Evaluation of Nordic heritage varieties and NILs for resistance to common bunt (*Tilletia caries* syn. *T.tritici*)

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NordGen is the genebank covering the Nordic countries, storing and distributing germplasm of Nordic origin to potential users. Allkorn is a Swedish seed saver organisation making their own maintainance of cereals mainly of Nordic origin. Accessions from Allkorn may therefore be different from the accessions from NordGen with the same name.

Prof. James MacKey started a program many years ago, developing a differential set of NILs (Near Isogenic Lines) with *Bt*-resistance genes against common bunt based on the variety Starke II, backcrossing resistant lines with Starke II. However, he didn't manage to finish his work, and the material was stored at NordGen.

The aim of this stydy was to evaluate accesions from NordGen and Allkorn for the susceptibility of common bunt.

Materials and methods

In 2014-15, 165 lines from NordGen was tested at Agrologica by contaminating ~50 seed with abundance of spores of either a race with low frequency of virulence, or a mixture of different races [1]. The idea of testing with these two different spore samples was that if a variety has any resistance, they will have low level of infection when contaminated with spores without virulence, but a higher infection with spores of mixed virulence, and no infection at all if the variety has a resistance against all virulence races. Spikelets of spelt and emmer was dehulled before spore application to ensure contact between spore and kernel.

The expected NILs were provided by NordGen along with the original source of resistance. The NILs were contaminated with spores with and without virulence

Table 1: Study 2015 of the MacKey NILs from NordGen, developed from Starke II and lines with known Bt resistance genes against common bunt (Tilletia caries)

Accession	Spores used	Percent infected heads	95% conf interval
NGB11503 (NIL Bt1)	Vr: 1	8,3	(0,0 - 17,4)
NGB11503 (NIL Bt1)	Vr: 8	0,0	(0,0 - 0,0)
NGB11503 (NIL Bt1)	Vr: wes	0,0	(0,0 - 0,0)
NGB21283 (source Bt1)	Vr: 1	16,7	(0,0 - 37,8)
NGB21283 (source Bt1)	Vr: 8	0,0	(0,0 - 0,0)
NGB21283 (source Bt1)	Vr: wes	0,0	(0,0 - 0,0)
NGB11506 (NIL Bt10)	Vr: 1	5,9	(0,0 - 17,1)
NGB11506 (NIL Bt10)	Vr: 8	12,0	(0,0 - 24,7)
NGB11506 (NIL Bt10)	Vr: wes	54,2	(34,2 - 74,1)
PI-554-118 (Bt10)	Vr:1	0,0	(0,0 - 0,0)
PI-554-118 (Bt10)	Vr: 8	0,0	(0,0 - 0,0)
PI-554-118 (Bt10)	Vr: wes	53,6	(35,1 - 72,0)
Weston (Bt10)	Vr:1	0,0	(0,0 - 0,0)
Weston (Bt10)	Vr: 8	13,6	(0,0 - 28,0)
Weston (Bt10)	Vr: wes	52,6	(30,2 - 75,1)
NGB16160 (NIL Bt???)	Vr: 1	21,6	(8,4 - 34,9)
NGB16160 (NIL Bt???)	Vr: 8	12,9	(1,1 - 24,7)
NGB16160 (NIL Bt???)	Vr: wes	23,8	(5,6 - 42,0)
PI-554-114 (Bt7)	Vr:1	30,0	(9,9 - 50,1)
PI-554-114 (Bt7)	Vr: 8	3,7	(0,0 - 10,8)
PI-554-114 (Bt7)	Vr: wes	22,2	(6,5 - 37,9)
PI-554-100 (Bt7)	Vr:1	4,5	(0,0 - 13,2)
PI-554-100 (Bt7)	Vr: 8	9,1	(0,6 - 17,6)
PI-554-100 (Bt7)	Vr: wes	45,7	(29,2 - 62,2)
NGB16105 NIL Bt12)	Vr: mix	35,7	(21,2 - 50,2)
PI-119-333 (Bt12)	Vr: Mix	0,0	(0,0 - 0,0)
NGB11504 (NIL Bt6)	Vr: mix	3,4	(0,0 - 10,1)
Rio (Bt6)	Vr: Mix	0,0	(0,0 - 0,0)
NGB11505 (NIL Bt9)	Vr: mix	0,0	(0,0 - 0,0)
NGB21193 (Source Bt9)	Vr: mix	0,0	(0,0 - 0,0)
NGB16106 (NIL Bt5)	Vr: mix	0,0	(0,0 - 0,0)
NGB16106 (NIL Bt5)	Vr: 5	0,0	(0,0 - 0,0)
PI-554-104 (Bt5)	Vr: Mix	0,0	(0,0 - 0,0)
Promesse (Bt5)	Vr: 5	0,0	(0,0 - 0,0)

against the *Bt*-resistance-gene the NIL was expected to have. For one NIL there were no information of which resistance gene it should have. This line was therefore tested with different races hoping to gain information to give hints of the resistence gene in question.

