

Characterization of Two Wheat Doubled Haploid Populations for Resistance to Common Bunt and Its Association with Agronomic Traits

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(Received 1 July 2013; Accepted 1 October 2013;
Communicated by P.S. Baenziger)

Two segregating populations of doubled haploid (DH) wheat lines derived androgenetically from crosses ‘Svilena’ (susceptible) × A-38b-4-5-3-3 (highly resistant) and ‘Svilena’ × WWRN (moderately resistant to moderately susceptible) were characterized for resistance to common bunt. Disease incidence was evaluated after inoculation of seeds with a mixture of *Tilletia foetida* teliospores in two autumn sown field experiments. Two-gene model of inheritance of resistance in line A-38b-4-5-3-3 was suggested. The transgressive segregation in the latter population was indicative for a quantitative mode of inheritance. The DH lines were assessed for plant height, heading time and important yield components in a three-year field experiment without bunt infection. In both populations, transgressive segregation was observed for all agronomic characteristics. Although the disease incidence was positively correlated with most of the agronomic traits, genotypes combining bunt resistance with good yield potential were isolated from ‘Svilena’ × A-38b set of lines. These genotypes are valuable for breeding varieties designed for growing in low-input and organic farming systems. The two DH populations are suitable to be used for further studies on the genetic basis of bunt resistance.

Keywords: common bunt, doubled haploid population, genetic control, *Tilletia* sp., wheat

Abbreviations: DH – doubled haploid; QTL – quantitative trait locus; TKW – thousand-kernel weight

Introduction

Common bunt is among the most destructive fungal diseases in wheat worldwide and probably has existed since wheat domestication (Fischer and Holton 1957). Even contamination levels as low as 0.1% can cause dramatic losses in grain yield and quality (Liatukas and Ruzgas 2005). The disease is caused by two soil borne heterobasidiomycete fungi – *Tilletia caries* (DC) Tul. & C. Tul. [syn. *T. tritici* (Bjerk.) G. Winter] and *T. foetida*

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(Wallr.) Liro (syn. *T. laevis* JG Kühn). *T. caries* is more prevalent in North-Western Europe, while *T. foetida* is more common to Eastern Europe (Matanguihan et al. 2011), including Bulgaria (Atanassov 1929; Dodov and Todorova 1974).

The chemical treatment of seeds introduced at the beginning of 20th century contributed to the rapid control over the disease. Thereafter, the research on the causal agents and resistance genetics, as well as the search for new resistance sources and breeding for resistance have been largely abandoned. This is one of the factors explaining the low number of commercial varieties of high bunt resistance (Liatukas and Ruzgas 2005; Bonman et al. 2006; Ciuca and Saulescu 2008; Dumalasová and Bartoš 2013). The disease has recently re-emerged in bio-controlled farming systems in Europe where chemical disease management is restricted or not allowed (Matanguihan et al. 2011). Bunt resistant varieties could be a good alternative in low-input and organic wheat production. Growing such varieties would greatly reduce the expenses of the intensive agricultural practices and diminish the risk for environmental pollution.

There are 15 known major genes (*Bt* genes) for race-specific bunt resistance (reviewed in Matanguihan et al. 2011), but quantitative mode of inheritance associated with field non-race specific resistance was also reported (Fofana et al. 2008; Dumalasová et al. 2012). The latter type is considered valuable in providing durable resistance against the dynamic pathogen population and is a highly desirable trait for environmentally safe wheat production. Genetic analysis and linkage mapping of novel resistance genes would facilitate their use for breeding new resistant varieties. Doubled haploid (DH) populations are particularly valuable for the needs of genetic mapping. As these populations are largely homozygous, they can be replicated and therefore evaluated repeatedly in different years or in variable environments, thus being useful for quantitative genetic analysis (Collard et al. 2005).

