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Microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*

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Abstract The *S1*, *S2* and *S3* genes of the induced sphaerococcoid mutation in common wheat (*Triticum aestivum*) were mapped using three different F_2 populations consisting of 71–96 individual plants. Twenty-four microsatellite markers from homeologous group 3 of *T. aestivum* were used to map the *S1*, *S2* and *S3* genes on chromosomes 3D, 3B and 3A, respectively. The *S1* locus was found to be closely linked to the centromeric marker *Xgwm456* of the long arm (2.9 cM) and mapped not far (8.0 cM) from the *Xgdm72* marker of the short arm of chromosome 3D. The *S2* gene was tightly linked to 2 centromeric markers (*Xgwm566*, *Xgwm845*) of chromosome 3B. *S3* was located between *Xgwm2* (5.1 cM), the marker of the short arm, and *Xgwm720* (6.6 cM), the marker of the long arm, both of chromosome 3A. Mapping the *S1*, *S2* and *S3* loci of the induced sphaerococcoid mutation near the centromeric regions supports the hypothesis that the sphaerococcum type may be due to gene duplication resulting from DNA recombination in the centromeric region.

Key words *Triticum aestivum* · Sphaerococcoid mutation · *S1*, *S2*, *S3* · Microsatellite map

Introduction

The sphaerococcum effect has been the distinguishing feature of a single hexaploid wheat, *Triticum sphaerococcum* Perc. Sears (1947) reported that the sphaerococcum gene (*s1*) is a hemizygous-ineffective recessive gene located on chromosome 3 of the D genome. The gene affects a set of characters formed during development and inherited as a unit. This set includes rigid short culm, straight flag leaf, dense spike, hemispherical glume, and small, spherical grains.

Sphaerococcum-like plants are also induced by mutation, especially by chemical mutagens, neutrons and X-rays. In contrast to the hemizygous-ineffective recessive gene *s1* of *T. sphaerococcum*, the mutant character of some sphaerococcum-like plants is under the control of a single gene, or gene block, with an incompletely dominant effect. Induced sphaerococcoid mutations have been observed in tetraploid wheat, thereby indicating that they may occur in the A and/or B genome (Bozzini 1965; Gupta and Swaminathan 1967; Schmidt and Johnson 1966). Sphaerococcoid mutations also occur in common wheat (Zschege 1963; Schmidt et al. 1963; Zoz 1971). Melnik (1988) obtained three independent mutants with morphological features resembling *T. sphaerococcum* after treating of the *T. aestivum* varieties Saratovskaya 29 and Skala with chemical mutagens. One of the induced mutants was allelic to the natural *Sphaerococcum* wheat. On the basis results from monosomic analysis, the genes for sphaerococcoidy were designated as *S1*, *S2*, *S3* and located on chromosome 3D, 3B and 3 A, respectively (Maystrenko et al. 1998). The genes for the sphaerococcoid mutations have not been mapped, so far. The close linkage of *T. sphaerococcum* gene *s1* to the centromere of chromosome 3D has been estimated to be 5.7% by Rao (1977) and as 5.0+2.0% by Koba and Tsunewaki (1978). These authors disagreed on the location of the gene – either on the long (Koba and Tsunewaki 1978) or short (Rao 1977) arm of chromosome 3D.

The aim of the study presented here was to map the *S1*, *S2* and *S3* genes of the induced sphaerococcoid mutation in *T. aestivum*. The more recent development of more than 300 polymorphic microsatellite markers and their integration into a genetic framework map (Röder et al. 1998) has allowed these markers to be used for molecular mapping of the wheat genes.

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Fig. 1 Spikes, and glumes grains were recognisable in the segregants from the cross of the sphaerococcoid mutant lines and the common wheat variety Novosibirskaya 67. From *left* to *right*: homozygous (mutant phenotype), heterozygous (intermediate phenotype), normal common wheat