In 2015-16, lines that in the first trial indicated some degree of resistance was tested again, this time with 8 different virulence races, and for comparison also a number of wheat varieties with known *Bt*-resistance genes.

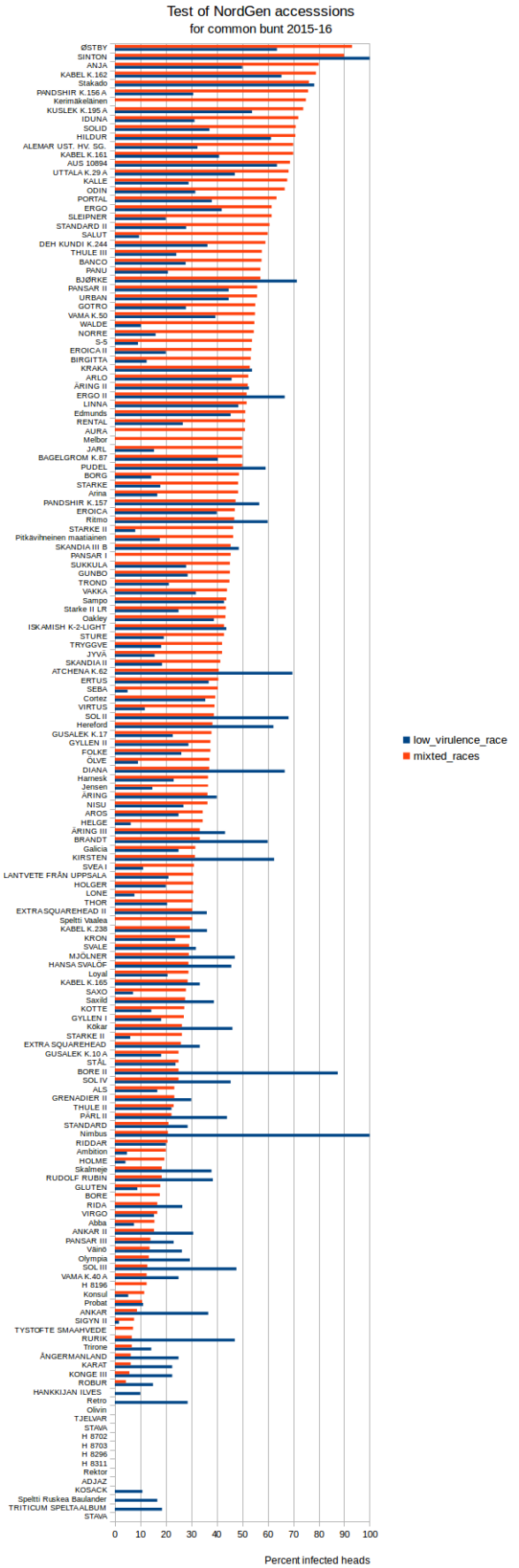
From MacKey´s NILs, 6-10 heads were collected and sown in head rows contaminated with spores of a race without virulance against the *Bt*-gene in question, in order to evaluate if the lines were homogenous.

In 2016-17, lines from Allkorn was tested in the same way with spores of low virulence. One line, Jacoby, was on beforehand expected to be resistant, and was therefore also tested with spores virulent to *Bt2*.

Results and discussion

The result of the 165 wheat accessions from NordGen showed infection leves from 0 to 100% (Figure 1). The infection level was in general higher using the mixed races compared with the race with low virulence, but there were exceptions most likely caused by the low number of tested plants. 16 accessions were selected for further studies the following year.