The objectives of the present study were: 1. to characterize two androgenetically derived DH populations developed from crosses between a susceptible wheat variety and two lines of very high and moderate resistance/susceptibility, respectively, for non-race specific common bunt resistance; 2. to determine the trait inheritance mode, and 3. to specify the association between bunt resistance and important agronomic traits.

Materials and Methods

Plant material

A Bulgarian commercial winter wheat (*Triticum aestivum* L.) variety 'Svilena' (susceptible to common bunt) was hybridized with two breeding lines: A-38b-4-5-3-3 (very resistant, A-38b) and WWRN (moderately resistant to moderately susceptible depending on the climatic conditions). Both lines were kindly provided by the author of the former line, H. Kurzhin, Plant Protection Institute, Agricultural Academy, Sofia, Bulgaria. Based on the pedigree (Fig. 1), the resistance of line A-38b can be traced back to the highly resistant variety 'Hope' and further back to the moderately resistant variety 'Marquis' (Gaines 1923). The genealogy of line WWRN is unknown.

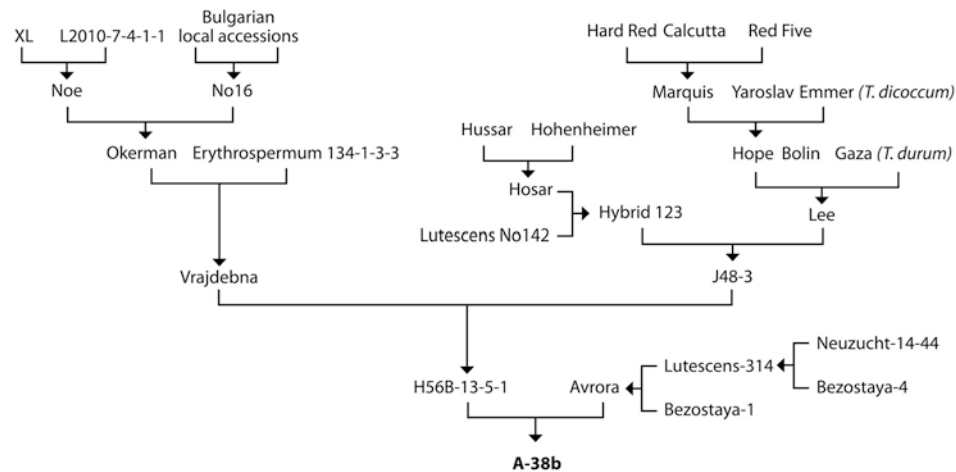


Figure 1. Pedigree of the highly resistant to common bunt (*Tilletia foetida*) line A-38b-4-5-3-3 (A-38b)

Doubled haploid (DH) populations were androgenically generated from the F₁s at Dobrudja Agricultural Institute, General Toshevo, Bulgaria. Forty-eight lines of the ‘Svilena’ × A-38b population and 115 lines of the ‘Svilena’ × WWRN population were studied for bunt resistance and agronomical performance.

Disease evaluation

Parents and DH populations were evaluated for bunt reactions in a three-year field experiment in 2006, 2007 and 2008. For each genotype, two replications of 0.5 m² were sown in the experimental field of the former Institute of Genetics (now Institute of Plant Physiology and Genetics), Bulgarian Academy of Sciences, Sofia, Bulgaria. Prior to sowing, the seeds were inoculated with a mixture of bunt teliospores obtained from infected spikes from Sofia region (42°41’N, 23°19’E). To guarantee optimal conditions for pathogen development, sowing was performed during second halves of October. After harvest, all spikes were evaluated for bunt infestation. The percentage of infected spikes was determined for each replication. The DH lines and parents were classified according to Liatukas and Ruzgas (2005): 0.0% infected spikes = very resistant (VR); 0.1–5.0% infected spikes = resistant (R); 5.1–10.0% = moderately resistant (MR); 10.1–30% = moderately susceptible (MS); 30.1–50.0% = susceptible (S); >50% = very susceptible (VS).

