but some lines with low priority has been exchanged with parental or genebank material and other germplasm of more relevance to the analysis.

Based on the new genotyping, we expect to be able to improve the markers developed so far and to find positions of new genes. However, there will still be a long way to go before MAS for bunt resistance is working properly for the breeders. Therefore, more trials are needed.

More consortium partners than expected have agreed to financially contribute to the work. This has made it possible to genotype not only most of the phenotyped lines from 2022, but also to continue with new phenotyping in 2023. Some partners have therefore provided in total 219 new lines for phenotyping, including Sz-Donau (12), KWS (25), Cultivari (45), Dottenfelder Hof (25), NARDI (14), and BOKU (58). These will be phenotyped using 5-10 different virulence races selected based on the expected resistance in the material. Additionally 119 spelt lines are sown with just a single virulence race for a pre-screening to find new resistances. Some of the material (in particular from BOKU) has already been genotyped, and I hope funding for genotyping the rest can be organised during 2023.

Status for virulence races in Europe

In 2022, Agrologica tested 39 bunt races collected by JKI and Agrologica for virulence pattern. The results were presented at the workshop in Denmark in July. Spore samples of all the 39 races has been sent to SLU for genetic analysis, and SLU has added its own collection of Swedish bunt samples. The research is coordinated with previous similar research at JKI.

In many cases, a race will be virulent against none, 1, 2 or more resistance genes, but in some cases, there are minor infections of maybe just a single infected plant. In these cases, it is not possible to conclude if the race may include virulent spores, or it may be is a sign of the differential line not being 100% pure line. Therefore, spores have been collected from different varieties with different resistance genes to be able to conclude if rare virulences are present in the spore samples. These selected spores are now being tested for virulence on selected resistant varieties by Agrologica.

With the new virulences identified in the pan-European screening, the scary conclusion is that we expect to have virulence in Europe against all single genes, except maybe Bt11, Bt12 and BtP. Also virulence to Bt9 needs to be confirmed. As the differential lines for Bt11, Bt12 and BtP have multiple resistance genes, it is still uncertain if virulence to the single genes exist within Europe.

Status for development on marker intervals for each Bt-gene

Based on data from previous work, Dennis is working on mapping the different genes including the known Bt-genes. It is still a bit unclear how many genes are actually involved, as some of the genes may actually be the same genes, while other “genes” have turned out to be more than a single gene. The brutto list of the status of this work is summarised in the table.

		Interval			
Gene	**Chromosome**	**Min [bp]**	**Max [bp]**	**Size [bp]**	**Evaluation of the current mapping**
Bt1	2B	755.889.858	772.760.826	16.870.968	Poor
Bt2	7A?	Unknown	Unknown	Unknown	NA
Bt3	1A	498.451.021	506.854.738	8.403.717	Medium
Bt4	1B	Unknown	Unknown	Unknown	NA
Bt5	1B	92.540.335	319.569.717	227.029.382	Medium
Bt6	1B	16.381.317	34.942.421	18.561.104	Good
Bt7	2D	621.068.156	624.830.049	3.761.893	Good
Bt8	Unknown	Unknown	Unknown	Unknown	NA
Bt9	6D	490.337.515	491.431.473	1.093.958	Good
Bt10	6D	0	Unknown	Unknown	Unknown
Bt11	3B	498.268.609	523.277.044	25.008.435	Poor
Bt12	7D	0	21.000.000	21.000.000	Poor
Bt13	7D	0	4.631.914	4.631.914	Poor
Bt14	Unknown	Unknown	Unknown	Unknown	NA
Bt15	Unknown	Unknown	Unknown	Unknown	NA
Btp	Unknown	Unknown	Unknown	Unknown	NA
BtZ	Unknown	Unknown	Unknown	Unknown	NA
BtQ_5A	5A	Unknown	Unknown	Unknown	NA
BtQ_7A=BtH=Bt2?	7A	671.343.067	676.631.073	5.288.006	Good
Bt_Blizzard_7A	7A	717.923.349	735.886.510	17.963.161	Good
Bt_PI119333_4B	4B	Unknown	Unknown	Unknown	Poor
Erythrospermum 5221	Unknown	Unknown	Unknown	Unknown	NA
Lutescens 6028	Unknown	Unknown	Unknown	Unknown	NA
Hope-resistance	Unknown	Unknown	Unknown	Unknown	NA

New genes are likely to show up, and in particular from synthetic hexaploid wheat it is expected that unknown genes can be introduced into bunt resistance breeding.

Updating the use of Differential Lines

Based on genetic information and segregation studies, it has turned out that the original Bt-genes denominated by Blair Goates needs to be revised. The existing set of differential lines has a few problems including that

- The differential line for Bt5 (Hohenheimer) probably has not only Bt5 but also Bt7.
- The differential line for Bt11: (M82-2123/PI 554119) also has an additional gene, probably Bt9.
- The differential lines for Bt12 (Pi119333 and Pi554106) has also a factor at 4B.
- The differential line for BtP seems to segregate by crossing into at least two different unidentified genes.
- Some differential lines, and in particular Pi173439 (BtP), Thule-III (Bt13) and Pi119333 (Bt12) have extremely poor agronomic performance which makes field trials and maintenance of purity difficult.