The result of the first test of the NILs is presented in Table 1. NGB11506 reacts in somewhat the same way as Weston and PI554118 both having *Bt10*. However, the line was also tested at Lantmännen in Sweden and at BOKU in Austria concluding that the line did not have *Bt10* (data not shown). The line NGB16160 with unknown resistance did not show any conclusive results, but there is some similarity with *Bt7*. NGB16105 has high infection to s mix of virulences of which none had virulence to *Bt12*. Therefore it is unlikely that this line has *Bt12*. NGB11504, NGB11505 and NGB16106 had low infection to the mixed races of which none had virulence to *Bt6, Bt9* or *Bt5*. Therefore it is likely that these lines indeed have the resistance genes indicated. However, the low infection in



NGB11504 may indicate that this line was not quite pure. Based onthese results, further studies the following year with head rows gave more detailed information. The results shows that the line

Table 2. Test of resistant wheat accessions against 8 different virulence races. Similar reaction to different virulence races indicates that the lines may have the same resistance genes.

	Vr: 1	Vr-2	Vr-G	Vr: 10	Vr: Q	Vr-5	Vr-13	Vr: 4
Stava	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PI-554-117 (Bt6)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Rio (Bt6)	0,0	0,0	2,9	0,0	1,7	4,2	0,0	0,0
NGB-11504 NIL Bt6	6,5	2,2	3,7	0,0	7,7	0,0	5,0	7,8
NGB-11505 (NIL Bt9)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PI-554-112 (Bt9)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Pi 554099 (Bt9)	0,0	0,0	1,5	0,0	0,0	0,0	0,0	0,0
PI-554-098 (Bt11)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PI-554-119 (Bt11)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PI-554-106 (Bt12)	2,2	0,0	0,0	0,0	2,1	0,0	0,0	0,0
PI-119-333 (Bt12)	2,9	0,0	0,0	0,0	0,0	0,0	0,0	17,5
Hohenheimer Ci-11458 (Bt5)	0,0	7,4	8,3	7,0	4,3	0,0	4,8	0,0
NGB-16106 NIL Bt5	0,0			0,0	1,7		0,0	
PI-554-104 (Bt5)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,9
Nebred (Bt4)	3,0	0,0	12,9	0,0	0,0	0,0	0,0	1,1
Promesse (Bt5)	0,0	0,0	34,3	0,0	0,0	0,0	0,0	0,0
PI-554-115 (Bt4)	7,8	0,0	26,1	5,7	0,0	13,5	0,0	3,0
PI-554-110 (Bt8)	16,7	0,0	10,0	16,7	0,0	0,0	0,0	0,0
PI-554-111 (Bt8)	66,7	2,7	55,0		30,0	17,9	0,0	0,0
NGB-17140 Speltti Ruskea Baulande	52,3	2,1			44,2	18,8	5,7	23,6
Pi173437 (Btp)	2,8	0,0	0,0	21,1	0,0	37,1	0,0	0,0
Thule III (Bt13)	0,0	0,0	0,0	3,3	0,0	0,0	45,7	0,0
Weston	0,0	5,9	5,9	43,8	0,0	2,8	0,0	0,0
PI-554-118 (Bt10)	0,0	1,3	11,3	38,8	3,8	4,1	0,0	2,8
PI-554-109 (Bt10)	0,0	0,0	25,0	28,6	0,0	16,7	0,0	0,0
NGB-11506 NIL Bt10	43,8	44,4	42,1	42,9	50,0	42,1	50,0	20,4
Inna (BtZ)	0,0	0,0	53,0	69,4	1,7	0,0	0,0	0,0
Nemchinovskaya 25 (BtZ) PI 591981	14,7	0,0	19,6	25,0	4,7	18,1	0,0	33,3
PI-554-101 (Bt1)	37,8	54,5	3,3	0,0	0,0	13,5	0,0	0,0
NGB-11503 NIL Bt1 (Albit)	81,3	63,6	0,0	7,3	0,0	6,3	0,0	31,9
PI-554-103 (Bt2)	14,6	44,4	0,0	4,0	0,0	0,0	2,0	22,0
PI-554-097 (Bt2)	17,4	43,6	0,0	30,0	24,0	0,0	5,0	0,0
NGB-7484 Rubrik	50,0	63,2	30,4	11,1	72,1	12,1	8,7	0,0
NGB-26 HELGE	48,7	29,5	28,6	19,0	72,1	31,7	5,5	39,6
Pi554-121 (Bt3)	5,8	0,0	0,0	0,0	61,3	3,6	0,0	5,9
PI 554-116 (Bt3	5,9	10,0	0,0	0,0	50,0	0,0	0,0	10,0
Ridit (Bt3)	0,0	3,2	0,0	0,0	27,5	0,0	0,0	0,0
NGB-473 SIGYN II	42,4	8,9	45,6	17,6	43,8	19,4	4,9	36,2
Erythrosperum 5221 (Bt14)	20,0	14,6	16,1	12,5	0,0	18,1	5,6	14,3
Lutescens	13,6	41,2	41,4	38,8	44,7	12,5	9,4	13,6
PI-554-114 (Bt7)	31,6	68,4	13,6	12,5	17,5	31,4	28,4	3,8
PI-554-100 (Bt7)	16,2	72,7	48,7	63,6	0,0	72,4	27,0	0,0
NGB-16160 NIL Bt?	22,7	29,0	21,2	32,1	51,7	37,8	31,1	39,3
PI 172201 (Doubbi resistens)	20,0	18,2	53,8	21,4	57,1	0,0	75,0	14,3
Gluten	40,7	12,2	61,2	26,4	75,4	49,0	22,7	59,6
NGB-347 AURA	60,7	45,5	65,2	34,0	38,7	42,9	26,5	37,3
NGB-16909 Probat	66,7	51,4	53,4	36,7	72,9	16,1	34,1	29,7
NGB-16852 Konsul	60,0	54,3	60,7	37,1	68,3	36,4	18,2	65,9
NGB6728 Seba	69,2	20,0	54,5	55,7	73,5	70,2	37,7	68,4
NGB-22 STARKE II	58,1	54,2	58,7	46,5	75,9	55,3	47,9	66,7
NGB-4 ANKAR	78,1	42,2	90,0	57,1	74,6	17,3	80,4	65,3
NGB-17139 Speltti Vaalea	65,4	57,4	86,1	67,6	83,3	83,3	41,0	33,3
NGB-13445 ÅNGERMANLAND	77,3	81,3		45,9	87,4	57,5	50,7	75,0
NGB-10883 TRITICUM SPELTA ALB	100,0	54,2	83,0	64,3	63,2	60,5	56,9	80,0