Agronomical evaluation

DH lines and parents were sown without bunt inoculation in 0.3 × 1.0 m plots in 2006 or in 1 m² plots in 2007 and 2008 at the same location as above. For each genotype, heading time was determined as number of days from 1 May, when ca. 50% of the spikes in the plots were half-emerged from the flag leaves. At harvest time, the following traits were measured on 5 plants for each genotype: plant height (from soil surface to the bottom of

spike), spike length, spikelet number, number and mass of grains per spike, thousand-kernel weight (TKW).

Statistical analysis

Data were analyzed (main effects ANOVA, correlation analysis and chi-square test) using the software package STATISTICA v. 7 (StatSoft 2005). Data on bunt incidence in 2008 were excluded because the conditions for the pathogen development were unfavorable and the infestation was much lesser.

Results

Resistance to common bunt

A mixture of *T. foetida* teliospores from races typical for fields in Sofia induced a differential reaction when inoculated onto parental lines, with A-38b being very resistant (average infection rate 0.0%) and 'Svilena' being susceptible (average infection rate 49.6%) (Table 1). An average infection rate of 24.1% was recorded in line WWRN.

Table 1. Bunt infection rate and agronomic traits in parents (mean and SD) on average over 2006 and 2007

Trait	'Svilena'	A-38b	WWRN
Infection rate (%)	49.6 ± 1.79	0.0	24.1 ± 2.53
Plant height (cm)	63.5 ± 14.5	88.3 ± 4.96	61.5 ± 9.78
Spike length (cm)	10.5 ± 0.82	9.8 ± 1.09	8.21 ± 1.37
Spikelet number	20.8 ± 2.24	21.1 ± 2.45	19.0 ± 1.06
Grain number / main spike	47.6 ± 14.8	49.3 ± 17.2	41.7 ± 3.87
Grain mass / main spike (g)	2.06 ± 0.47	1.97 ± 0.74	1.74 ± 0.44
TKW (g)	44.4 ± 3.99	39.8 ± 3.86	41.4 ± 11.8
Heading (days from 1 May)	15.5 ± 4.09	21.5 ± 3.21	13.3 ± 5.21

The infection rate in the DH population derived from the cross 'Svilena' × A-38b ranged from 0.0 to 81.4% in 2006 and from 0.0 to 78.0% in 2007, average range from 0.0 to 78.2% (Table 2). In the DH population derived from the cross 'Svilena' × WWRN, the disease incidence ranged from 1.6 to 100% infected spikes in 2006, and from 2.0 to 100% in 2007, on average from 5.7 to 92.8%. According to ANOVA results, the highly significant variations for bunt resistance were determined attributed to the genotypic background of the DH lines in both populations ($p < 0.001$), and the year ($p < 0.01$). The effect of year accounting for different climatic conditions was different in the two populations. In 'Svilena' × A-38b population, the disease incidence was higher in 2006, whereas in 'Svilena' × WWRN population it was higher in 2007. However, the year-to-year correlation analysis between bunt infection rates of individual DH lines showed highly consistent results for both populations ('Svilena' × A-38b: $r = 0.89$, $p < 0.001$, $N = 48$ lines; 'Svilena' × WWRN: $r = 0.64$, $p < 0.001$, $N = 115$ lines).

Table 2. Bunt infection rates and agronomic characteristics in two wheat androgenetically derived doubled haploid populations on average over two (bunt resistance) and three (agronomic traits) years

