Cytogenetical characteristic of the introgressive common wheat lines including and lacking the $4S^l$ chromosome

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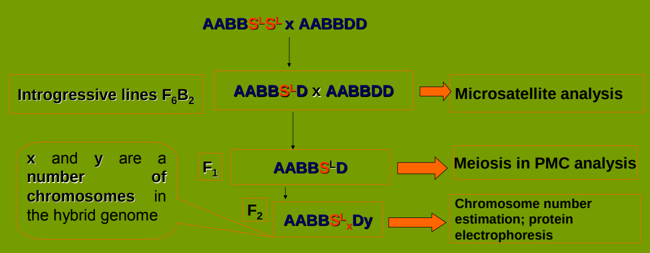
Abstract . The lines *Triticum aestivum/Aegilops sharonensis* were explored in regard to presence of introgressions in line genome, their amount and belonging to definite homoeologic group. The results of studying of chromosome associations in M1 of PMC in the hybrids between the lines with each other and with recurrent common wheat genotype Aurora were compared with the data of the line assessment for the chromosomal biochemical and morphological markers. 26 lines were distinguished between six groups with specific genome rearrangement regard to recurrent genotype.

Introduction. In recent years, introgressive plant material, including wheat plant material, is actively used for development of mapping populations. Using the mapping population for gene linkage determination, the comparison between the empirical ant theoretical rations of phenotype classes is carried out. When calculating the theoretical class volume we need to be sure that meiosis in the F_1 plants proceeds without abnormalities, otherwise, we can obtain the artifacts.

The group of introgressive lines *Triticum aestivum/Aegilops sharonensis* with certain alien characters was investigated. These lines are applicable to be used in genetic analysis of wheat for genes that control alien gradation of characters. Detailed genetic examination of introgressive lines concerning their cytological stability is the first and necessary step when using such lines for development of mapping population. Moreover, the cytogenetical peculiarities of the F_1 hybrids from crosses of the lines with each other, which are the direct source of the mapping population, should be also studied.

Experimental design and plant material development



AABBS^{L}S^L x AABBDD

Introgressive lines F_6B_2

AABBS^{L}D x AABBDD → **Microsatellite analysis**

x and y are a **number of chromosomes** in the hybrid genome

F_1 **AABBS^{L}D** → **Meiosis in PMC analysis**

F_2 **AABBS^L_xDy** → **Chromosome number estimation; protein electrophoresis**

Results and Discussion. Introgressive lines which phenotype differs from phenotype of variety Aurora concerning one of the spike morphology characters were selected for genome structure examination. Just these distinctions were considered by us as a proof of presence of the genetic material from *Ae. sharonensis* in the line genome. Screening of the lines studied for the mentioned below proteins shows the absence the diagnostic changes in the spectra of storage proteins, alpha-amylase, seed and leaf peroxidase. So, the lines could not be considered as carriers of whole alien chromosome of appropriate homoeologous group. Electrophoretic spectra of β-amylase, acid phosphatase, seed and leaf esterase in some lines studied differ from electrophoretic spectra of variety Aurora. The marker components controlled by genes β-*Amy-S^{l}1* and *Acph-S^{l}1*, located on long arm of chromosome $4S^l$, are inhered in 12 introgressive lines. Translocation with breakpoint between these genes is not detected: all lines are characterized by two alien components that are controlled by β-*Amy-Sl1* and *Acph-Sl1,* or two wheat components. Marker components *Est-S^{l}1,* controlled by chromosome $3S^l$, were found on spectra of nine lines. Components of electrophoretical spectra controlled by gene *Est-2,* localized on long arm of chromosome 3D, were absent on leaf esterase spectra in five lines; components, controlled by gene *Est-1,* which is localized on chromosome arm 3DS, are absent in 12 lines. This results can be considered as indirect argument of substitution of certain wheat chromosome segments for alien ones.

Evaluation of chromosome number revealed variation from 39 to 45 in F_2 generation (Table1).

Table1

Chromosome number in the primary roots of the F_2 plants

Hybrid number	**Chromosome number variation**	**Hybrid number**	**Chromosome number variation**
112	**39-44**	**96**	**42-44**
122	**40-42**	**106**	**39-42**
150	**41-44**	**136**	**42-44**
78	**39-43**	**143**	**40-42**
121	**40-43**	**185**	**42-45**

Progenies of different hybrids demonstrated distortions from the maternal genotype of *Aurora* cultivar: couple of dicentric chromosomes, four satellite chromosomes instead of two, up to four telocentric chromosomes, and very small chromosomes that are not typical for *Avrora* cultivar (**Fig.1, 2**). Therefore, one can assume that decreased fertility of F_1 obtained from crossing introgressive lines can be associated with both cytological instability of F_1 plants and parental introgressive lines.

Fig.1. Metaphase chromosome plate with four satellite chromosomes

Fig.2. Metaphase chromosome plate with dicentric chromosomes

In the F_1 hybrid between the chromosome substitution line and variety Aurora the chromosomes of the D and S genomes out of the same homoeologous group can not pair with each other and keep as univalents. Chromosomes of the A and B genomes should be paired without irregularities, although marginal probability of association between the S-chromosomes and the chromosomes from the A, B, and D genomes may be left. The triploid hybrid tetra-Aurora x *Ae. sharonensis* (ABS^l) was shown to form 0,06 trivalents per cell. Moreover, it is impossible to exclude possibility of D-S^L associations with forming of heteromorphic bivalents. Although, pairing between wheat and alien *(Ae. sharonensis)* chromosomes is extremely reduced, it occurs with minor frequency. So, number of bivalents and univalents under highest chromosome association in meiosis M1 of hybrid between the introgressive line and variety Aurora and knowledge about homoeologous belonging of introgressions remain to be major sources of information about line genome structure. The number of univalents divided at two points on amount of substituted chromosomes in line, and number of open bivalents indicate presence of translocations in line genome. These translocations can include alien genetic material, but they, also, can be extremely wheat-wheat. Some hybrids between introgressive lines and cultivar Aurora are characterized by greater number of closed bivalents, than in variety Aurora. It does not contradict the data about recessiveness of desynapsis genes, which presence was shown for variety Aurora.