NGB-11503 is homogeneous and is resistant most like having *Bt1*, NGB-16106 is homogeneous and is resistant most like having *Bt5* and NGB-11505 is homogeneous and is resistant most like having *Bt9*. None of the tested lines of NGB16105 had resistance, whereas NGB-11506, NGB-11504 and NGB-16160 seems to be heterogeneous, with some lines being resistant.

Accessions from NordGen that seems to have some degree of resistance including the NILs in the first trial was included in a more detailed study in 2015-6 and tested with 8 different virulence races, and the results are presented in Table 2. These results shows that the lines Stava, NGB-11504 (*Bt6*), NGB-16106 (*Bt5*) and NGB-11505 (NIL *Bt9*) are indeed very resistant against all the races. Stava has in other studies show presence of the marker for *Bt9* [2], but may also have resistance gene Bt8. NGB-11506 (*Bt10*) do not react similar to the the lines with *Bt10*, but as shown in Table 3, this is most likely caused by diversity in the sample. Selected lines with resistance are now purified and will be tested again.

NGB-11503 (*Bt1*) has similarities in reaction with differential line PI-554-101 having *Bt1* confirming that they both have *Bt1*.

NGB-16160 with an unknown resistance gene reacts somewhat similar to *Bt7*, but as the line is diverse (Table 3), the indication must be taken with precaution.

Table 3. NILs based on Starke II tested for presence of Bt-genes

	Virulence Race	Percent Infected	
NGB-11503 NIL Bt1	Vr13	0,0	Most likely having Bt1
2	Vr13	0,0	
3	Vr13	0,0	
4	Vr13	0,0	
5	Vr13	0,0	
6	Vr13	0,0	
7	Vr13	0,0	
8	Vr13	0,0	
9	Vr13	0,0	
10	Vr13	0,0	
NGB-16106 NIL Bt5	Vr:Mix	0,0	Most likely having Bt5
2	Vr:Mix	5,7	
3	Vr:Mix	0,0	
4	Vr:Mix	0,0	
5	Vr:Mix	0,0	
6	Vr:Mix	0,0	
NGB-11505 (NIL Bt9)	Vr:Mix	0,0	Most likely having Bt9
2	Vr:Mix	0,0	
3	Vr:Mix	0,0	
4	Vr:Mix	0,0	
5	Vr:Mix	0,0	
6	Vr:Mix	0,0	
7	Vr:Mix	0,0	
NGB16105 NIL Bt12)	Vr:Mix	46,2	None of the tested lines had Bt12
2	Vr:Mix	65,2	
3	Vr:Mix	53,7	
4	Vr:Mix	84,1	
5	Vr:Mix	35,9	
NGB-11504 NIL Bt6	Vr:Mix	24,4	There seem to be some diversity among the lines, so maybe some of them do indeed have Bt6.
2	Vr:Mix	14,7	
3	Vr:Mix	0,0	
4	Vr:Mix	2,0	
5	Vr:Mix	7,4	
6	Vr:Mix	14,8	
NGB-11506 NIL Bt10	Vr:2	3,7	There seem to be some diversity among the lines, so even most of them are susceptible, maybe some of them do indeed have Bt10.
2	Vr:2	71,7	
3	Vr:2	76,0	
4	Vr:2	42,2	
5	Vr:2	59,5	
6	Vr:2	65,0	
7	Vr:2	1,6	
8	Vr:2	54,2	
9	Vr:2	57,4	
NGB-16160 NIL Bt?	Vr:4	0,0	There seem to be some diversity among the lines, so maybe some of them do indeed have a resistane gene.
2	Vr:4	82,1	
3	Vr:4	94,1	
4	Vr:4	88,4	
5	Vr:4	79,1	
6	Vr:4	0,0	
7	Vr:4	84,6	
8	Vr:4	56,4	

