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Resistance genes and sources for the control of wheat common bunt (*Tilletia tritici* (DC.) Tul.)

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Resistance to common bunt is a highly desirable trait in wheat cultivars suitable for organic growing. Over 1000 germplasms were analysed for common bunt resistance during 2006–2008. In 2007, none of the 474 genotypes tested was found to be highly resistant, while 8 accessions were resistant. Investigations in 2008 showed two accessions to be highly resistant and 28 resistant out of the 714 modern germplasms tested. Among the tested genotypes with the known resistance *Bt1–15,Z* genes, only two single resistance genes conferred complete effectiveness in cultivars 'PI 554120' and 'Yayla 305' possessing *Bt8* and in 'Eryth-5221' possessing *Bt14*. The other single resistance genes considered as effective (infection up to 10.0%) were *Bt5, 9, 11, 12, 13, 15, Z*. Among the most resistant modern cultivars, none was free from infection during both years. Cultivars characterized as at least moderately resistant during both years were 'Sana', 'Penta' and 'Sj05–15' with the 0.0–8.5% infection level. The most susceptible cultivars 'Azimut' and 'Champion' were infected up to 99.0% on average, and in some replications infection was as high as 100.0%. Infection range from 0 to 100% proved that the infection of cultivars was adequate for the characterization of the test genotypes by resistance to common bunt. More than 800 breeding lines were tested during the two screening years. Only five lines were infected no more than 10% during both years.

Key words: wheat, common bunt, resistance genes, organic growing

INTRODUCTION

Wheat yield losses caused by common bunt (*Tilletia tritici* (DC.) Tul.) have been mentioned since the beginning of recorded history. Due to the perfect efficiency of synthetic pesticides, breeding for resistance to this disease has been very limited [1]. Despite availability of efficient chemical control, common bunt can still cause significant losses when treatments are not applied or incorrect. Organic farming, which prohibits the use of chemicals, might favour an increase of affected areas [2]. Highly resistant genotypes are available among world wheat germplasm [3], however, cultivars with an adequate resistance level are rare among European wheat germplasm [4, 5]. Moreover, among these resistant cultivars, only a few are suitable for organic farming by other traits [6]. Cultivars selected for organic growing should possess at least some resistance, as available organic treatments are not highly effective and only in combination with cultivar resistance can provide desirable effectiveness [7, 8].

Efficient resistance breeding can be done when information about the effectiveness of resistance genes is available. Recent investigations in Europe have shown that in Austria and Germany effective resistance genes among *Bt1–15* are *Bt4–6, 8–12, 14* [9, 10]. The hungarian *T. tritici* population showed avirulence on genes *Bt5, 6, 8–10* among the tested genes *Bt1–10* [11]. An investigation in Romania revealed that genes Bt5, 8–13 were effective against the local population [12]. A complex evaluation of the virulence of the European *Tilletia tritici* population showed the effectiveness of genes *Bt3, 5, 8, 9, 11–13* among genes *Bt1–13* [13].

The aim of the present study was to evaluate the effectiveness of resistance genes *Bt1–15, BtZ* in differentials, old cultivars and landraces as well as the resistance of modern winter wheat cultivars and advanced breeding lines against the local population of *Tilletia tritici*.

MATERIALS AND METHODS

During the period 2006–2008, experiments were carried out at the Lithuanian Institute of Agriculture (LIA) in a nursery with artificial infection. The material subjected to bunt resistance tests included mainly winter wheat cultivars used as the initial breeding material, a few cultivars of spring wheat, advanced breeding lines, old cultivars and differentials with known *Bt* genes. The information about resistance genes in the selected genotypes was taken from the publications of Bonman et al. [3], Wätchter et al. [10] Oncica & Saulescu [12] and Martynov et al. [14].

Inoculation was carried out by shaking seeds with teliospores of common bunt, collected in the local area (5 g

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spores / 1000 g seed) in a flask for 5 min. The inoculated seed was sown at a rate of 15 g per genotype per 3 m long row at a depth of 7–10 cm in three replications situated in different parts of the field at the beginning of October when the soil temperature was below 10 °C. The spring-type genotypes were sown at the beginning of April.