Trait	Group	'Svilena' × A-38b		'Svilena' × WWRN	
		Mean ± SD	Range	Mean ± SD	Range
Bunt infection rate (%)	VR	0.0	0.0	–	–
	R	2.4 ± 1.77	0.6–5.0	–	–
	MR	7.7 ± 2.09	6.2–9.2	7.6 ± 1.18	5.7–9.9
	MS	21.2 ± 6.55	10.7–27.8	20.2 ± 6.44	11.1–29.9
	S	38.2 ± 5.81	32.1–49.1	40.9 ± 5.31	30.8–49.9
	VS	66.1 ± 8.42	53.1–78.2	70.1 ± 11.51	50.8–92.8
Plant height (cm)	VR	68.3 ± 12.30	49.9–88.3	–	–
	R	70.2 ± 2.07	68.6–73.7	–	–
	MR	72.7 ± 4.43	67.2–77.8	62.3 ± 3.87	57.6–66.9
	MS	79.5 ± 11.30	63.9–96.4	64.9 ± 9.65	49.9–84.6
	S	77.1 ± 18.00	53.2–105.0	63.9 ± 8.32	48.5–80.0
	VS	69.9 ± 12.50	49.0–94.7	61.0 ± 8.98	43.9–84.8
Spike length (cm)	VR	9.2 ± 2.54	6.0–13.6	–	–
	R	10.1 ± 1.81	7.2–12.2	–	–
	MR	9.2 ± 0.66	8.5–9.9	9.7 ± 0.99	8.3–10.6
	MS	10.4 ± 0.87	9.4–12.0	9.2 ± 1.01	7.0–11.2
	S	10.9 ± 2.07	8.4–15.0	9.9 ± 1.19	7.8–12.5
	VS	10.7 ± 1.60	7.9–13.0	9.7 ± 1.02	7.5–12.1
Spikelet number	VR	19.4 ± 1.79	16.5–22.4	–	–
	R	20.7 ± 2.03	18.3–23.4	–	–
	MR	19.9 ± 2.37	17.0–22.2	20.1 ± 2.60	16.4–22.0
	MS	20.1 ± 2.47	17.1–24.8	20.1 ± 1.84	15.2–23.6
	S	21.5 ± 1.75	19.9–24.2	20.0 ± 1.56	16.4–24.4
	VS	21.1 ± 1.67	17.6–24.0	19.7 ± 2.19	15.2–29.0
Grain number / main spike	VR	35.2 ± 10.90	18.7–49.5	–	–
	R	43.7 ± 7.95	34.3–53.0	–	–
	MR	37.0 ± 9.61	28.3–47.3	33.8 ± 13.0	15.0–43.2
	MS	47.8 ± 10.60	38.2–69.0	36.1 ± 8.85	22.4–51.4
	S	45.3 ± 8.22	32.7–60.9	42.1 ± 10.8	24.4–60.8
	VS	44.3 ± 6.52	34.4–57.2	37.7 ± 8.29	18.8–56.0
Grain mass / main spike (g)	VR	1.5 ± 0.49	0.8–2.0	–	–
	R	1.6 ± 0.33	1.2–1.9	–	–
	MR	1.3 ± 0.50	1.0–2.1	1.4 ± 0.72	0.4–2.0
	MS	2.0 ± 0.71	1.3–3.4	1.5 ± 0.54	0.8–2.6
	S	1.9 ± 0.49	1.3–2.9	1.9 ± 0.57	0.9–2.9
	VS	1.9 ± 0.27	1.5–2.5	1.5 ± 0.52	0.4–2.5
TKW (g)	VR	39.4 ± 4.76	29.3–44.8	–	–
	R	36.9 ± 5.11	32.7–45.8	–	–
	MR	35.2 ± 7.36	27.4–43.6	41.3 ± 13.2	26.7–58.6
	MS	44.3 ± 4.11	39.5–49.9	41.8 ± 8.02	31.0–55.0
	S	41.4 ± 2.87	36.8–44.9	43.5 ± 7.74	27.1–62.5
	VS	41.8 ± 1.45	39.7–44.0	41.2 ± 8.48	23.5–59.0

Table 2 (cont.)

