DNA-markers for Resistance to Common Bunt Transferred from *Aegilops cylindrica* Host. to Hexaploid Wheat

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Abstract: A single dominant gene for bunt resistance from *Aegilops cylindrica* was transferred to common wheat. Microsatellite markers linked to the resistance gene were searched for. The marker Xgwm 259 was linked to the resistant gene with a map distance of 8.3 cM. Molecular mapping of the resistant gene was carried out. The gene for bunt resistance was located in intercalary region of chromozome 1 BL.

Keywords: common bunt; *Aegilops cylindrical*; wheat; resistance; DNA-markers

Common bunt, caused by *Tilletia caries* (DC.) Tul., is widespread disease of wheat (*Triticum aestivum* L.) that causes serious yield reduction and loss of quality of this crop. Majority of Ukrainian wheat varieties are susceptible to bunt. It is necessary to search for sources of effective resistance genes.

Aegilops cylindrica (2*n* = 28; genome CCDD) is a source of genes for some pest resistance in wheat Bochev *et al.* (1982). *Ae. cylindrica* carries resistance to leaf rust, stem rust, fusarium, septoria, powdery mildew and common bunt.

The identification of DNA-markers linked to bunt resistance and susceptibility alleles would greatly facilitate the screening of wheat introgressive genotypes and accelerate the development of new resistant varieties. Microsatellites or simple sequence repeat (SSR) is widely used to search for molecular markers linked to many economic traits genes McIntosh *et al.* (2003).

Present study reports markering and mapping with microsatellite markers of effective resistance gene to bunt in bread wheat introgressed from *Ae. cylindrica*.

MATERIALS AND METHODS

Plant material. Local population of *Ae. cylindrica*, recurrent parent wheat (*Triticum aestivum* L.) cv. Odesskaya polucarlikovaya and wheat line Lutestens 23397, introgressive wheat line 5/55-91 – [(Odesskaya polucarlikovaya × *Ae. cylindrica*) × Odesskaya polucarlikovaya]F_9 (2*n* = 42), introgressive line 378/2000 – (5/55-91 × Odesskaya polucarlikovaya) F_5 (2*n* = 42). BC_3F_2 population from crossing 378/2000 × Lutestens 23397 and derived BC_3F_3 families were used in the study.

Bunt evaluation. *Ae. cylindrica*, recurrent parent wheat cv. Odesskaya polucarlikovaya and line Lutestens 23397, introgressive lines 5/55-91, 378/2000 and BC_3F_2 – derived F_3 families were evaluated for resistance to bunt in field infection nursery. Bunt resistance in line 5/55-91 is controlled by a single dominant gene (Babayants *et al.* 2004).

Microsatellite marker analysis. For SSR-analysis were used 95 pair primers to 107 microsatellite loci with known localization on chromosomes of wheat (Röder *et al.* 1998). For identification microsatellite markers linked to the resistance gene was used bulked segregation analysis (BSA) as described by Michelmore *et al.* (1991). Microsatellite markers generating polymorphic fragments between bulks were further checked for their linkage to the resistance gene, using 170 plants of BC_3F_2 population.

Linkage analysis. Linkage between microsatellite markers and the bunt resistance gene were

calculated using "MAPMAKER" ver. 3.0 (Lander *et al.* 1987) and converted to cM using Kosambi mapping function. Molecular mapping of microsatellite markers was carried out using "JOINMAP" ver. 2.0 (Stam 1993) with using Kosambi and Haldane mapping functions. The linkage is reliable if the markers were placed with LOD (logarithm of odds ratio) threshold of 3,0.

RESULTS AND DISCUSSION

Common bunt resistance. *Ae. cylindrica*, introgressive lines /55-91, 378/2000 were resistant to bunt, recurrent parents wheat cv. Odesskaya polucarlikovaya, line Lutestens 23397 was susceptible to bunt. Total of 170 BC_3F_2 individuals were identified for resistance to bunt in the field. Observed segregation of 138 resistant and 32 susceptible individuals fitted 3:1 segregation ratio ($\chi^2 = 2.13$). This result showed that resistance to bunt BC_3F_2 population is controlled by a single dominant gene. 138 BC_3F_2 – derived F_3 families were tested for resistance to bunt to determine genotype BC_3F_2 individuals. 34 families were homozygous resistant, 104 families were heterozygous resistant. Observed segregation did not fit 1:2:1 segregation ratio ($\chi^2 = 7.66$). The factor of segregation distortion was possibly linked with location of the resistance gene in alien translocation as result of which may be certain distortions.

