Common Bunt Resistance of Czech and European Winter Wheat Cultivars and Breeder Lines

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Abstract

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Winter wheat cultivars recently registered in the Czech Republic were tested in three-year field tests for resistance to common bunt. Seeds were inoculated with a mixture of local strains of *Tilletia tritici* and *T. laevis*. None of the cultivars displayed a higher level of resistance compared with the resistant checks. The mean percentage of bunted ears in the three test series including checks was 39%. Mean bunt infection in resistant and susceptible checks was 2% and 63%, respectively. In the European *Tilletia* cooperative test performed in Prague-Ruzyně, thirty-five winter wheat cultivars from six countries were tested during 2007–2013. The cultivars Bill, Nadro, Quebon, Samurai, Stava and Tommi exhibited infection levels below 10% in the respective years of the test. Additionally, 75 breeding lines from six countries were tested. Infection levels below 1% were recorded in 56% of the lines and 1–10% levels in 19% of the lines. A close relationship between the resistant cvs. Tommi and Globus was confirmed using SSR allelic markers.

Keywords: *Bt* genes; SSRs; *Tilletia tritici*; *Tilletia laevis*

Although the chemical treatment of wheat seed for the control of common bunt (*Tilletia tritici* /Bjerk./ Wint. and *T. laevis* /Kühn/) is widely used, genetic resistance of wheat is an important part of the bunt control in many countries, particularly on organic farms. Previous studies have demonstrated a low level of resistance to common bunt among cultivars registered in the Czech Republic (Dumalasová & Bartoš 2007, 2010). The present paper describes the bunt resistance of winter wheat cultivars registered in the years 2009–2011 and among European cultivars.

Microsatellite (SSRs) markers are widely used in plant breeding and genomic research (Guo *et al*. 2011; Mir *et al*. 2012; Son-mezoglu *et al*. 2012; Simpfendorfer *et al*. 2013). Concerning the wheat variability analysis, Roussel *et al*. (2005) reported that a genetic distance exists between wheat cultivars from the western part of Europe (France, The Netherlands, Great Britain, Belgium, Germany) and those from northern and central European countries. An attempt to determine a relationship between bunt resistant cultivars Tommi, Globus and Quebon based on SSR allelic diversity of west European cultivars is presented.

MATERIAL AND METHODS

Seed and inoculum. Seed of wheat cultivars registered in the Czech Republic was kindly supplied by the Central Institute for Supervising and Testing in Agriculture, Brno, http://www.ukzuz.cz/ (Table 1). Seed of the cultivars/lines tested in the European *Tilletia* cooperative tests was supplied by the cooperating institutions from Denmark, France, Finland, Germany, Lithuania, Romania, Switzerland and Ukraine (Table 2). The inoculum employed in all years was a mixture of Czech isolates of *T. tritici* and *T. laevis.* The ratio of *T. tritic*i and *T. laevis* samples in the mixture was 1:1. Samples of *T. tritici* were obtained from Červený Újezd, Jičín, Kroměříž, samples of *T. laevis* from Kralovice, Prague-Ruzyně, Úhřetice and Kroměříž. A mixture of *T. tritici* and *T. laevis* samples was used for inoculation in all experimental years. For inoculation, 0.1 g of teliospores was applied to 250 seeds. Seed and inoculum were shaken by hand in a glass flask for 1 min. The inoculum mixture was tested on controls consisting of lines/cultivars possessing resistance genes (Goates 1996) kindly supplied by Dr. B.J. Goates, USDA, Aberdeen, USA.

Field trials. Inoculated seed was sown in Prague-Ruzyně in October after the usual winter wheat sowing period. Each seed sample was sown in plots consisting of 1 m rows long, 0.2 m apart. There were four replications arranged in a randomized complete block design. Healthy and diseased ears were scored in July. The reaction to bunt was expressed as a percentage of all the spikes in the row exhibiting bunt. The known resistant checks Globus and Bill and the susceptible check Batis (Dumalasová & Bartoš 2007) were included in the tests of registered cultivars. In the European *Tilletia* cooperative tests only a susceptible check Batis was included. Registered cultivars were tested for three years, duration of the tests of cultivars/lines in the cooperative test varied (Table 2). Analysis of variance was employed to determine if statistical differences between treatment means were observed and Fisher's Least Significant Difference (LSD) was employed to separate means (UNISTAT 5.0 package, UNISTAT Ltd., London, UK).