Disease incidence was measured after harvesting at the medium milk development stage as the number of infected ears from the total ears harvested. The following scale was used to estimate varietal resistance: infected ears 0.0% = very resistant, 0.1–5.0% = resistant, 5.1–10.0% = moderately resistant, 10.1–30.0% = moderately susceptible, 30.1–50.0% = susceptible, 50.1–100.0% = very susceptible [11, 15]. Resistance genes were considered effective when the infection of genotypes possessing them was no higher than 10% [16]. Statistical analysis involved calculation of mean values $(\overline{X})$ and standard deviation (SD). The means were compared with Duncan's Multiple Range test at the level of significance P = 0.05.

RESULTS

The distribution of winter wheat genotypes by common bunt resistance has revealed that only the minority of modern germplasm can be used for resistance breeding (Table 1). In 2007, none of the 474 genotypes tested was found to be highly resistant, and 8 accessions were resistant. Screening in 2008 showed two accessions to be highly resistant and 28 resistant out of the 714 modern germplasms tested. Out of the 106 accessions of old cultivars, landraces and differentials, 56 genotypes were highly resistant and 13 resistant.

An international differential set and other cultivars with known *Bt* genes consisting of 46 genotypes were evaluated for resistance to *Tilletia tritici*. The mean percentage of infected spikes and the corresponding standard deviations are listed in ascending order of genotypes with resistance genes in Table 2. Investigation of the winter wheat germplasm for resistance to *T. tritici* resulted in a broad range of variation

Table 1. **The distribution of winter wheat genotypes by common bunt resistance, 2007–2008**

Year	Resistance groups												Total No.
	0		0.1–5.0		5.1–10.0		10.1–30.0		30.1–50.0		≥50.1		
	No.*	%**	No.	%	No.	%	No.	%	No.	%	No.	%	
2007	0	0.0	8	1.7	10	2.1	48	10.1	55	11.6	353	74.6	474†
2008	2	0.3	28	3.9	36	5.0	88	12.3	79	10.6	484	67.8	714†
2008	56	52.8	13	12.3	6	5.7	17	16.0	5	4.7	9	8.5	106††

* – Number of genotypes per group. ** Percentage of genotypes per group.

† Modern cultivars and breeding lines. †† Differential lines, cultivars with known resistance genes, resistance sources, etc.

Table 2. **Reaction of wheat genotypes with Bt resistance genes to common bunt, 2008**