Microsatellite markers linked to introgression DNA fragments. Based on the microsatellite map of wheat (Röder *et al.* 1998), 107 microsatellite (MS) markers were used to search of introgression fragments in backcrossing progenies of *Triticum-Aegilops* hybrids (lines 5/55-91 and 378/2000). SSR-analysis allowed to characterize the genome variability caused introgression of the alien genetic material of *Ae. cylindrica* into wheat and detected eight introgression DNA fragments in the line 5/55-91 BC_1F_9. Introgression DNA fragments revealed in the line 5/55-91 show stability for the line 378/2000 BC_2F_5. SSR-analysis has allowed also locating introgression DNA fragments in the genome of wheat.

Microsatellite markers linked to the resistance gene. Eight MS markers to stabile introgression DNA fragments were screened to discriminate polymorphic MS markers between the resistant and susceptible DNA bulks. One primer pair to MS locus Xgwm 259 generated polymorphic DNA fragment between the bulks with size 99 bp. The allele 99 bp Xgwm 259 was in the resistance pool, but absent in the susceptible pool. The marker Xgwm 259 was linked to the resistant gene with a map distance of 8.3 cM. The resistance gene is located in telomere region of long arm of chromosome 1B according to location of the marker Xgwm 259 on MS map of wheat (Röder *et al.* 1998).

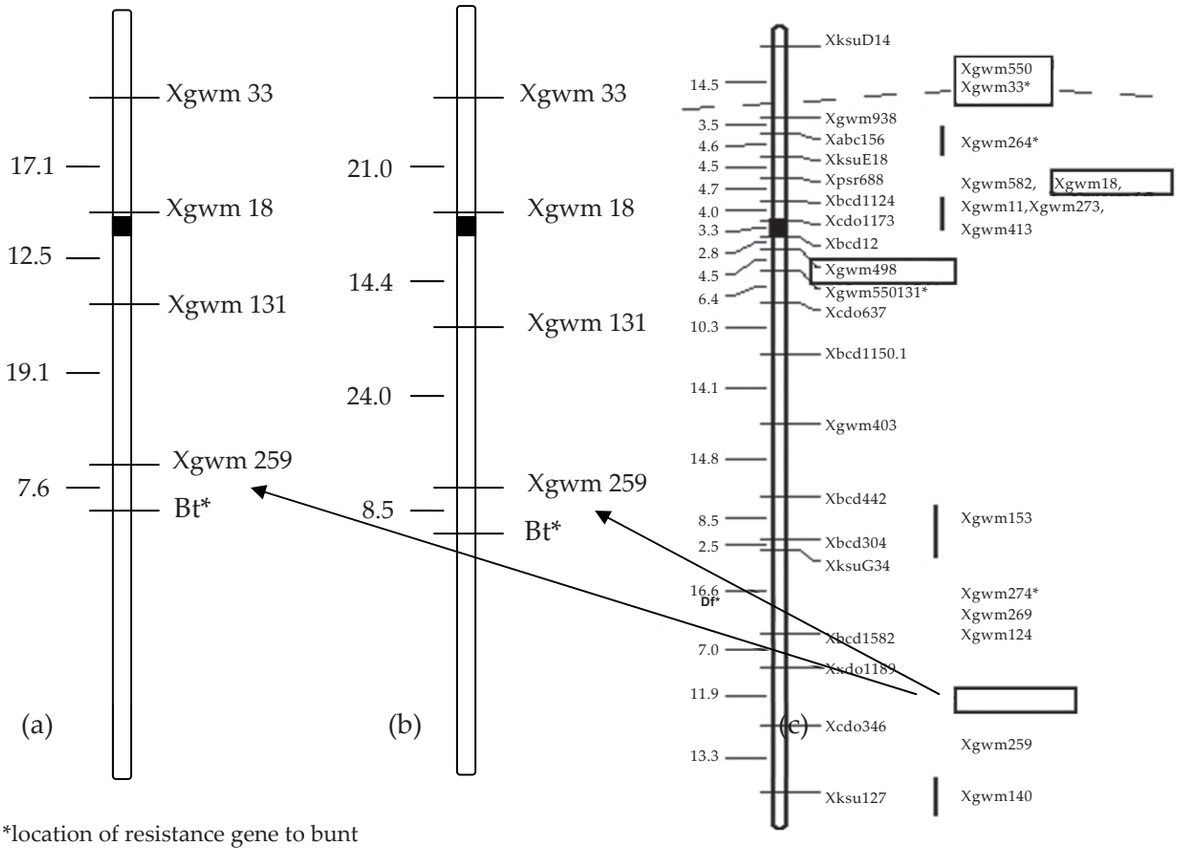
Molecular mapping of the bunt resistance gene. The resistance gene to bunt was mapped with four MS markers (Xgwm 33-1BS, Xgwm 18-1BS, Xgwm 131-1BL and Xgwm 259-1BL). These MS markers were used for segregation analysis of 170 plants of BC_3F_2 population (Table 1). Molecular mapping of microsatellite markers and the bunt resistance gene carried out using "JOINMAP" ver.2.0 (Stam 1993) with using Kosambi (Figure 1a) and Haldane (Figure 1b) mapping functions. MS markers reveal that resistance gene is located in intercalary region of long arm of chromosome 1B of wheat.