Trait	Group	'Svilena' × A-38b		'Svilena' × WWRN	
		Mean ± SD	Range	Mean ± SD	Range
Heading (days from 1 May)	VR	18.4 ± 2.55	15.4–22.0	–	–
	R	18.4 ± 3.59	13.5–21.8	–	–
	MR	16.8 ± 2.42	14.8–20.3	22.2 ± 1.89	21.0–25.0
	MS	17.2 ± 3.15	14.4–23.0	21.6 ± 1.82	18.0–25.0
	S	19.6 ± 2.67	16.8–23.0	21.1 ± 1.35	19.0–25.0
	VS	17.4 ± 3.51	10.5–23.0	20.5 ± 2.23	15.0–25.0

The lines are classified in groups, according to their infection rate: 0% – very resistant (VR), 0.1–5.0% – resistant (R), 5.1–10.0% – moderately resistant (MR), 10.1–30.0% – moderately susceptible (MS), 30.1–50.0% – susceptible (S), >50.0% – very susceptible (VS)

On average over the two experimental years, 9 of the 48 DH lines of the cross 'Svilena' × A-38b had 0.0% disease incidence, classified as very resistant (VR), and 14 showed over 50.0% infection classified as very susceptible (VS, average infection rate 66.1%) (Table 2). The rest of DH lines formed one group of intermediate resistance (9 lines) consisting of resistant (R) and moderately resistant (MR) lines, and another group of intermediate susceptibility (16 lines), consisting of moderately susceptible (MS) and susceptible (S) lines (Fig. 2, Table 2). The occurrence of two highly distinguishable and two interme-

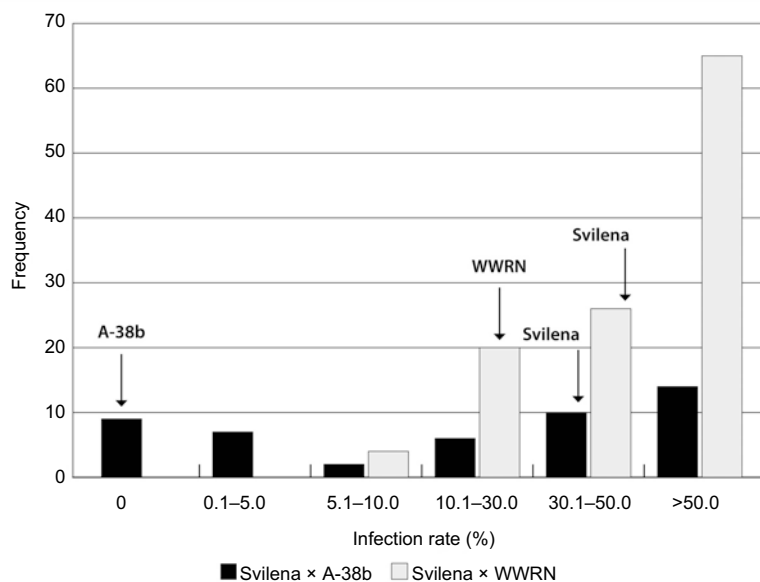


Figure 2. Distribution of wheat doubled haploid lines from 'Svilena' × A-38b and 'Svilena' × WWRN populations according to the infection rates after inoculation with a mixture of *Tilletia foetida* teliospores averaged over 2006 and 2007 field experiments. Arrows indicate parents

diate groups of lines in segregation of 9 VR : 9 R + MR : 16 MS + S : 14 VS suggests that the resistance of line A-38b is probably conferred by two major genes of additive action. The chi-square test against an expected ratio of 1:1:1:1, i.e. 12 VR : 12 R + MR : 12 MS + S : 12 VS, for two independent loci for resistance failed to reject the H_0 ($\chi^2 = 3.16$, $p = 0.05$, $df = 3$) thus being in favour of the above suggestion.

The DH population derived from the cross 'Svilena' \times WWRN consisted of only moderately resistant and moderately susceptible to highly susceptible lines when averaged over the two experimental seasons (Table 2, Fig. 2), although single resistant lines were observed in both 2006 and 2007. The infection rate within the population followed continuous distribution and displayed transgressive segregation (Fig. 2) with 16 lines showing higher resistance than the moderately susceptible parent WWRN thus suggesting that the bunt reaction of line WWRN is probably under polygenic control with minor effects of the individual genes.