Genome structure of the lines can be detailed using as a base the meiosis M1 pattern in hybrids F_1 between lines with each other. These results demonstrated what kind of introgression characterizes the line, chromosome substitution or translocation. In addition to cytological characterization, the line were analyzed with the use of biochemical markers that are specific for definite homoeologous chromosome group of *Triticeae.* Lines with similar genome structure form 21 closed bivalent. If four univalents occur in metaphase 1 of meiosis in the F_1 hybrid between lines, each out of the lines has alien chromosome substitution, but the lines differ from each other concerning homoeologic belonging of alien chromosomes. Our resalts allow us to characterize each line regarding alien chromosome presence, their homoeologic belonging, and translocation presence. Homoeologic belonging of translocations was only to be presumed in most of cases. It suggests that efficiency of the biochemical marker method decreases when not the whole alien chromosome but only its portion as a translocation on the wheat chromosome or as a telocentric chromosome is present in the line genome. This portion may not contain the gene that marks definite chromosome. That is why microsatellite markers attract our attention, as they may be used as markers for different parts of alien chromosomes. It is essential that the use of microsatellite markers requires meticulous prior selection of chromosome specific primers.

Two primer pairs, *Xcfd89* and *Xcfd106,* for the long (L) and the short (S) arm of chromosome 4D, respectively, were used to screen introgressive lines, cultivar Aurora and genome substitution form Aurorsis (**Fig. 3, 4**).

Primer *Xcfd89* for the 4DL long produced electrophoretic band (amplicon) when using DNA of variety Aurora and some lines as a matrices. This primer produced no amplicon by using DNA of form Aurosis and certain lines. All the lines studied were identified by us earlier as to presence or absence of the $4S^l$ chromosome with the use of some marker genes. These genes were genes β-*Amy1* and *Acph1* which are situated on the long arm of the $4S^l$ chromosome. And gene *Hs-S^l* (hairy sheath, which was identified by us earlier as a morphological marker of chromosome 4Sl, though the arm localization of the gene was not determined. The certain lines are characterized by the presence of amplicon in the spectrum and their leaf sheath have no hairiness. The other ones have no amplicon in spectrum and were characterized with the nonhairy leaf sheath (**Fig. 3**). So, primer *Xcfd89* is really specific for wheat chromosome 4D. This primer can be used for detection of substitution 4DL/$4S^l$.

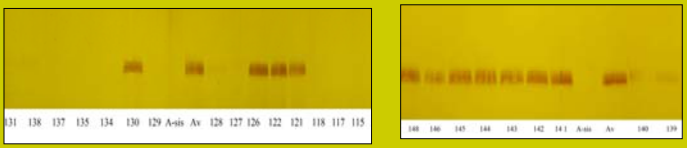


Fig.3. Electrophoresis pattern of PCR products amplified with *Xcfd89* in introgressive lines, Avrora and Avrorsis, where 115–148 are introgressive lines, AV–Aurora, Asis – Aurosis.

The primer *Xcfd106* (**Fig.4**) produces amplicon when using DNA from the all strains studied. However, variety Avrora and form Avrosis differ from each other in intensity of the band. The amplicon of form Avrosis was weaker. Precisely the same difference is observed between amplicons of the lines without chromosome 4Sl and with one. So, primer *Xcfd106* is not specific for chromosome 4D because produce amplicon under absence chromosome 4D when the 4A and (or) 4B chromosomes were used as a matrices. Through the difference in amplicon intensity primer *Xcfd106* can be used for verification of the arm 4DS presence in the line genome.

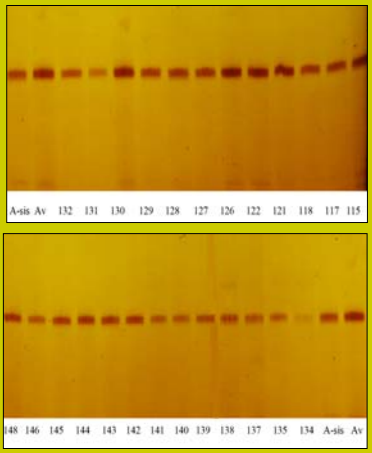


Fig.4. Electrophoresis pattern of PCR products amplified with *Xcfd106* in introgressive lines, Aurora and Aurorsis, 115–148 are introgressive lines, AV–Aurora, Asis– Aurosis

Conclusions

Data presented concerning the chromosome association in M1 of PMC in F_1 hybrids from crosses between introgressive lines and variety Aurora and with each other, and electrophoretic spectra of proteins prove the changes of 1–4 chromosomes in the line genomes in comparison with the variety Aurora genome.

The number of chromosome rearrangement in the introgressive wheat line with or without $4S^l$ chromosome were showed. Wheat chromosomes are either substituted with chromosome S^l from the same homoeologous group or a rearrangement through translocations (alien-wheat or wheat-wheat) takes place.

Owing to the use the microsatellite markers specific for the 4D chromosome it was proved that determination of translocation arm specificity is possible only for gametocidal chromosome $4S^l$.