According to MS map of wheat (Röder *et al.* 1998), marker Xgwm 259 is located in telomere region of chromosome 1BL (Figure 1c), while this marker in our research was located in intercalary region of chromosome 1BL of wheat introgression lines. It is possible to assume, that occurred: translocation of introgression fragment terminal

Table 1. Segregation analysis for the resistance gene to common bunt

Gene or MS marker	Number of BC_3F_2	Observed number	Expected number	χ^2	*P* (df = 2)
Resistance gene to common bunt	170	34^1:104^2:32^3	42^1:86^2:42^3	8.1	0.025–0.010
Xgwm 259	170	40:92:38	42:86:42	0.9	0.75–0.50
Xgwm 131	170	33:96:41	42:86:42	4.0	0.25–0.10
Xgwm 18	170	30:102:38	42:86:42	5.0	0.10–0.05
Xgwm 33	170	31:102:37	42:86:42	5.8	0.10–0.05

[1]homozygous resistant; [2]heterozygous resistant; [3]homozygous susceptible



*location of resistance gene to bunt

Figure 1. Genetic linkage maps of bunt resistance gene and the linked MS markers on wheat chromosome 1B

The genetic distances between resistance gene to bunt and MS markers were based: a – on data calculated with Kosambi mapping function; b – Haldane mapping function; c – on published data by Röder *et al.* (1998); short arms of chromosomes are at the top; the centromeres are indicated in black

into intercalary region long arm chromosome 1B of wheat; inversion of chromosome 1BL fragment; or location of marker Xgwm 259 in intercalary region characteristic of research to wheat cultivars.

The allele 99 bp Xgwm 259 is present in twelve introgressive lines BC_1F_9 that have resistance to bunt transferred from *Ae. cylindrica* and absent in twenty-seven Ukrainian wheat varieties. This fact shows that the allele 99 bp is connected to bunt resistance gene specific to *Ae. cylindrica* and introgression lines carrying it.

SSR-analysis of population BC_3F_2 of 378/2000 × Lutestens 23397 revealed linkage of microsatellite locus Xgwm 259 to common bunt resistance gene. The resistance gene was located in intercalary region of chromosome 1B long arm distal on 7.6–8.5 cM from Xgwm 259. Microsatellite locus Xgwm 259 can be used in wheat breeding for selection of genotypes resistant to common bunt that is controlled by discovered gene.

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