Agronomic evaluation

The parental genotypes of the two DH populations were semi-dwarf and highly productive. 'Svilena' and WWRN were earlier and had shorter culms (Table 1). In both populations, transgressive segregation was observed for all traits (Table 2).

According to the correlation analysis, higher infection rate in 'Svilena' \times A-38b population was associated with higher values of spike length, spikelet number and grain set (Table 3). In the progeny of 'Svilena' \times WWRN cross, higher disease incidence was recorded in plants with longer culms, more spikelets in the main spike and later heading time. Despite this general trend of positive correlation between disease infection and yield components, there were genotypes among the very resistant and resistant lines of the 'Svilena' \times A-38b population that combined high resistance and high productivity as can be seen by the wide agronomical variation within these two groups (Table 2). Within the 'Svilena' \times WWRN population, only few lines of moderate resistance (infection rate <10%) had good production potential (Table 2).

Table 3. Correlations (r) between bunt infection rate (averaged over two years) and agronomic traits (averaged over three years) in two wheat androgenetically derived doubled haploid populations

Population	Plant height	Spike length	Spikelet number	Grain number / main spike	Grain mass / main spike	TKW	Heading
'Svilena' \times A-38b	0.14	0.53**	0.41*	0.39*	0.25	0.21	0.07
'Svilena' \times WWRN	0.24**	-0.12	0.21*	-0.05	0.03	0.10	0.23*

*,** significant at $p < 0.05$ and $p < 0.01$, respectively

Discussion

The most effective approach to control bunt incidence in wheat is the use of varieties combining high disease resistance with a set of agronomic characteristics resulting in adequate grain yield and ecological plasticity. The current trend to reduce or eliminate chemical

treatments in bio-controlled farming systems relies mostly on the inherent resistance of the wheat varieties. Screening of large pools of genotypes comprising commercial and historical varieties, breeding lines, and old local accessions revealed very low levels of resistance (Liatukas and Ruzgas 2005, 2008; Bonman et al. 2006; Dumalasová and Bartoš 2006, 2013; Rajković and Dolovac 2006). The search for new sources of resistance would enhance the genetic diversity available for breeding purposes.

The genealogy of the very resistant line A-38b suggests two possible sources of bunt resistance – the variety ‘Hope’ and/or ‘Hybrid 123’ (see Fig. 1). ‘Hope’ appears to have gained this quality from its moderately resistant parents ‘Marquis’ (Gaines 1923) and/or ‘Yaroslav Emmer’ (Martynov et al. 2004). McFadden (1930) explains the high resistance of ‘Hope’ with recombination events and transgressive segregation. The field resistance of ‘Hope’ is non-race-specific and is probably conferred by two major recessive genes and two other genes with minor effects (McKenzie 1964). It is expressed at high temperatures (20–22°C at spring sowing), but at autumn sowing the resistance is suppressed by the low winter temperatures (Gaudet and Puchalski 1989). The other suggestive resistance donor is ‘Hybrid 123’. This line has probably obtained its resistance from its ancestor ‘Hussar’. Both ‘Hussar’ and ‘Hybrid 123’ were reported to be highly resistant to the prevailing *T. foetida* races in Bulgaria (Dodov and Todorova 1974). According to Gaines (1923) the resistance of ‘Hussar’ was determined by the additive effect of a large number of genes, while Briggs (1930) attributed it to the effectiveness of one dominant and one incompletely dominant genes. Until recently, three genes – *Bt1*, *Bt2* and *Bt5*, have been identified in ‘Hussar’ (Metzger 1970; Hoffman and Metzger 1976; Martynov et al. 2004).