The variety Sigyn II does not react as any other variety, and this may indicate that this line do not have any of the known *Bt*-genes. Helge and Rubrik has some similarities with lines having *Bt2*. Speltti Ruskea Baulander (NGB-17140) has some resistance which is interesting because little has been done to identify resistance genes in spelt wheat. There are inconsistencies in the reactions between the lines with *Bt8*, and it is therefore not conclusive that Speltti Ruskea Baulander has indeed *Bt8*, and these results should be confirmed before any final conclusions are taken.

Heritage varieties maintained by the Allkorn also showed high susceptibility to common bunt as shown in Table 4. Only the German variety Jacoby and the Swiss spelt variety Oberkulmer Rotkorn can be considered resistant whereas all the other varieties of Nordic origin were susceptible. Jacoby has previously been shown to have resistance, and the test with spores virulent to *Bt2* indicate, that the line has resistance gene *Bt2*.

Conclusion

Most accessions from NordGen and Allkorn were susceptible to common bunt which is in line with other screening experiments of randomly selected varieties. However, a few lines not previously known to be resistant were shown to have different resistance genes. None of them however were resistant to all the virulence races tested. The MacKey´s NILs were evaluated and there seem to be a potential for using some of them as differential lines for resistance gene *Bt1*, *Bt5*, and *Bt9*. Purification based on resistant head rows may also develop NILs with resistance to *Bt6* and *Bt10* and possible also *Bt7* from NGB16160. A crossing program of the remaining *Bt*-genes was started in 2016. The NILs may be used also to support development of genetic markers of the resistance genes.

Table 4. Susceptibility of heritage varieteis maintained by Allkorn

	Infected heads %	95% Conf interval
Jacoby	0,0	(0,0 - 0,0)
Jacoby (infected with spores virulent to Bt2)	31,8	(12,4 - 51,3)
Oberkulmer Rotkorn (spelt)	3,3	(0,0 - 9,8)
Odin	21,2	(7,3 - 35,2)
Walde	23,5	(9,3 - 37,8)
Holger	25,0	(14,7 - 35,3)
Eroica	26,1	(13,4 - 38,8)
Eroica, white kernels	46,9	(29,6 - 64,2)
Red Prolific	29,6	(12,4 - 46,9)
Spelt Gotland	34,2	(19,1 - 49,3)
Lv Gotland	34,9	(20,6 - 49,1)
5113	37,5	(20,7 - 54,3)
Rauweizen	38,5	(12,0 - 64,9)
Erbe (white kernels)	38,5	(23,2 - 53,7)
Erbe (red kernels)	41,0	(25,6 - 56,5)
Holger brun	40,9	(26,4 - 55,4)
Börstvete Gotland	43,3	(25,6 - 61,1)
Robur	43,5	(23,2 - 63,7)
Svart Emmer (Hulless seed)	43,5	(23,2 - 63,7)
Svart Emmer	53,8	(38,2 - 69,5)
Starke	46,3	(31,1 - 61,6)
Svale	50,0	(33,7 - 66,3)
Aros	52,6	(36,8 - 68,5)
Banko	55,3	(41,1 - 69,5)
Sol	58,7	(44,5 - 72,9)
Sol (large kernels)	72,2	(51,5 - 92,9)
Ure	61,3	(44,1 - 78,4)
Lv Halland	78,4	(65,1 - 91,6)

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