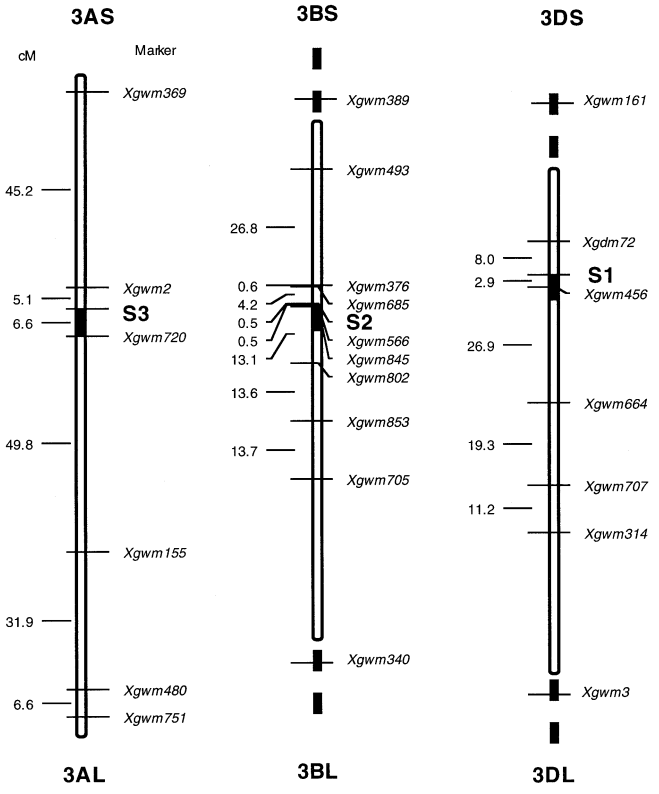

Fig. 2 Genetic microsatellite map of homeologous group 3 derived from the F_2 of the cross of the sphaerococcoid mutant lines of the *T. aestivum* varieties Saratovskaya 29, Skala×Novosibirskaya 67 showing the location of *S3*, *S2* and *S1*. Genetic distances are given in centiMorgans (cM). The centromeres are indicated in *black*. The markers placed on the *interrupted line* are genetically independent

Materials and methods

Plant material

The induced sphaerococcoid mutant lines of the *T. aestivum* varieties Saratovskaya 29 (MS 3287, MS 1453) and Skala (MSK 2454) are phenotypically similar and their genotypes are *S1/S1*, *S3/S3* and *S2/S2*, respectively (Maystrenko et al. 1998). To map the *S1*, *S2* and *S3* genes, we developed three F_2 populations by crossing the sphaerococcoid mutant lines and the common wheat variety Novosibirskaya 67.

Individuals of the F_2 generations from the combination sphaerococcoid mutant lines and common wheat variety Novosibirskaya 67 were classified according to mutant, intermediate or normal phenotype (Fig. 1). The morphological features taken into account in phenotype determination were height and rigid stem, dense spike, glume and grain shape. Seventy two F_2 individuals (18 plants of genotype *S1/S1*; 36, *S1/s1* and 18, *s1/s1*) of the MS 3287×Novosibirskaya 67 cross, 71 F_2 plants (15, *S3/S3*, 39; *S3/s3*; 17, *s3/s3*) of the MS 1453×Novosibirskaya 67 cross and 96 F_2 plants (25, *S2/S2*; 46, *S2/s2*; 25, *s2/s2*) of the MSK 2454× Novosibirskaya 67 cross were used.

Mapping technique

Nuclear DNAs were isolated from leaves of single F_2 plants following the procedure of Plaschke et al. (1995).

The wheat microsatellites located on chromosome 3 A, 3B and 3D were chosen to map the *S3*, *S2* and *S1* genes. The microsatellite markers with numbers from *Xgwm2* to *Xgwm674* have been developed for *T. aestivum* and described by Röder et al. (1998). The microsatellites marked as *Xgdm...* have been developed for *Aegilops squarrosa* (unpublished data). Unpublished primer sequences are available upon request.

Microsatellite analysis was conducted according to Röder et al. (1998). Fragment analysis was carried out on automated laser fluorescence (ALF) sequencers (Pharmacia) using short gel cassettes.

Multipoint linkage values in centiMorgans (cM) were calculated using the MAPMAKER 2.0 computer program (Lander et al. 1987).

Results

Mapping of the *S3* gene (the cross between MS 1453×Novosibirskaya 67)

In a previous paper, the *S3* gene for the induced sphaerococcoid mutation in MS 1453 was located on chromosome 3A (Maystrenko et al. 1998). Subsequently, 71 F_2 plants from the MS 1453×Novosibirskaya 67 cross were used for microsatellite mapping. Of these, 15 individuals have the sphaerococcoid phenotype (*S3/S3*), 39 are phenotypically intermediate (*S3/s3*), and 17 have the normal phenotype (*s3/s3*) (Fig. 1). Sixteen microsatellite probes, mapped on chromosome 3 A (Röder et al. 1998 and unpublished mapping data), were used to identify polymorphism between MS 1453 and Novosibirskaya 67. Six polymorphic markers which detect a single locus (with the exception of the *Xgwm2* marker which detects two loci) were used to map the *S3* gene on chromosome 3A (Fig. 2). The segregation ratios of the chromosome microsatellite probes conformed to the expected 1:2:1, as the χ^2-values demonstrate ($P>0.9$ for *Xgwm2*, *Xgwm720*, $P>0.25$ for *Xgwm480*, *Xgwm751*, $P>0.1$ for *Xgwm369*, *Xgwm155*). The genetic map location of the *S3* locus is

shown on the left side of Fig. 2, located between the centromeric markers *Xgwm2* (5.1 cM) and *Xgwm720* (6.6 cM). The chromosomal arm locations of these microsatellites were determined using the respective ditelosomic lines of Chinese Spring. *Xgwm2* and *Xgwm720* are located on the short and long arms of chromosome 3A, respectively.