Genotype	Bt genes	$\overline{X}$, %	SD, %	Genotype	Bt genes	$\overline{X}$,%	SD, %
'Red Bobs'**	*Bt0*	80.3 p*	5.5	'PI 554120'	*Bt8*	0.0 a	0.0
'B. C. Elgin'	*Bt0*	96.0 q	2.8	'Yayla 305'	*Bt8*	0.0 a	0.0
'White Odessa'	*Bt1*	37.5 j	3.5	'M85-22'	*Bt8.9*	0.0 a	0.0
'Kooperatorka'	*Bt1*	57.0l m	9.9	'Weston'	*Bt8.9.10*	0.0 a	0.0
'Hussar'	*Bt1.2.5*	0.0 a	0.0	'Hansel'	*Bt8.9.10*	0.0 a	0.0
'Columbia'	*Bt1.4.6*	25.0 de	5.0	'Andrews'	*Bt9*	0.0 a	0.0
'Burt'	*Bt1.4.6*	61.3 n	3.2	'PI 554099'	*Bt9*	6.3 bd	1.5
'Martin'	*Bt1.7*	10.7 cd	3.1	'M85-2124'	*Bt9.10*	0.0 a	0.0
'PI 554097'	*Bt2*	65.0 o	5.0	'R63-6982'	*Bt10*	7.7 bd	2.5
'Ridit'	*Bt3*	12.7 cd	5.9	'PI 554118'	*Bt10*	18.8 d	3.4
'Malkesi'	*Bt3.9*	25.0 de	6.0	'F00281G2-11'	*Bt11*	0.0 a	0.0
'VH078373'	*Bt3.9.10*	0.0 a	0.0	'PI 544119'	*Bt11*	0.0 a	0.0
'Nebred'	*Bt4*	8.7 bd	3.2	'F94976G-M28'	*Bt11*	2.8 b	0.7
'CI 1558'	*Bt4*	35.9 fj	5.5	'F94895G-M31'	*Bt12*	0.0 a	0.0
'PB82-187'	*Bt4.5*	0.0 a	0.0	'P78-24'	*Bt12*	0.0 a	0.0
'Oro'	*Bt4.7*	0.0 a	0.0	'PI 119333'	*Bt12*	4.5 bc	0.5
'Promesse'	*Bt5*	0.0 a	0.0	'Lut.6028'	*Bt12.13*	0.0 a	0.0
'F94889G-M7'	*Bt5*	0.8 ab	0.2	'F95601G-M37'	*Bt13*	0.0 a	0.0
'Rio'	*Bt6*	29.2 f	6.2	'Thule III'	*Bt13*	9.9 bd	2.6
'Rex'	*Bt6*	45.0 jl	5.0	'Eryth-5221'	*Bt14*	0.0 a	0.0
'Sel. 50077'	*Bt7*	45.0 jl	5.5	'Carleton'**	*Bt15*	3.3 b	1.5
'Cheyenne'	*Bt7*	56.0 l	5.3	'Kalininskaya 27'	*BtZ*	1.7 b	0.3
'PI 554100'	*Bt7*	77.0 p	2.6	'Nemchinovskaya 25'	*BtZ*	9.0 bd	1.4

* Means followed by the same letters do not differ according to Duncan's Multiple Range Test at 5% of significance. ** Spring type.

from as low as 0.0% of diseased spikes up to 96.0% for the cultivar 'B. C. Elgin' without *Bt* genes. Only two single resistance genes conferred complete effectiveness in cultivars 'PI 554120', 'Yayla 305' possessing *Bt8* and 'Eryth-5221' possessing *Bt14*. Other single resistance genes considered as effective were *Bt5* in 'Promesse' – 0.0% and F94889G-M7 – 0.8%, *Bt9* in 'Andrews' – 0.0% and 'PI554099' – 6.3%, *Bt11* in 'F00281G2-11' and 'PI 544119' – 0.0%, 'F94976G-M28' – 2.8%, *Bt12* in 'F94895G-M31' and 'P78-24' – 0.0%, 'PI 119333' – 4.5%, *Bt13* in 'F95601G-M37' – 0.0% and "Thule III' – 9.9%, *Bt15* in 'Carleton' – 3.3%, *BtZ* in 'Kalininskaya 27' – 1.7% and 'Nemchinovskaya 25' – 9.0%. Combinations of genes *Bt1.2.5*, *Bt3.9.10*, *Bt4.5*, *Bt4.7*, *Bt8.9*, *Bt8.9.10* and *Bt12.13* showed a complete effectiveness. Only gene combination *Bt1.4.6* was inefficient as cv. 'Columbia' and 'Burt' possessing this combination were infected 25.0% and 61.3%, respectively. Ineffective single genes were *Bt1* – 37.8% and 57.0%, *Bt2* – 65.0%, *Bt3* – 12.7%, *Bt4* – 8.7% and 35.9%, *Bt6* – 29.2% and 45.0%, *Bt7* – 45.0%, 56.0% and 77.0%, *Bt10* – 7.7% and 18.8% of infection in cultivars possessing these genes.

None of the most resistant cultivars was free from infection during both years (Table 3). Cultivars characterized as at least moderately resistant during both years were 'Sana', 'Penta' and 'Sj05–15' with the infection level 0.0–8.5%. The other cultivars showed a variable resistance level from resistant to medium susceptible with infection 0.3–17.3%. The most susceptible cultivars 'Azimut' and 'Champion' were infected up to 99.0% on average, and in some replications infection was as high as 100.0%. The infection range from 0 to 100% proved that infection of cultivars was adequate for characterization of the test genotypes by resistance to common bunt.

