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# The Identification of D-Genome in Some Spring Triticale Lines

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**Abstract**: Main problems limiting spring triticale cultivation are poor grain quality, plant height, low plant productivity and preharvest sprouting. One of the suitable way to improve this situation is application of wheat D-genome into triticale. During the secondary spring hexaploid triticale development it is available to observe the genomic reorganization and elimination of D-genome material. In order to resolve the problem regarding the identification of breeding samples bearing D-genome we carried out PCR analysis with chromosome specific SSR-markers and specific STS-marker of locus *Sec2* (2RS). Further for precise identification of triticale lines 2R/2D-substituted we applied genomic *in situ* hybridization (GISH). So it has been shown that 7 lines of spring triticale (of 15 ones) bear 2R/2D substitution, results derived from SSR-PCR and STS-PCR analysis being approved by GISH. It means that PCR-analysis alone is enough to select D-genome bearing plants. Spring triticale forms L 8-4 and L 8-6 are segregating in rye chromosome number, among them there being 2R/2D-substituted, non-substituted plants and hybrids between them. In this respect, it is necessary to make individual selection based on the molecular marker application. Such amazing large ratio of 2R/2D-substituted forms in the collection investigated may be explained by the preliminary selection under field conditions according to low plant height and short growing period. Spring triticale forms appeared to be 2R/2D substituted are involved into breeding process.

**Key words**: secondary spring triticale, D-genome, 2R/2D substitution, chromosome specific SSR-marker, STS-marker, preliminary selection under field conditions, D-genome elimination, short growing period.

## Introduction

Spring triticale is of great interest for cultivation in Europe due to its capacity to produce high grain yield under the various humid conditions (http://mshp.minsk.by/; http://www.zeus.cz/; Salmon et al., 2004). Main limiting problems are poor grain quality, low plant productivity, preharvest sprouting. One of the suitable ways to improve the problems mentioned above is application of wheat D-genome (Inagaki et al., 1997; Cheng et al., 2002; Mergoum et al., 2004; Budzianowski et al., 2004; Benmebarek et al., 2006; Wos et al., 2006; Monneveux et al., http://ressources.ciheam.org/). During the secondary hexaploid triticale development observation of the genomic reorganization and elimination of D-genome (http://www.fao.org/; Ma and Gustafson 2006) is made available. The problem of identifying breeding samples with D-genome arises. There are different methods to identify alien genetic material in the analyzed genome, such as the differential staining, *in situ* hybridization (FISH, GISH), analysis of $F_1$ hybrid meiosis, molecular markers and protein markers (Ribeiro-Carvalho et al., 2001).

Molecular marker application allows to fasten the isolation of the D-genome carrier with the 100% authenticity under the condition of absence of the cross amplification with other genomes. Microsatellite SSR-markers have a number of advantages: codominance, technical simplicity, rapidness, presence of wide databases and abundance of other laboratory researches, being not time- and money-consuming ones (Röder et al., 1998a, 1998b; Song et al.., 2005).

At the same time DNA-PCR-marker application alone is not enough to establish the cytogenetic constitution of a sample because along with substitutions and addition involving genome D high frequency of translocations takes place. That is why the combination of the cytogenetic methods (GISH, FISH, chromosome number count) and the molecular markers is of great importance. There is a wide collection of spring triticale samples, some of them being obtained from international centers (VIR, CIMMYT, USDA). Some low-stemmed and early-maturing triticale forms have been developed at the Department of Genetics as a result of breeding programs (Soloviev 2007). The aims included *(i)* screening of the spring triticale collection of the Department of Genetics (RSAU-MTAA) to define D-genome material using SSR-markers represented here; *(ii)* investigation for the lines with genomic substitutions by means of genomic *in situ* hybridization (GISH); *(iii)* improvement of the selected strains in the breeding programs.

