

Minimization of alien chromatin volume in the introgressive common wheat lines.

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ABSTRACT

Among developed by us introgressive hexaploid common wheat lines, the ones resistant to powdery mildew owing to involving *Aegilops speltoides* and *Ae. sharonensis* in their pedigrees were found. Only the D chromosomes were involved in genome rearrangement. Four means of minimizing alien chromatin, namely, irradiation of hybrids F₁ from crossing these lines with promising common wheat varieties with γ -rays, crossing the same hybrids with the Chinese Spring *ph1* and *ph2* mutants, action of *Ae. speltoides* genotype, and action of gametocidal 4S¹ chromosome were used. There was 80% loss of hybrids after irradiation. Four out of 21 F₂ populations contained several resistant plants. No success was achieved by using *ph*-mutants, perhaps, due to winter nature of introgressive lines. Out of five F₂ populations related to *Ae. speltoides*, the segregation 3 susceptible : 1 resistant was found in one event. The resistance gene of *Ae. speltoides* is thought to be novel for common wheat. One line out of 16 resistant ones originated from *Ae. sharonensis* was found to contain a single dominant gene for resistance in small segment of alien chromatin. The resistance genes from both *Aegilops* species are believed to be situated in the 3rd homoeologous group of *Aegilops*. Four microsatellite primers on chromosome 3D were successfully used for search molecular evidence of alien chromatin presence in this chromosome in resistant lines.

INTRODUCTION

Practically all the useful genes of alien origination are transferred into genome of planted wheat species by the methods of chromosome engineering. Success in transfer of alien gene governing a useful character from the wild related species into the bread wheat genome should be supported by the mendelian type of gene behavior, i.e. the stable transfer through the both kind of gametes and involving in recombination with homologous wheat chromosome including crossing-over. In addition, its full expression should be happened in the wheat genome. For that, it is essential that the volume of alien chromatin was decreased to the minimum, under which the maintenance of useful character in the line is not accompanied with the presence of the characters unwanted in the crop. In our paper, results of use of four different means of minimizing of alien chromatin included into common wheat introgressive lines originated from two *Aegilops* species are presented. Two means (use of *ph*-mutants and action of *Ae. speltoides* genotype) are based on suppression of *Ph1* (*Ph2*) gene arresting the pairing of homoeologous chromosomes in polyploid wheat species. The other two ones (irradiation and action of 4S¹ chromosome) promotes different meiotic breaches and casual processes seem to be resulted in insertion of a small alien chromosome fragment into wheat chromosome.

MATERIALS AND METHODS

As an object of our investigation, the common wheat variety Aurora, (genome formula AABBDD) as recipient of alien genetic material, the genome-substituted forms Aurosis (AABBS¹S¹, AABB is tetra-Aurora, S¹S¹ is genome of *Ae. sharonensis*) and Auroides (AABBSS, SS is genome of *Ae. speltooides*) as well as several common wheat lines resistant to powdery mildew were used. The resistance genes were passed from the resistant alien species into the wheat lines through the forms Aurosis and Auroides. According to our data, the volume of alien chromatin in the line genomes varies from one whole chromosome to one or several alien translocations per line (Antonyuk, 1996). In so doing, owing to the particular mean of developing introgressive lines, alien genetic material has substituted for the chromosomes out of the D sub genome of wheat. To minimize the alien chromatin fragments and include simultaneously the useful genes into genotypes of the modern promising wheat variety, several methodical approaches were carried out: (i) 134 F₁ hybrids from crossing resistant lines with the promising bread wheat varieties were pollinated with the Chinese Spring mutant lines, *ph1b* (125 crosses) and *ph2b* (9 crosses). Seed sets are obtained from 52 crosses, total 2215 kernels; (ii) The same hybrids were irradiated with γ -rays in doses 2·10⁴ rad (there were 31 lines, 189 plants, 2294 seeds, 12.4 seeds per plant), 5·10⁴ rad (there were 31 lines, 249 plants, 457 seeds, 1.84 seeds per plant), and 10⁵ rad (there were 30 lines, all 255 plants were perished before heading). In addition, several F₂ populations developed from crossing the recipient variety Aurora with resistant lines sharing 4S¹ chromosome (16 populations) were assessed as for segregation into susceptible and resistant to powdery mildew plants against artificial infection background.