Genetic diversity among cultivars. Thirty-seven bread wheat accessions, mostly from Germany, were chosen from our database of simple sequence repeats (SSRs) of previously processed European cultivars to evaluate the probability of the same pedigree and the same resistance genes in cvs. Tommi, Globus and Quebon. Plants were grown in greenhouse conditions and about 30 plants per accession were pooled and frozen at –80°C. Genomic DNA was extracted using CTAB method according to the optimised protocol of Saghai-Maroof *et al.* (1984). For genotyping, 42 microsatellite loci were selected (Roussel *et al.* 2004). PCR with fluorescently labelled primers (6-fam, vic, ned and pet) was performed in a reaction volume of 15 μl according to the optimised protocol. Reactions were run in the UNO II cycler (Biometra, Goettingen, Germany). Products of PCR reactions were separated using capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems, Foster City, USA). A multiplexed configuration of four reactions was used in one analysis. LIZ500 (Applied Biosystems) was used as the internal size standard. Electrophoretograms were evaluated using the GeneMapper software (Applied Biosystems). For each locus, the presence or absence of bands in each size category was scored for all genotypes. Data were set in a binary matrix. The neighbour-joining cluster analysis based on the Jaccard dissimilarities computed from microsatellite analysis data was performed using the DARwin software (http://darwin.cirad.fr/darwin; Perrier & Jacquemoud-Collet 2006) to visualize

Table 1. Analysis of variance for common bunt infection for the Czech cultivars evaluated in Prague-Ruzyně from 2009–2013

Experimental series	Source of variability	Sum of squares	% variation	df	Mean square	*F*-value	*P*-value
1 (2009–2011)	cultivar	60283.13	65.69	9	6698.13	53.31	< 0.001
	year	8005.24	8.72	2	4002.62	31.85	< 0.001
	cultivar × year	12173.98	13.27	18	676.33	5.38	< 0.001
	error	11308.99	12.32	90	125.66		
	total	91771.33	100.00	119	771.19		
2 (2010–2012)	cultivar	53989.87	57.14	15	3599.33	23.94	< 0.001
	year	3070.77	3.25	2	1535.38	10.21	< 0.001
	cultivar × year	15768.88	16.69	30	525.63	3.50	< 0.001
	error	21654.59	22.92	144	150.38		
	total	94484.12	100.00	191	494.68		
3 (2011–2013)	cultivar	64789.10	58.69	12	5399.09	64.22	< 0.001
	year	25728.31	23.31	2	12864.15	153.01	< 0.001
	cultivar × year	10037.98	9.09	24	418.25	4.98	< 0.001
	error	9836.53	8.91	117	84.07		
	total	110391.91	100.00	155	712.21		

df – degree of freedom

the genetic distances among isolates. The robustness of the nodes in each tree was assessed through repeated bootstrap resampling 1000 times.

RESULTS

Bunt resistance of registered cultivars. Common bunt infection levels were significantly affected by genotypes and years (environments), and by genotype-by-environment interactions ($P \leq 0.001$) (Table 1). Mean bunt infection levels in the susceptible check Batis was 67% in 2009, 70% in 2010, 63% in 2011, 42% in 2012 and 87% in 2013, suggesting that there was a sufficient disease pressure in all years of the study. Mean bunt infection in the inoculated, resistant checks was 2% suggesting that resistance in these cultivars could be expressed under the prevailing environmental conditions. None of the cultivars tested were as resistant as the checks Globus and Bill. In the first series of the trials (Table 2), cultivars Brilliant and Secese had less than a half of the bunt levels compared to the susceptible check cv. Batis. In the second series, cv. Iridium exhibited a similar level of resistance compared to cv. Batis. In the third series (Table 2), cv. Feria exhibited the lowest bunt infection levels; however there were no significant differences in bunt infection among the cultivars Feria, Matylda, Sorrial, Elan, Beduin and Potenzial (Table 2).

Data based on susceptibility of the differential lines to the race mixture were obtained in 2012 and 2013, respectively. The following infection with a mixture of Czech isolates, the % of infected ears was recorded: *Bt0* – 37.4, 78.1; *Bt1* – 15.0, 14.2; *Bt2* – 35.6, 54.2; *Bt3* – 17.5, 28.4; *Bt4* – 0.8, 0.6; *Bt5* – 0.9, 5.0; *Bt6* – 0.0, 0.3; *Bt7* – 19.2, 29.0; *Bt8* – 0.0, 2.0; *Bt9* – 0.0, 0.3; *Bt10* – 0.0, 0.0; *Bt11* – 0.0, 0.0 and *Bt13* – 0.0, 0.0.