The most common bunt-resistant advanced breeding lines have shown that resistant cultivars can be developed from modern European winter wheat cultivars (Table 4). However, during two screening years, more than 800 lines were tested and only 5 lines were infected no more than 10% in both years. This suggests that less than 1% of the genotypes tested possessed an adequate resistance level. The majority of the most resistant lines contained in their pedigree parents possessing various resistance levels, which was confirmed by research in Lithuania and abroad [4, 6, 10]. The most susceptible lines showed a reaction similar to that of the most susceptible cultivars.

DISCUSSION

The experimental years were very favourable for common bunt resistance screening. Our previous long-term research had shown that under Lithuanian climatic conditions a high common bunt infection can happen when plants are not damaged by winter frost [17]. Such conditions occurred during the 2006–2008 study period.

A very small percentage (less than 1%) of resistant genotypes shows the necessity to screen hundreds of modern cultivars to find a suitable source of resistance for breeding. Only the minority of modern winter wheat cultivars possess a complex of traits allowing their cultivation under organic conditions [18].

Investigation of old genotypes with various resistance genes showed a promising use of these germplasms as a source of resistance. More than half of the genotypes were very resistant or resistant, although the main constraint in many cases is the poor agronomic characteristics of these genotypes. Their undesirable traits are a high susceptibility to lodging, fungal diseases, and a low yielding capacity. Therefore, transfer of resistance genes from these genotypes to breeding material with desirable traits will take a commercially unacceptable period. The low efficiency of single resistance genes shows that the local bunt population possesses virulence to the majority of the study genes. Cultivars with the same genes or their combinations were infected differently. The highest contrast was found among the cultivars possessing *Bt4* (8.7% and 35.9%) and *Bt13* (0.0% and 9.9%). Such

Table 3. **Modern cultivars of winter wheat most divergent by resistance to common bunt**

Genotypes	2007		2008		2007–2008
	$\overline{X}$, %	SD	$\overline{X}$, %	SD	$\overline{X}$, %
			Most resistant		
'Sana	3.0 a*	2.0	2.9 a-c	0.9	3.0
'Penta'	8.5 a-c	2.5	0.0 a	0.0	4.3
'Sj05-15'	7.5 ab	2.3	4.9 b-d	1.8	6.2
'Sj05-18'	13.3 b-d	4.2	0.9 ab	1.0	7.1
'Quebon'	13.5 b-d	5.6	1.5 a-c	1.5	7.5
'Donskaya Niva'	11.3 b-d	3.5	4.8 b-d	0.3	8.1
'Tommi'	14.8 d	3.3	7.7 de	0.6	11.3
'Patria'	17.3 d	3.8	5.9 cd	4.2	11.8
'BC Antea'	14.3 cd	4.5	13.2 f	0.8	13.8
'Bill'	15.8 d	3.8	12.0 ef	8.0	13.9
			Most susceptible		
'Ebi'	93.5 ef	4.1	95.8gh	2.0	94.7
'Dromos'	96.8 ef	4.6	96.3 gh	1.5	96.6
'Alitis'	96.3 ef	1.5	97.7 gh	3.2	97.0
'Azimut'	96.5 ef	1.5	99.0 h	1.0	97.8
'Champion'	98.3 f	2.1	99.0 h	1.0	97.8

* Means followed by the same letters do not differ according to Duncan's Multiple Range Test at 5% of significance.