The excellent resistance of line A-38b might be explained by a combination of resistance genes that are highly effective towards the pathogen racial composition in Bulgaria. The results of the present study suggested an inheritance model in line A-38b involving two resistance genes of additive action that had probably been transferred from both potential resistant ancestors: one dominant gene originating from ‘Hussar’, and one ‘Hope’-derived recessive gene. This assumption could explain the occurrence of lines with 0.0% disease incidence (carrying two resistance genes), lines with >50% disease incidence (no resistance gene), and two intermediate groups carrying either gene. The relatively high autumn temperatures recorded during the two experimental seasons were likely to allow the expression of the ‘Hope’-derived non race-specific resistance.

The composition of the pathogen races in Bulgaria has not been monitored during the last few decades because of the effective chemical control over the disease. Earlier studies on the composition of the pathogen races showed little dynamics during the period from 1940s to 1970s, as well as the prevalence of races I Ib, I f and I Ic (Krustev 1946; Dodov and Todorova 1974). The obstruction to bunt spread by fungicide seed treatments, as well as the use of susceptible varieties could explain the low variability in the pathogen population. This, in turn, could account for the preserved effectiveness of the resistance genes originated from the old germplasm that seem to have been further transferred to line A-38b.

Line WWRN showing 24% infection rate on average over two experimental years was classified as moderately susceptible based on the scale proposed by Liatukas and Ruzgas

(2005) although in other seasons the line exhibited lower infection level and was described rather as moderately resistant. The transgressive segregation observed in the DH population derived from the cross between this line and the susceptible variety 'Svilena' is indicative for a quantitative mode of inheritance. Very few works are available on identifying sources of non-race specific resistance that can provide durable resistance. Appearance of progenies with higher resistance compared to that of both parents was reported earlier by McFadden (1930) suggesting polygenic control of resistance. Recently, three QTL for bunt resistance were identified on chromosomes 1B and 7A using a segregating DH population derived from a cross between lines with 12% and 30% disease incidence, respectively (Fofana et al. 2008). Dumalasoová et al. (2012) mapped QTL for bunt resistance on the same chromosomes, and additional loci on 5B and 7B.

Most sources of resistance are found within older germplasm and are generally of inferior agronomic performance. Thus, they are not suitable for direct use in wheat production but can serve as donors of resistance genes in breeding programs (Ruzgas and Liutukas 2009; Matangiuhian et al. 2011). In compliance with this observation, the present study showed that bunt incidence was positively correlated with yield components (spike length, spikelet number and grain number in the main spike) in the progeny of 'Svilena' × A-38b cross. These associations cannot be attributed to the effects of the pathogen infection because the agronomic performance of the DH lines was evaluated on non-infected control plants. Nevertheless, recombinant lines were observed within the 'Svilena' × A-38b population where high and very high resistance was combined with good agronomic potential. These genotypes are a promising source for breeding resistant varieties of acceptable yield capacity and could be of value for growing in organically oriented farming systems. For breeding using doubled haploids, it is essential to have an estimate of the anther culture responsiveness of the parental genotypes and the hybrid material (Lantos et al. 2013). In our case, 'Svilena' is a highly responsive variety in terms of high rate of callus and embryoid induction, high rate of green plantlets regeneration and relatively low occurrence of albinism (Belchev et al. 2000; Lantos et al. 2013). This quality is expected to have been transferred in some of the 'Svilena'-derived DH lines. Therefore, both sets of doubled haploids could be screened for anther culture response. Lines with good androgenic response could be used in wheat biotechnology to facilitate the development of breeding material that combines high resistance and adequate productivity. The two DH sets of lines are also suitable to be used for further studies on the genetic basis of bunt resistance aiming at gene identification and localization, and mapping of associated QTL.

Acknowledgements

The authors thank Ms T. Georgieva (Plant Protection Institute, Kostinbrod, Bulgaria) for providing pathogen teliospores. The technical assistance of Mrs. K. Prokopova and Mrs. P. Popova is gratefully acknowledged.

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