## Materials and Methods

Tab. 1. The lines of spring triticale, wheat and rye being investigated are listed below

| № | Sample name | Origination | Species | Genetic constitution | Chromosome number, 2n= |
|---|---|---|---|---|---|
| 1 | PI 587512 | USDA collection | *Triticosecale* Wittm. | No information | No information |
| 2 | L 8-1 | Department of Genetics | | | |
| 3 | L 8-3 | | | | |
| 4 | L 8-4 | | | | |
| 5 | L 8-6 | | | | |
| 6 | L 12 | | | | |
| 7 | L 13 | | | | |
| 8 | L 15 | | | | |
| 9 | L 22 | | | | |
| 10 | L 24 | | | | |
| 11 | L 26 | | | | |
| 12 | 131/16-2 | | | | |
| 13 | S-17 | | | | |
| 14 | S-17-4 | | | | |
| 15 | k - 1433 | VIR (All-Union Institute of Plant Industry) | | | |
| 17 | k – 1185 | | | AABBRR 2R/2D-замещение | 42 |
| 16 | Selenga | | *Secale cereale* L. | RR | 14 |
| 17 | Ivolga | Department of plant breeding and seed science | *Triticum aestivum* L | AABBDD | 42 |
| 18 | Chinese Spring | Nemchinovka | | AABBDD | 42 |
| 20 | Bezenchukskaya amber | Department of plant breeding and seed science | *Triticum durum* Dest. | AABB | 28 |

DNA extraction and PCR were conducted according to the standard procedure (Divashuk et al., 2007), genomic *in situ* hybridization procedure was carried out in lines with standard procedure (Karlov et. al., 1999).

Chromosome-specific microsatellite (SSR) markers used in the paper are cited in the Tab. 2.

Tab. 2. Primer name, chromosome arm, genetic map position, annealing temperature, $MgCl_2$ concentration and typical fragment size

| № | Marker name | Chromosome arm | Annealing temperature,˚C | $MgCl_2$ concentration, мM/l | Amplified fragment size, bp |
|---|---|---|---|---|---|
| 1 | Xbarc271 | 1DL | 56 | 1,5 | 158 |
| 2 | Xbarc149 | 1DS | 54 | 2,5 | 249 |
| 3 | Xwmc111 | 2DS | 60 | 2,5 | 243 |
| 4 | Xgwm261 | 2DS | 60 | 2,5 | |
| 5 | Xbarc168 | 2DS | 58 | 1,5 | 174 |
| 6 | Xgwm102 | 2DS | 60 | 1,5 | 145-153 |
| 7 | Xgwm484 | 2DS | 60 | 1,5 | 143-153 |
| 8 | Xwms157 | 2DL | 62 | 1,5 | 110 |
| 9 | Xbarc228 | 2DL | 62 | 1,5 | 177 |
| 10 | Xbarc1143 | 2DL | 57 | 1,5 | 193 |
| 11 | Xgwm539 | 2DL | 62 | 1,5 | 143-157 |
| 12 | Xgwm301 | 2DL | 62 | 1,5 | 171 |
| 13 | Xgwm349 | 2DL | 60 | 2,5 | 243 |
| 14 | Xbarc270 | 3DL | 58 | 1,5 | 249 |
| 15 | Xbarc6 | 3DS | 56 | 2,5 | 471 |
| 16 | Xbarc1183 | 4DL | 60 | 1,5 | 254 |
| 17 | Xwmc285 | 4DS | 62 | 2,5 | 289 |
| 18 | Xbarc110 | 5DL | 56 | 1,5 | 200 |
| 19 | Xwmc233 | 5DS | 62 | 2,5 | 260 |
| 20 | Xbarc1030 | 6DL | 56 | 1,5 | 197 |
| 21 | Xbarc196 | 6DS | 60 | 1,5 | 163 |
| 22 | Xbarc53 | 7DL | 60 | 1,5 | 298 |
| 23 | Xwmc506 | 7DS | 62 | 2,5 | 216 |

To identify chromosome 2R we carried out amplification with the STS-marker for locus Sec2, mapped in the short arm of this one (2RS) (Lee et al., 1994).

Each primer was synthesized by «Sintol» (Moscow).

## Results and discussions

PCR-analysis conducted with two markers located in each chromosome arm of D-genome has shown that 7 samples from the 15 represented here contain genetic material of chromosome 2D (Tab. 3. and Tab. 4.).