Table 4. **Advanced breeding lines of winter wheat most divergent by resistance to common bunt**

No. in LIA catalogue	Pedigree	2007		2008		2007–2008
		$\overline{X}$,%	SD	$\overline{X}$,%	SD	$\overline{X}$,%
		Most resistant				
5055–2	'WW2498 / Corvus'	2.5 a*	1.3	3.3 a	1.5	2.9
5017–2	'Dream / 91002G.2.1'	5.0 a	2.0	3.7 a	0.6	4.4
6040–4	'Tarso / Bussard'	5.5 a	1.3	10.0 b	1.0	7.8
5059–2	'WW2498 / Sj965491'	5.9 ab	2.1	9.7 b	2.5	7.8
6062–1	'Bill / Dream'	6.0 ab	2.0	10.0 b	5.0	8.0
5017–1	'Dream / 91002G.2.1'	12.1 bc	5.2	9.3 b	2.1	10.7
5059–3	'WW2498 / Sj965491'	15.4 c	5.0	9.7 b	4.5	12.6
5368–9	'Dream / Pesma'	22.8 d	3.3	14.0 b	4.0	18.4
6040–2	'Tarso / Bussard'	27.3 d	6.0	20.3 e	0.6	23.8
5060–47	'Flair / Lut. 9–329'	25.8 d	5.5	28.7 f	2.9	27.3
		Most susceptible				
5060–2	'WW2498 / Aspirant'	86.0 e	3.6	94.3 cd	4.0	90.2
6031–4	'STH1096 / Bussard'	93.7 f–h	4.7	95.3 cd	2.5	94.5
5005–3	'Pegasos / Lasta'	91.3 ef	1.5	97.7 cd	1.5	94.5
5417–10	'Olivin / Aspirant'	94.7 f–h	1.5	98.0 d	0.8	96.4
6038–2	'STH1096 / Nord34/106'	99.3 h	1.2	97.7 cd	1.5	98.5

* Means followed by the same letters do not differ according to Duncan's Multiple Range Test at 5% of significance.

differences can be influenced by environmental factors [11] as well as by different levels of partial resistance [19]. Effects of the environment on the efficiency of winter wheat resistance genes has been studied quite well. Oone of the key factors for an adequate infection level is cold damage during winter. Winter wheat cultivars differ by cold resistance very significantly. Our research period was very fovarouble for plant overwintering; however, some genotypes could be slightly damaged. Winter wheat infection with *Tilletia tritici* decreases its cold resistance. Therefore, the low percentage of perished infected plants of more cold-susceptible winter wheat genotypes could be one of the reasons why accessions with the same resistance genes showed a different level of disease. Also, wheat varieties differ in their tillering capacity. Accessions with a higher tillering could be infected less, as the pathogen did not penetrate to all tillers. The least investigated area is wheat partial resistance to common bunt. At present, only several significant publications are available. The situation with wheat partial resistance to other fungal diseases may indicate that in wheat should be present numerous polygenes responsible for common bunt resistance.

The high efficiency of gene combinations consisting of inefficient genes suggests that the *T. tritici* population used has no complex virulence. Similar results had been obtained earlier in Europe [9, 10, 13]. This allows the use of cultivars with single defeated resistance genes for breeding purposes in the absence of other genotypes with more effective genes.

The *T. tritici* pathogen is a biotrophic fungus. Genes conferring resistance against biotrophic pathogens confer complete resistance without obvious symptoms of infection. Conversely, effective resistance genes against necrotrophic pathogens allow developing a certain level of infection, while genes conferring partial resistance to both types of pathogens allow some disease development. This suggests that *Bt* genes act rather like partial resistance genes or genes conferring resistance to necrotrophic fungi than genes conferring resistance to biotic fungi. This may be one of the reasons why *T. tritici* populations change more slowly than do other pathogens.

In some European countries, winter wheat breeders have achieved some progress in developing common bunt resistant material. Danish, German, and Croatian cultivars are among the most resistant ones. Resistant genotypes from Denmark and Germany are very well adapted to an intensive growing system but possess very poor traits for organic growing. Resistant cultivars from Croatia are not adapted to Lithuanian climate as they are too susceptible to fungal diseases and winter damage. Bonman et al. [3] report that in former Yugoslavia many resistant accessions were found. These accessions are old landraces. A high resistance level in modern cultivars from the same region shows a probability that these cultivars have inherited resistance from the mentioned landraces.

