Abstract

Chromosome 2C of *Aegilops cylindrica* induces chromosomal rearrangements in alien chromosome addition lines, as well as in euploid lines, of common wheat. To induce chromosomal rearrangements in barley chromosome 7H, reciprocal crosses were made between a mutation-inducing common wheat line that carries a pair of 7H chromosomes and one 2C chromosome and a 7H disomic addition line of common wheat. Many shrivelled seeds were included in the progeny, which was an indication of the occurrence of chromosome mutations. The chromosomal constitution of the viable progeny was examined by FISH (fluorescence in situ hybridization) using the barley subterminal repeat HvT01 as a probe. Structural changes of chromosome 7H were found in about 15% of the progeny of the reciprocal crosses. The aberrant 7H chromosomes were characterized by a combination of N-banding, FISH and genomic in situ hybridization. Mosaicism for aberrant 7H chromosomes was observed in seven plants. In total, 89 aberrant 7H chromosomes were identified in 82 plants, seven of which had double aberrations. More than half of the plants carried a simple deletion: four short-arm telosomes, one long-arm telosome, and 45 terminal deletions (23 in the short arm, 21 in the long arm, and one involving both arms). About 40% of the aberrations represented translocations between 7H and wheat chromosomes. Twenty of the translocations had wheat centromeres, 12 the 7H centromere, with translocation points in the 7HS (five) and in the 7HL (seven), and the remaining four were of Robertsonian type, three involving 7HS and one with 7HL. In addition, one translocation had a barley segment in an intercalary position of a wheat chromosome, and two were dicentric. The breakpoints of these aberrations were distributed along the entire length of chromosome 7H.

Introduction

Many studies have been conducted with the aim of transferring alien genes from distantly related species into wheat (see Jiang et al. 1994). Barley carries many useful genes, such as resistance genes for viruses and nematodes (Islam and Shepherd 1992) and a gene for early heading (Murai et al. 1997). Since Islam et al. (1981) produced six of the possible barley-wheat disomic addition lines of common wheat Chinese Spring (CS; the CS 1H addition line is missing), the manipulation of barley chromosomes in wheat genomes has become possible. Attempting to transfer genes from barley to wheat, Islam and Shepherd (1992) and Murai et al. (1997) isolated wheat-barley recombinant chromosomes induced by homoeologous pairing in the absence of the *Ph1b* gene. However, the pairing between wheat and barley chromosomes was low (Murai et al. 1997). In addition, segments of barley chromatin recombined by crossing over would generally carry deleterious genes in addition to the target gene, and it would be difficult to break the linkage between the deleterious genes and the target gene by the use of the *Ph1b* gene.

Chromosome maps are necessary for chromosome-mediated transfer, as well as for map-based cloning, of agronomically important genes. Various genetic linkage maps have been constructed in barley by the use of different DNA polymorphisms, e.g., RFLP (restriction fragment length polymorphism) (Kleinhofs et al. 1993), AFLP (amplified fragment length polymorphism) (Qi et al. 1998) and RAMP (random amplified microsatellite polymorphism) (Becker and Heun 1995). Künzel et al. (2000) have constructed a physical map of barley using microdissected translocation chromosomes for polymerase chain reaction (PCR) amplification with STS (sequence tagged site) primers derived from genetically mapped RFLP probes. However, it is preferable to have more detailed linkage and physical maps for the efficient isolation of genes, as well as general genetic studies. Direct physical mapping in barley, as conducted in wheat using the deletion stocks (Endo and Gill 1996), would...
provide a virtually unlimited number of physical landmarks (breakpoints) and allow a greater number of DNA markers to be mapped since deletion mapping does not require markers to be polymorphic. Barley, however, is diploid and thus deletion stocks of this species are unlikely to be viable.

The above-mentioned problems could be overcome by the use of the gametocidal chromosomes that have been introduced into common wheat from related wild species, such as *Aegilops triuncialis* (Endo and Tsunewaki 1975; Endo 1978) and *Aegilops cylindrica* (Endo 1979, 1988). When the gametocidal chromosome is present in a monosomic condition, chromosomal structural changes occur in those wheat gametes that lack the *Aegilops* chromosome. Depending on the genotype of common wheat, such mutations are lethal or sublethal. Gametes with sublethal mutations are viable and after fertilization frequently give rise to plants carrying chromosomal aberrations (Endo 1990). Even though the mechanism of the gametocidal action is not yet understood, this genetic chromosome mutation inducing system has been successfully used to produce an array of terminal deletion lines in common wheat (Endo and Gill 1996). Also this system could induce structural changes in the rye chromosome 1R substituted for wheat chromosome 1B (Endo et al. 1994).