Mapping of the S2 gene (the cross between MSK 2454×Novosibirskaya 67)

Monosomic analysis of the Skala mutant, namely MSK 2454, demonstrated that the *S2* gene for sphaerococcoidy is located on chromosome 3B (Maystrenko et al. 1998). Twenty-five F_2 individuals showing the sphaerococcoid phenotype (*S2/S2*), 46 of an intermediate phenotype (*S2/s2*) and 25 with a normal phenotype (*s2/s2*) were included in the microsatellite mapping (Fig. 1).

The total number of microsatellite markers of chromosome 3B tested for identification of polymorphism between MSK 2454 and Novosibirskaya 67 was 26. Of the 12 polymorphic markers 10 were chosen to map the *S2* gene. All the probes used for mapping were not significantly different from the expected 1:2:1 ratio, as tested by the χ^2 ($P>0.75$ for 9 markers, $P>0.1$ for *Xgwm 853*).

As shown in Fig. 2, the *S2* gene is tightly linked to 2 centromeric markers (*Xgwm566*, 0.5 cM; *Xgwm845*, 0.5 cM) and by 4.2 cM to *Xgwm376*, which was placed on chromosome 3BS. The centromere was positioned according to the microsatellite map of Röder et al. (1998).

Mapping of the S1 gene (the cross between MS 3287×Novosibirskaya 67)

The *S1* gene is allelic to the gene in the natural group of *sphaerococcum* and located on chromosome 3D (Maystrenko et al. 1998). Seventy-two plants of the F_2 population were taken for microsatellite mapping. The phenotype is sphaerococcoid (*S1/S1*) in 18 individuals, intermediate (*S1/s1*) in 36 and normal (*s1/s1*) in 18 individuals (Fig. 1). Of the 21 markers mapped on chromosome 3D 7 polymorphic microsatellites were chosen for further analysis. No deviations from the expected 1:2:1 ratio were found by the χ^2-test ($P>0.1$). The results of microsatellite mapping of *S1* are shown in Fig. 2 (right). The *S1* locus is closely linked to the centromeric marker *Xgwm456* of the long arm (2.9 cM) of chromosome 3D and mapped not far (8.0 cM) from the *Xgdm72* marker of its short arm. The location of the *Xgdm72* and *Xgwm456* were checked also by corresponding ditelosomic lines.

Discussion

There have been attempts to localize the genes for sphaerococcoidy. As a result, the *s1* gene of natural s*phaerococcum* wheat was placed near the centromere of chromosome 3D, but the localization data for the long or short arm disagreed (Rao 1977; Koba and Tsunewaki 1978). As for the genes of the induced sphaerococcoid mutation designated as *S1*, *S2* and *S3*, attempts to localize them by the telosomic method were unsuccessful (Maystrenko et al. unpublished data).

Microsatellites are good tools as wheat genetic markers for mapping agronomically and botanically valuable genes. They are abundant, are evenly distributed throughout the genome and have a higher polymorphism than restriction fragment length polymorphism (RFLP) markers. We identified the level of allelic variation in the microsatellites of chromosomes 3 A, 3D (between the mutant lines of the *T. aestivum* varieties Saratovskaya 29 and Novosibirskaya 67), and chromosome 3B (between MSK 2454 and Novosibirskaya 67). Seven of the sixteen (44%) microsatellite loci of chromosome 3A, about 46% of the markers of chromosome 3B and 7 of the 21 microsatellites (33%) of chromosome 3D were polymorphic. It should be noted that lower the polymorphism level of the D genome was also identified by microsatellite and RFLP mapping of the International Triticeae Mapping Initiative (ITMI) population (Marino et al. 1996; Röder et al. 1998).

Microsatellite markers enables us to map the *S1*, *S2* and *S3* genes on chromosome 3D, 3B and 3 A, respectively. All of the genes for sphaerococcoidy were closely linked to 2–4 microsatellites and located near the centromeric region between the markers of the short and long arms.

No crossovers were recovered in attempts to localize the *S1* gene using ditelo- 3AS and ditelo- 3AL (Maystrenko et al. unpublished data), and this confirms again that the gene for the induced sphaerococcoid mutation lies close to the centromere.

The sphaerococcum genes, like the other wheat genes controlling spike shape (Q-speltoid type, C-compactoid type), have a gene dosage effect (Muramatsu 1963; Zschege 1963). One hypothesis explains the mutagenic induction of sphaerococcum types by an increased recombination in the centromeric region, which may give rise to gene duplication (Zoz 1971).

It is well-known that in wheat recombination is suppressed around the centromeres and increasing towards the telomeres (Dvorak and Chen 1984; Curtis and Lukaszewski 1991; Werner et al. 1992). The sphaerococcoid wheat lines MS 3287 and MS 1453 (Melnik 1988) were induced by the chemical mutagen *N*-nitroso-*N*-methylurea (NMU), which was shown to increase the recombination rate (Efremova 1968). The mapping of genes for NMU induced sphaerococcoidy in mutant wheat lines near the centromeric regions supports the hypothesis that the sphaerococcum type may be due to DNA recombination in the centromeric region resulting in gene duplication.

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