Anders, Dennis and Magdalena Ehn are therefore working on developing a new set of differential lines with single gene resistances and improved growth habit. At present, we are working on 21 major resistance genes and have already proposals for new differential lines for most of them.

New line development

With the data available, it has been possible to map some of the resistance genes into intervals of markers, but not actually found the gene, and the intervals are in most cases too wide to be used for practical MAS or sequencing. Often this is due to chromosomes of the parental lines being monomorphic within the intervals. To decrease the intervals and come closer to the actual genes, two things can be done. Either screen a huge mass of lines to hope for recombination events, or to make new crosses with parents that are polymorphic within the specific interval in question.

All partners are encouraged to contribute with genomic data to identify recombination events and to find breeding lines, genebank accessions or varieties that are polymorphic to the resitant lines within the identified intervals. Please contact Dennis if you have datasets that can be screened to search for contrast.

Agrologica has good facilities for field trials, and other partners have better facilities for speed breeding. A cooperation between partners could optimise the process. Breun and SLU have so far volunteered to make new crosses. We would like to discuss a detailed plan with you at the online meeting in December.

For each gene, we need to find an interval, and for each interval, we need to make crosses to reduce it. So don’t worry. A lot of work needs to be done in the time to come, and there will be plenty of work to be done new projects and for everybody who wish to be included in future bunt research.

A crossing plan could be to deliver F6 RILs from 5 populations to Anders for sowing in field trials in the spring of 2024. The plan could be:

F1: April 2023 - October 2023 (optimal conditions)
F2: October 2023 – January 2024 (speed breeding)
F3: January 2024 – April 2024 (speed breeding)
F4: April 2024 – July 2024 (speed breeding)
F5: July 2024 – December 2024 (optimal conditions in order to produce 25 g of seeds)

Crosses are spring x spring or winter x spring to get spring lines for rapid advancement.

Prioritizing crosses can be done based on a number of criteria

- The effectiveness of the gene
- The amount of material present in the pipeline
- If multiple genes present in the cross - can all gene combinations be detected in phenotyping

Genes can roughly be divided into classes depending on how much known virulence exist against them

Gene Effectiveness Classes		**Genes**
A	No known virulence	Bt12
B	No known virulence on continent	Bt8,Bt9
C	Known virulence	Bt1,Bt3,Bt4;Bt5,Bt6,Bt10,Bt13,BtZ
D	Much virulence	Bt2,Bt7,Bt14,Bt15,BtHope
E	Unknown	Bt11,BtP

We have 835 lines in the genotypic pipeline from this years phenotyping. A further 450 resistant breeding lines from Agrologica are available. They have not been phenotyped with a full set of virulence races.

Based on material in the pipeline a rough estimate for the need of new material could look like this

Pipeline Need	
Bt1	2
Bt2	2
Bt3	2
Bt4	2
Bt5	3
Bt6	3
Bt7	1
Bt8	3
Bt9	3
Bt10	2
Bt11	2
Bt12	2
Bt13	2
Bt14	5
Bt15	5
BtP	4
BtZ	2
Bt_4B	2
BtB_7A	2
BtH	1
BtHope	5
BtX	5

Not all gene combinations can be separated in phenotyping. If no virulence exists against a gene, this gene will hide all other genes. With the new virulences identified in the pan-European screening, we have expect to have virulence against all single genes, except maybe Bt11, Bt12 and BtP. Also virlence to Bt9 needs to be confirmed. As the differential lines for Bt11, Bt12 and BtP have multiple resistance genes, it is still uncertain if virulence to the single genes exist within Europe.

This means that crosses involving, Bt9, Bt11, Bt12 and BtP should ideally only have these genes. For many two-gene combinations all four combinations can be distinguished in phenotyping by their infections patterns and in this case we get twice the value for our effort.

For crosses involving genes with known intervals maximal or at least good marker contrast should be present.

For crosses involving unmapped genes maximal or good genome wide marker contrast should be present (= minimal genomic relation ship)

Most of the genotyped lines are resistant winter types and for picking better crossing partners we need to genotype more susceptible spring (and winter) lines.

A prioritized spreadsheet with candidates for future crossings has so far been maintained by Dennis.

How to prioritise crossings should be discussed by the entire consortium in general terms, but also specifically about which crosses to do this year.

Plan for file/data sharing infrastructure

Sharing files by email is possible, but it is much better to have a common repository with version control. In this way, all can upload their data and we have a complete overview over who did what and when. The system must work for all kinds of files. It must be possible to keep data private to consortium members.
I (Dennis) can think of these systems:

1) Github
2) SourceForge
3) GoogleDocs

We must investigate them and others and pick one.