Tab.3. The amplification results for the long arm of the D-genome chromosomes (1-7 DL)

| № | Sample name | 1 DL | 2 DL | 3 DL | 4 DL | 5 DL | 6 DL | 7 DL |
|---|---|---|---|---|---|---|---|---|
| | | Xbarc 271 | Xgwm 349 | Xbarc 270 | Xbarc 1183 | Xbarc 110 | Xbarc 1030 | Xbarc 53 |
| 1 | L 8-1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | L 8-3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | L 8-4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 4 | L 8-6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | L 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | L 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | L 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | L 22 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 9 | L 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | L 26 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 11 | 131/16-2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | S 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | S 17-4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | PI 587512 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | k-1433 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

Tab. 4. The amplification results for the short arm of the D-genome chromosomes (1-7 DS)

| № | Sample name | 1 DS | 2 DS | 3 DS | 4 DS | 5 DS | 6 DS | 7 DS |
|---|---|---|---|---|---|---|---|---|
| | | Xbarc149 | Xwmc111 | Xbarc6 | Xwmc285 | Xwmc233 | Xbarc196 | Xwmc506 |
| 1 | L 8-1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | L 8-3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | L 8-4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 4 | L 8-6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | L 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | L 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | L 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | L 22 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 9 | L 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | L 26 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 11 | 131/16-2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | S 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | S 17-4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | PI 587512 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | k-1433 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

There is locus Xgwm 349 cited in the 2DL and Xwmc 261 (2DS) in triticale lines L 8-1, L 8-3, L 8-4, L 8-6, L 22, L 26, that were developed in the genetic department and selected as low-stemmed and early-maturing forms (Soloviev 2007), and k – 1433, that was obtained from All-Russian Institute of Plant Industry (VIR).

The presence of the 2D-chromosome-specific loci evidences the availability of the genetic material of this chromosome in the represented lines genomes. These 7 lines were investigated by means of set of the additional SSR-markers for the chromosome 2D. The results are shown in Tab. 5. These loci are spread along the length of this chromosome. That allows to state the presence of the almost whole chromosome 2D in the triticale lines genome.

Tab. 5. The amplification results for the short and long arms of the chromosome 2D

| № | Sample name | 2DS | | | | | 2 DL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xbarc 168 | Xbarc 102 | Xbarc 484 | Xgwm 261 | Xwmc 111 | Xgwm 349 | Xgwm 157 | Xbarc 228 | Xbarc 1143 | Xgwm 539 | Xgwm 301 |
| 1 | L 8-1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | L 8-3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 3 | L 8-4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 4 | L 8-6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 5 | L 22 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | L 26 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 7 | k-1433 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| 8 | k-1185 | 1 | 1 | 1 | 1 | - | 1 | 1 | 1 | 1 | 1 | 0 |

Using the SSR-PCR analysis the existence of 3 2D chromosome variations was established in these strains (Tab. 5.):

- the first variation – L 8-1, L 22, L 26;
- the second variation – L 8-3, L 8-4, L 8-6, k-1185;
- the third variation – k – 1433.

To sum up, 7 samples of the spring triticale were found to have genetic material of chromosome 2D as the substitution. The substitutions were known to happen between the homeological groups, the most common substitution in the breeding samples being 2R/2D (Cheng et al., 2002). To approve of this hypothesis STS-PCR-analysis for the locus Sec2 in the 2RS was applied (Fig. 1.). As a result, the triticale lines possessing chromosome 2D appeared not to have chromosome 2R, showing 2R/2D-substitution presence.

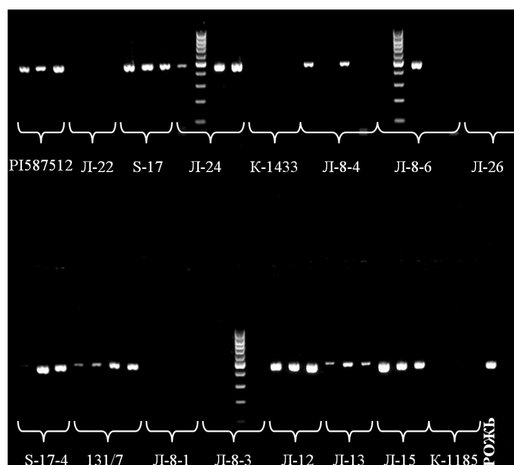


Fig. 1. Electrophoresis of the amplified products of the STS-markers for Sec-2 locus.

Precise 2R/2D-substitution identification with the genomic *in situ* hybridization (GISH) technique was conducted for the same lines: L 8-1, L 8-3, L 8-4, L 8-6, L 22, L 26, k – 1433. We have detected that k-1433, L 8-1, L 8-3, L 22, L 26 were found to have 12 rye and 30 wheat originated chromosomes (Fig. 2.), in other words they bear the substitution.

Fig. 2. Genomic in situ hybridization on the metaphase chromosomes of triticale sample L 8-1 (30 wheat+12 rye chromosome). Genetic material of rye is lightly grey, wheat – taupe.