Four microsatellite primers located in chromosome 3D of bread wheat (Röder, 1998) were used for amplification of genomic DNA of the bread wheat variety Aurora (this is a recipient of alien chromatin when developing the introgressive lines) and genome substitution form Aurosis, which served as donors of alien chromatin.

RESULTS AND DISCUSSION

Loss of the F₁ plants when growing the irradiated seeds was on the average 80%. The plants, which survived, were characterized with poor development and produced the small number of seeds F₂, from 16 to 112. Additional loss was observed when germinating the F₂ seeds. Among 21 populations F₂, 17 ones did not include the resistant plants. In the rest four populations, there were two resistant plants out of 74 ones, four out of nine, one out of 28, and two out of 12. Simultaneously, five F₂ populations developed from crossing five unprogressive resistant lines of a list of the same 21 lines, which were irradiated, with the Aurora variety were obtained under absence of irradiation of the F₁ hybrid seeds. The progeny numbers in F₂ was from 120 to 200. In one out of these five populations, the segregation as for resistance to powdery mildew was observed. The plant group was discovered, which did not differ for their reaction to disease from the parent introgressive line. The segregation ratio was 3 susceptible : 1 resistant (91 and 30 plants, respectively, $\chi^2 = 0,003$). None of these five introgressive parental lines was characterized by appearance of resistant plants in F₂ population obtained out of irradiation of F₁ hybrids. Obviously, the last circumstance is the consequence of small number of the plant samples, from 6 to 23 plants obtained from the F₁ plants after irradiation the hybrid seeds. It is possible, there is the selection against gametes and zygotes with unbalanced genome, and so, it acts against the cells sharing alien genetic material. The sample volumes do not allow one to determine the inheritance pattern for the gene caused resistance in the population developed from the

irradiated seeds. However, these genes originate from other unprogressive lines as compared with origination of the revealed by us recessive gene for resistance. To the present day, only one gene of resistance to powdery mildew transferred into common wheat from *Ae. speltoides* is known (Miller et al., 1987). This is dominant gene *Pm12*. The recessive resistance gene, *Pm5*, was transferred into common wheat from *Triticum dicoccum* (Lebsock, Briggie, 1974). The gene of resistance to powdery mildew transferred by us into common wheat is thought to be novel.

Populations F_2 from crossing spring wheat lines *ph1b* and *ph2b* with winter resistant lines on the base of winter variety Aurora had a quarter of unheading plants. From the rest there were a portion of resistant plants, from which the F_3 populations were obtained. The observed segregation ratio as and in F_2 was characteristic for event of inheritance of dominant genes through the complete alien chromosome. Thus, no success was achieved by using *ph*-mutants, perhaps, due to winter nature of introgressive lines.

For genetic analysis of the introgressive lines resistant to powdery mildew, the F_1 and F_2 hybrids from crosses of the lines one with another and with the bread wheat variety Aurora were used. The lines 936, 937, and 1166 were revealed to have dominant genes for resistance. The lines 834, 1025, 1077, 788, and 1096 involved the semidominant genes for resistance. All the F_1 plants from diallel crosses of resistant lines were resistant. In F_2 from crosses with the variety Aurora (susceptible cross component), the segregation in ratio 3 resistant : 1 susceptible was revealed only in cross with the line 788. In F_2 from other crosses, the susceptible plants were absent. It must be considered as a result of negative selection against the gametes lacking the gametocidal 4S¹ chromosome that decreases significantly the sample of susceptible plants in population under study.

Using the genes controlling the several proteins in wheat, the introgressive lines were characterized earlier by us regarding to homoeologous belonging of alien genetic material including the resistance gene *Pm* (3D chromosome) (Antonyuk, 1996). For the arm allocation of the resistance gene in the chromosome, the DNA sequences flanking microsatellite repeats (SSR-markers) of known chromosome location were used. Four pairs of primers specific for chromosome 3D of common wheat, two primer pairs for each arm, were used. Each tested primer provided the individual spectrum of amplified fragments for Aurora and Aurosis. The components of amplified DNA fragment spectrum specific for the genome-substitution form and corresponding introgressive resistant lines can serve as the markers of chromosome segment contenting the resistance gene. The SSR-markers seem to fit for study of large body of plant material with respect to its resistance to powdery mildew as well as genetic structure regarding the alien insertions

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