With the aim of generating deletions and translocations in barley chromosomes, Shi and Endo (1997) produced the chromosome mutation inducing lines for the six wheat/barley addition lines. These lines carry a pair of homologous barley chromosomes and one 2C gametocidal chromosome derived from *Ae. cylindrica*. A preliminary study revealed that chromosome 2C induces deletions and translocations in every barley chromosome at frequencies greater than 10% (Shi and Endo 1999). In the present study we extensively investigated the progeny of the chromosome mutation inducing line carrying barley chromosome 7H. We employed a combination of N-banding, fluorescence in situ hybridization (FISH), and genomic in situ hybridization (GISH) to identify and characterize the aberrant chromosomes involving chromosome 7H.

### Materials and methods

**Plant materials**

The chromosome mutation inducing line used in this study had a pair of barley 7H chromosomes and one 2C chromosome added to common wheat (*Triticum aestivum*, 2n=6x=42, genome symbol AABDD) cultivar CS (Shi and Endo 1997). Chromosome 2C is derived from *Ae. cylindrica* and, when present in CS, induces chromosome mutations in the gametes lacking it (Endo 1988). This chromosome mutation inducing line (21′+1′7H+1′2C) was reciprocally crossed with the disomic 7H addition line (21′+1′7H) of CS. The disomic 7H addition line was chosen in order to ensure the transmission of aberrant 7H chromosomes with terminal deletions to the next generation. The normal 7H chromosome from the disomic 7H addition line was expected to pair during meiosis with the normal chromosome arm of 7H deletion chromosomes.

### Cytogenetics

Structural changes of chromosome 7H were identified essentially as described by Shi and Endo (1999). Briefly, first, FISH using a probe of the barley-specific subtelomeric sequences (HvT01) was conducted to select plants with aberrant 7H chromosomes that lacked terminal FISH signals in either of the chromosome arms. Selected plants carrying aberrant chromosomes were further examined using other root tips from the same plants by N-banding, followed by a combination of FISH and GISH in the same metaphase cells. The combined FISH and GISH analysis was conducted with a hybridization mixture containing the HvT01 and barley total genomic DNA probes, and wheat DNA for blocking: the concentrations of the probes and blocking DNA were the same as those when FISH and GISH were conducted separately. The FISH and GISH probes were labeled with digoxigenin and biotin, respectively, as described by Schubert et al. (1998). A mixture of appropriately diluted anti-digoxigenin FITC (fluorescein isothiocyanate) and anti-avidin FITC was applied to detect the signals of the combined FISH and GISH analysis. The barley subtelomeric regions showed much stronger signals than the other barley chromosomal regions. This analysis was useful in identifying the 7H chromosome arm involved in minor terminal deletions because chromosome 7H is metacentric and has no diagnostic terminal N-bands. The N-banding, FISH, and GISH images were photographed and processed as described by Shi and Endo (1999). The size of the deletions and translocations of chromosome 7H (fraction length, FL) was estimated based on the length of the remaining arm relative to the length of the intact opposite arm and the arm ratio of normal chromosome 7H, as described by Endo and Gill (1996). The FL values were calculated with five or more chromosomal images for each of the deletions or translocations.

### Results and discussion

The cross between the chromosome mutation inducing line as female and the disomic 7H addition line yielded 533 plump and 100 shrivelled seeds. Of the plump seeds 438 (82.2%) germinated (shrivelled ones were not used). From the reciprocal cross 300 plump and 60 shrivelled seeds were obtained. Two-thirds of the plump seeds and 18.3% of the shrivelled seeds germinated (Table 1). This germination rate is relatively low compared with that (95.0%) of the progeny between the monosomic 2C addition (as female) and euploid lines of CS (Endo 1990). The high frequency of inviable progeny from the reciprocal crosses in this study is probably due to the severer gametocidal action of the 2C chromosome in the 7H addition line. A similar reduction in the viability of progeny was observed in other barley chromosome addition lines with the 2C chromosome (Shi and Endo 1999). This implies that the gametocidal action of chromosome 2C is influenced by the addition of barley chromosomes.

The FISH analysis was conducted with all the germinated seedlings from the reciprocal crosses (438+211). Chromosome 7H has two prominent FISH signals at the terminals of both arms, and, therefore, chromosomes with single FISH signals were judged to be aberrant 7H chromosomes. Some of the plants, 54 out of the 438 progeny and 22 out of the 211 progeny, showed only two FISH signals that were located at both ends of one chromosome and were assumed to possess only one normal 7H chromosome. These individuals probably resulted from the pairing failure of chromosome 7H in meiosis of