European *Tilletia* cooperative test. Of the tested cultivars, Bill, Nadro, Quebon, Samurai, Stava and Tommi exhibited the highest levels of resistance with bunt infection averaging below 10% (Table 3). All of the above-mentioned cultivars were tested only for one year with the exception of Bill and Quebon. Bill was tested for two years and had average bunt infection 7%. Quebon was tested for three years and had average bunt infection 1%. In the years 2007–2012 bunt infection on the susceptible check cv. Batis varied between 37% in 2008 and 87% in 2013.

Most of the breeding lines (Table 4) displayed high levels of bunt resistance. Infection levels below 1% were recorded in 56% of the tested lines and levels from 1 to 10% were observed in 19% of the tested lines. Only 25% of lines showed infection above

Table 2. Mean levels of common bunt infection observed in field trials at Prague-Ruzyně from 2009 to 2013

Exp.	Cultivar	Registration*	% bunted ears
1 (2009–2011)	Globus (check)	2003	2.7^{a}
	Bill (check)	2002	3.3^{a}
	Brilliant	2009	26.5^{b}
	Secese	2009	29.0^{b}
	Manager	2007	36.5^{bc}
	Hermann	2007	39.0^{bcd}
	Seladon	2009	45.3^{cd}
	Bagou	2009	51.7^{d}
	Batis (check)	2001	66.4^{e}
	Federer	2009	74.2^{e}
	mean		37.5
2 (2010–2012)	Bill (check)	2002	0.6^{a}
	Globus (check)	2003	1.3^{a}
	Iridium	2009	25.4^{b}
	Elly	2010	38.7^{c}
	Graindor	2010	38.7^{c}
	Salutos	2009	39.3^{c}
	Bodyček	2010	41.0^{c}
	RW Nadal	2010	42.6^{cd}
	Jindra	2010	45.3^{cde}
	Fortis	2009	45.3^{cde}
	Henrik	2010	48.6^{cdef}
	Brentano	2010	48.7^{cdef}
	Aladin	2010	54.8^{def}
	Preciosa	2009	55.2^{ef}
	Magister	2009	55.2^{ef}
	Batis (check)	2001	58.3^{f}
	mean		39.9
3 (2011–2013)	Globus (check)	2003	1.2^{a}
	Bill (check)	2002	2.7^{a}
	Feria	2011	35.2^{b}
	Matylda	2011	38.1^{b}
	Sorrial	not registered	38.7^{b}
	Elan	2012	38.7^{b}
	Beduin	2011	39.7^{b}
	Potenzial	2012	42.6^{b}
	Carroll	2011	59.1^{c}
	Altigo	2011	61.0^{c}
	Batis (check)	2001	63.9^{c}
	JB Asano	2012	63.9^{c}
	Athlon	2013	64.1^{c}
	mean		42.2

Means in columns followed by the same letter are not significantly different from each other (LSD, $P < 0.05$); Exp. – experimental series ; *year of registration in the Czech Republic

Table 3. Common bunt infection levels in the European *Tilletia* cooperative test conducted at Prague-Ruzyně from 2007 to 2013

Cultivar	Supplied from	% bunted ears (year)
Arolla	CH	40.3 (2007)
Batis	CZ	59.1 (2007); 37.2 (2008); 66.5 (2009); 66.9 (2010); 63.1 (2011); 42.2 (2012); 86.5 (2013)
Bill	CZ	7.9 (2007); 6.0 (2008)
Butaro	DE	25.4 (2009)
Camedo	CH	45.9 (2009)
Claro	CH	11.2 (2008); 21.5 (2009); 44.7 (2011)
Delloro	CH	14.2 (2008)
Forel	CH	40.4 (2007); 36.3 (2009)
Greina	CH	51.8 (2012)
Hanswin	CH	43.3 (2013)
Haven	CH	26.6 (2013)
Chaumont	CH	66.1 (2013)
Levis	CH	3.7 (2008); 34.8 (2010)
Lona	CH	58.7 (2012)
Lorenzo	CH	19.5 (2012)
Molinera	CH	22.5 (2011); 61.5 (2013)
Nadro	CH	8.0 (2012)
Nara	CH	21.3 (2009)
Quebon	FR	1.6 (2007); 1.2 (2008); 1.1 (2013)
Rehti	FI	12.7 (2007)
Runal	CH	19.7 (2008); 41.5 (2011); 42.5 (2012)
Samurai	CH	4.2 (2008)
Sankara	FR	28.9 (2007)
Sertori	CH	62.1 (2010); 23.2 (2012)
Siala	CH	38.8 (2007); 30.6 (2009); 53.3 (2010)
Skagen	DK	15.1 (2007); 9.2 (2008)
Solution	FR	15.8 (2009)
Stava	DK	0.0 (2007)
Suretta	CH	41.7 (2011); 9.6 (2012); 41.2 (2013)
Tambor	DE	15.8 (2007)
Titlis	CH	43.8 (2007); 15.0 (2011); 7.6 (2012)
Togano	CH	19.9 (2010); 0.0 (2012); 44.1 (2013)
Tommi	DK	7.2 (2007)
Torrild	DK	12.8 (2007)
Urho	FI	34.6 (2007)
Zinal	CH	38.8 (2007); 11.3 (2008); 56.2 2010)