Material available for pre-breeding

To start up a new breeding program for bunt resistance, resistant lines must be available for crossings. We have therefore made an annotated list of material with known resistance genes that can be used for pre-breeding. Possible lines to be used as new differential lines are highlighted in green (Work in progress, to be discussed)

Name	ID	Source	Gene	Habit	Agro-nomic Perfor-mance	Comment
PG3540		Agrologica	Bt1	Winter	5	Very tall. Poor baking quality
Starke Bt1 NIL		Agrologica / NGB	Bt1	Winter	6	Tall and late, but healthy.
Selection 1102	PI 554097	GRIN	Bt2	Facultive	2	Differential line, Compactum
Bussard			Bt2	Winter	6	Variety developed in 1980'ies. Good baking quality
Skagen		Nordic Seed	Bt2	Winter	7	Short variety developed by Nordic Seed in the 1990'ies. Good baking quality.
Quebon			Bt2+Bt5	Winter	5	OK, but susceptible to mildew
M85-9	PI 554121	GRIN	Bt3	Winter	2	Differential line, Compactum
Ridit		GRIN	Bt3	Winter	3	Tall. From 1930'ies
Blizzard			Bt3+Bt6+Bt_7A	Winter	5	
Bonneville			Bt3+Bt6+Bt_7A	Winter	5	
Bonneville*Rainer		BOKU	Bt3+Bt6+Bt_7A	Winter	6	
M81-152	Pi 554115	GRIN	Bt4	Spring	2	Differential line, Weak straw.
Nebred			Bt4	Winter	3	Weak straw.
Starke Bt5 NIL		Agrologica	Bt5	Winter	6	Tall and late, but healthy.
Tommi			Bt5	Winter	6	Variety developed in 1980'ies. Good baking quality
Bosporus			Bt5	Winter	7	
Tillsano		Saatzücht Donau	Bt5	Winter	7	Developed by Saatzücht Donau. Good baking quality
Rio		GRIN	Bt6	Winter	2	Tall, weak straw
Starke Bt6 NIL		Agrologica	Bt6	Winter	6	Tall and late, but healthy.
Selection 50077	PI 554100	GRIN	Bt7	Winter	2	Differential line, tall with weak straw
Tambor			Bt7	Winter	6	Variety developed in 1980'ies.
Quarna		DSP	Bt7	Spring	6	Spring wheat varieties from DSP. Good baking quality
Fiorina		DSP	Bt7	Spring	6	Spring wheat varieties from DSP. Good baking quality
XeWes		Agrologica	Bt7+Bt10	Winter	6	Spring wheat
Fritop		Nordic Seed	Bt7+BtZ	Winter	7	
M72-1250	PI 554120	GRIN	Bt8	Winter	2	Differential line, Compactum
Amy x Økilde		Agrologica	Bt8	Spring	7	
Stava		Nordgen	Bt8+Bt9	Winter	7	
6256	PI 178383	GRIN	Bt8+Bt9+Bt10+ BtX	Winter	1	Famous Donor
R63-6968	PI 554099	GRIN	Bt9	Winter	2	Differential line, Compactum
Starke Bt9 NIL		Agrologica / NGB	Bt9	Winter	6	Tall and late, but healthy.
P68-1336-7	PI 554098	GRIN	Bt9+Bt11	Winter	2	Differential line, Compactum
(Coops Purple Marker x Penta) x Pi		Agrologica	Bt9+Bt11	Winter	6	
702-1102-C		Erik Tybirk	Bt9+Bt11 (?)	Winter	6	good for northern European conditions, short straw
Ark		GRIN	Bt1+Bt4+Bt6+Bt 7+Bt9+Bt10	Winter	3	
Starke Bt10 NIL		Agrologica / NGB	Bt10	Winter	6	Tall and late, but healthy.
Tillexus		Saatzücht Donau	Bt10	Winter	7	Good baking quality
Tillstop		Saatzücht Donau	Bt10	Winter	7	Good baking quality
M-822123	PI 554119	GRIN	Bt11	Winter	1	Bt11 differential, durable resistance in BOKU trials
Starke Bt12 NIL		Agrologica	Bt12	Winter	6	Tall and late, but healthy.
1696/Pi 119333 * Rainer	IFA-P106.30	BOKU	Bt12 + Bt_4B	Winter	4	OK for pre-breeding
1696	PI 119333	GRIN	Bt12+Bt_4B	Winter	1	Differential line, extremely weak straw.
Thule III	PI 181463	GRIN	Bt13	Winter	1	Differential line, Extremely early with very poor agronomic performance.
Segor*Thule-III*Ciaveza-sel.-2		Agrologica	Bt13	Spring	5	Early spring wheat.
7838	PI 173437	GRIN	BtP	Winter	2	Differential Line, Extremely weak straw
Starke II * 7838 / PI 173437		Agrologica	BtP	Winter	4	
Tilliko			BtZ	Winter	7	Good for organic conditions

Future perspectives

The ongoing research projects BOOST, DIVERSILIENCE, ECOBREED have limited resources left for continued research, but new projects in Germany and Sweden are starting up. It seems that the consortium contains a strong group of partners within both research and business.

One way ahead is to apply for a common European research project, stacking up the ongoing research plans. Please come forward if you see funding opportunities for such a project. Another (not excluding the first) could be to form a member based global or European society for bunt research. The current consortium can be seen as a first step in this direction. We should discuss to include research groups from other countries and continents to strengthen the research platform, and to discuss leadership of governance of the consortium/society. Please consider this.