Breeders in Romania developed some resistant breeding material, but the samples received from them were susceptible to lodging and leaf diseases under Lithuanian conditions. These accessions were similar to the old cultivars and landraces tested for resistance to common bunt.

Testing of breeding lines showed that among more than 800 accessions less than 1% were resistant or medium resistant. Such a low percentage of resistant material hardly allows selecting lines with all desirable traits for organic growing. Almost all lines possessed in their pedigree parental cultivars with a certain level of resistance, suggesting that the European winter wheat germplasm possesses common bunt resistance genes which alone confer a low resistance level, whereas in combinations these genes can be practically exploited.

Investigations on common bunt resistance breeding at the LIA have been carried out since 1992 [17]. Long-term investigations enabled us to optimize resistance screening. Our experience shows that the breeding process can be improved, if selection of lines with traits desirable for organic growing is carried out at early breeding stages. Intensive screening of parental material for crossing can essentially accelerate selection of resistant lines. Such technology allows concentrating on the most promising derivatives, avoiding a futile screening of obviously susceptible lines.

Resistance to common bunt in modern cultivars used for intensive growing does not supply additional competitiveness with other cultivars as long as chemical seed treatment is effective and environmental safety standards allow it. The situation could change when other *Tilletia* species, for example, *T. contraversa*, spread in some regions. The situation with *T. contraversa* resistance sources is much harder than in the case of common bunt, and its effective chemical control is limited to several pesticides used for seed treatment [20]. However, resistance to this *Tilletia* species is conferred by the same resistance *Bt* genes [9, 21]. This relation enables screening for *T. contraversa* resistance using *T. tritici,* as this species is not so complicated in research. The resistant genotypes identified can later be re-tested with *T. contraversa* populations for the final evaluation of their resistance.

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KVIEČIŲ ATSPARUMO KIETOSIOMS KŪLĖMS (*TILLETIA TRITICI* (DC.)TUL.) KONTROLĖ

Santrauka

Kviečių atsparumas kietosioms kūlėms yra pageidaujamas veislių, skirtų auginti ekologinėmis sąlygomis, požymis. Lietuvos žemdirbystės institute 2006– 2008 m., dirbtinai užkrėtus kietosiomis kūlėmis, buvo ištirta daugiau kaip 1000 žieminių kviečių veislių bei linijų. Tarp 2007 m. tirtų 474 genotipų labai atsparių kietosioms kūlėms nebuvo nustatyta, o 8 buvo atsparūs. Tarp 2008 m. tirtų 714 šiuolaikinių genotipų buvo nustatyti 2 labai atsparūs ir 28 atsparūs genotipai. Tiriant genotipus su žinomais *Bt1–15, Z* atsparumo genais nustatyta, kad tik du genai buvo labai efektyvūs – tai *Bt8* genas ‘PI 554120’ ir ‘Yayla 305’ veislėse bei *Bt14* ‘Eryth-5221’ veislėje. *Bt5, 9, 11, 12, 13, Z* genai buvo pakankamai efektyvūs – pažeistų genotipų su šiais genais buvo ne daugiau nei 10%. Per dvejus tyrimo metus labai atsparių kietosioms kūlėms šiuolaikinių veislių nepasitaikė. 0,0–8,8% pažeistas ‘Sana’, ‘Penta’, ‘Sj05-15’ veisles galime laikyti vidutiniškai atspariomis. Jautriausios veislės ‘Azimut’ ir ‘Champion’ vidutiniškai buvo pažeistos 99%, o kai kuriais atvejais ir 100%. Veislių pažeidimo svyravimas nuo 0 iki 100% rodo, kad užkrėtimo lygio pakako diferencijuojant tirtus genotipus pagal atsparumą kietosioms kūlėms. Iš daugiau nei 800 2006–2008 m. tirtų selekcinių linijų tik penkios linijos buvo pažeistos mažiau nei 10%.

Raktažodžiai: kviečiai, kietosios kūlės, atsparumo genai, ekologinis auginimas