SSR-PCR and STS-PCR-analysis and GISH (Fig. 1. and Fig. 2.) have shown, that L 8-4, L 8-6 are segregating in the rye chromosome number.

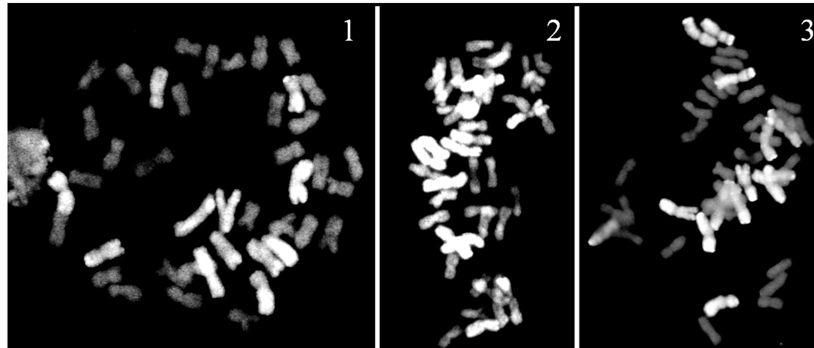


Fig. 3. Genomic in situ hybridization on the metaphase chromosomes of triticale sample L 8-6 (30 wheat+12 rye chromosome(1), 29+13 (2), 28+14 (3)). Genetic material of rye is lightly grey, wheat – taupe.

## Conclusions

The search model for D-genome of wheat in triticale genome including the consecutive application of the SSR-, STS-markers, genomic *in situ* hybridization (GISH) was developed. The results of the combined application of the SSR-PCR and STS-PCR-analysis were approved by means of GISH. It means that one should use only SSR-PCR and/or STS-PCR-method during the routine analysis.

The chromosome constitution of the 15 spring triticale samples was established by SSR- and STS-markers and GISH (2n=6x=42). There appeared to occur a 2R/2D-substitution in the genome of these 7 lines. That was verified by the SSR-, STS- analysis and GISH.

L 8-4 and L 8-6 are segregating in rye chromosome number, among them there being 2R/2D-substituted, non-substituted plants and hybrids between them. It is necessary to make individual selection based on the meiotic analysis of these lines and their hybrids with tester-lines.

Selection under the field conditions (plant height, maturing duration) may appreciably decrease the number of analyzed samples. The great amount of 2R/2D-substituted forms in the collection of the Department of Genetics is likely to be explained by the selection pressure.

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# IDENTIFIKACIJA D-GENOMA KOD NEKIH LINIJA JAROG TRITIKALE

- originalni naučni rad -

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## Rezime

Osnovni problemi koji ograničavaju gajenje jarog tritikale su slab kvalitet zrna, niska produktivnost biljke i proklijavanje semena pred žetvu. Jedan od pogodnih metoda za prevazilaženje ovih nedostataka je ugradnja D-genoma pšenice u tritikale. U toku razvoja sekundarnih jarih heksaploidnih tritikale moguće je primetiti pojavu genomske reorganizacije i eliminacije materijala D-genoma. Da bi se prevazišao problem koji se odnosi na identifikaciju selekcionog materijala koji sadrži D-genom upotrebljena je PCR analiza za SSR markere specifičnim za hromozome, kao i STS markere za lokus Sec (2RS). Za preciznu identifikaciju 2R/2D supstitucionih linija primenjena je genomska in situ hibridizacija (GISH). Na osnovu rezultata SSR – PCR i STS – PCR analize, kao i GISH pokazano je da 7 linija jarog tritikale (od odabranih 15) sadrži 2R/2D supstituciju. To znači da je sama PCR analiza dovoljna za selekciju biljaka koje sadrže d-genom. Forme jarog tritikale L 8-4 i L 8-6 se gregiraju u pogledu broja hromozoma raži: postojale su biljke koje su imale 2R/2D supstituciju, biljke koje nisu imale, kao i hibridi između njih. S obzirom na to, neophodno je primeniti individualnu selekciju baziranu na upotrebi molekularnih markera. Ovakva iznenađujuće velika proporcija 2R/2D supstitucionih formi u ispitivanoj kolekciji može biti objašnjena prethodnom selekcijom u poljskim uslovima usmerenoj prema biljkama niže stabljike i kraćeg vegetacionog perioda. Forma jarog tritikale koje su nosile 2R/2D supstituciju su uključene u program oplemenjivanja.