DE – Germany; DK – Denmark; CH – Switzerland; FR – France; CZ – Czech Republic; FI – Finland

10%. Resistance was evident among lines from all six participating countries. The genetic nature of the resistance is unknown but based on infection results of lines inoculated with inoculum with known virulence, it is unlikely to be connected with the presence of *Bt1*, *Bt2*, *Bt3* and *Bt7*.

Genetic diversity using SSR markers. Quebon, Tommi and Globus originated from the same plant breeding company Nordsaat Saatzucht GmbH (Germany). For this reason we tried to establish the relationship between these three bunt resistant cultivars using SSR markers. Results (Figure 1) confirmed a

Table 4. Common bunt infection levels among breeding lines entered in the European Tilletia cooperative test conducted at Prague-Ruzyně from 2007 to 2013

Origin	Year	No. of tested lines			
		% bunted ears			total
		< 1	1–10	> 10	
DE	2007	2	1	4	7
	2008	8	–	1	9
	2009	4	1	1	6
	2010	6	–	1	7
	2011	5	–	–	5
	2012	–	3	2	5
	2013	1	4	1	6
UA	2007	4	–	1	5
	2008	5	1	–	6
	2009	3	–	–	3
DK	2007	1	–	–	1
	2008	1	–	–	1
RO	2009	1	2	–	3
LT	2009	–	1	6	7
CH	2009	1	1	2	4
Σ		42	14	19	75
%		56.0	18.7	25.3	100

DE – Germany; DK – Denmark; CH – Switzerland; LT – Lithuania; RO – Romania; UA – Ukraine

close relation of Tommi and Globus and a distant relationship between these two cultivars and Quebon.

DISCUSSION

Among the winter wheat cultivars recently registered in the Czech Republic, none was resistant to common bunt. Although the Danish cv. Bill is considered resistant, with infection levels below 10% during 7 years of our experiments, it was deregistered in 2012. Cv. Bill originates from a multiple cross 891088, double haploid (http://genbank.vurv.cz/genetic/resources/). The bunt resistance of cv. Bill was previously reported (Borum 2001, cit. after Fischer *et al* 2002; Váňová *et al.* 2006). Liatukas and Ruzgas (2007, 2008) recorded the bunt infection of 10–22% and 9% on the cv. Bill in 2007 and 2008, respectively. Collectively, the results demonstrate that the resistance expression in cv. Bill varies considerably across environments. Different proportions of virulent races in the composite mixture used in individual tests may also account for variation in the resistance reactions.

The only registered common bunt resistant cultivar at present is Globus, which was shown to be resistant to bunt strains originating from Czech Republic and Germany in 2006–2007. During 2006, maximum levels of 2% bunt were observed following

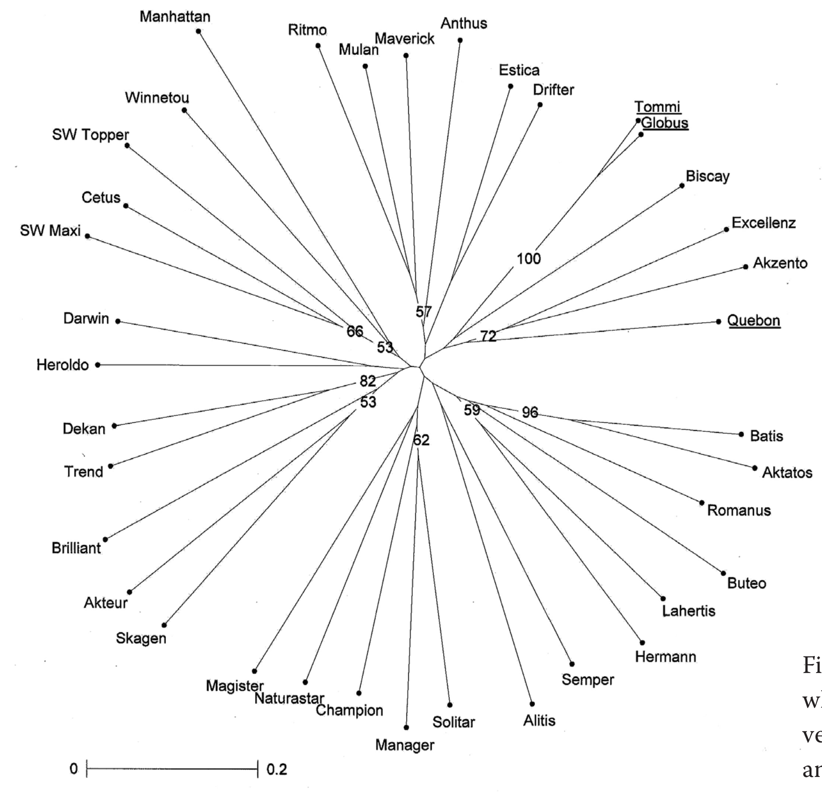

Figure 1. Relationship between European wheat cultivars based on SSR allelic diversity using neighbour-joining cluster analysis on the Jaccard dissimilarities

inoculation with the bunt strain from Berlin-Dahlem (Dumalasová & Bartoš 2007). Bunt infection levels in the cv. Globus of 3% or less have been reported (Huber & Buerstmayr 2006; Liatukas & Ruzgas 2008; Fontaine *et al.* 2009). Globus was also resistant in a 2004/2005 trial at Pfaffenwald (Germany) (Dr. H. Spiess, personal communication). However, when bunted ears from the cv. Globus were used as inoculum in 2007 and the average bunt incidence was higher, a susceptible reaction of 25% infection was observed on Globus (Dumalasová & Bartoš 2007). This suggests that races virulent on Globus may be present in Europe. Cv. Globus originates from the cross Ralf/Astron//Haven (O. Unger, personal communication). Cvs. Astron and Haven are susceptible to common bunt (Dumalasová & Bartoš 2006; Dumalasová, unpublished results). The resistance level in cv. Ralf remains to be established.

The present study has identified cultivars that are resistant to the virulence spectra of the bunt inoculum used in our trials. In a different test (Dumalasová, unpublished results), registered cultivars such as cv. Alibaba and lines possessing *Bt2* (Sel. 1102) and *Bt3* (Ridit) were resistant following inoculation with strains originating from Kroměříž but were susceptible to the inoculum from the Czech Republic. This suggests that cv. Alibaba possesses resistance genes *Bt2* and/or *Bt3* that are ineffective in our present trials. Similar results were reported by Dumalasová and Bartoš (2006) regarding German cultivars Euris and Bussard that were resistant to *T. tritici* from Kroměříž but susceptible to *T. laevis* from Prague-Ruzyně. The European *Tilletia* cooperative test from all cooperating institutions is not generally published so it is not possible to determine if resistance is effective against all prevalent European races. Fontaine *et al.* (2009), who presented partial results from six locations in Europe in 2007, demonstrated that cv. Quebon was resistant at all locations except Romania where it was susceptible to one of the three bunt strains tested. The ancestry of cv. Quebon is unknown. These results suggest that variability in the virulence spectra among common bunt strains occurs across Europe and that widespread testing over years should be done prior to selecting resistance sources for incorporation into breeding programs. Cv. Tommi, tested in Germany with common bunt proveniences originating from five locations (Wächter *et al.* 2007), was included in the group with the lowest bunt infection levels that ranged from 0 to 2%. German cvs Fakir and Zeppelin possessing cv. Tommi in their pedigrees were recently registered in the Czech Republic and are currently being evaluated for bunt resistance.

Breeding for common bunt resistance has resulted in the release of the resistant cultivars Tjelvar and Stava in Sweden (Jönsson 1991). Resistant European cultivars originating from Germany, Romania, Switzerland and Ukraine have been reported (Bänziger *et al*. 2003; Wächter *et al*. 2005; Dumalasová & Bartoš 2006). Though no resistance was found in cultivars recently registered in the Czech Republic, the results of the present study indicate that there is excellent resistance in European wheat cultivars available for incorporation. Variability in virulence among bunt strains originating from across Europe must also be considered in resistance breeding programs because of their potential introduction into the Czech Republic.

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