
Review

Cytological dissection of barley genome by the gametocidal system

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Barley is one of the major cereals in the world. The analysis of genomes is important in modern breeding, but the barley genome is too huge and complicated that it is still difficult to arrange sequenced pieces of the genome in order. Each of the barley chromosomes or chromosome arms has been added to common wheat and such barley chromosome addition lines have been used in determining the chromosomal regions of genes and DNA markers. It is undoubtedly desirable to have the genome divided into smaller pieces in separate common wheat lines. There is a unique genetic system in common wheat that induces frequent chromosomal structural rearrangements. This system is called the gametocidal (Gc) system involving alien chromosomes called Gc chromosomes, which were introduced into common wheat from certain wild species belonging to the genus Aegilops. When the Gc chromosome exists in a common wheat plant in monosomic condition, the plant produces two types of gamete, one with the Gc chromosome and the other without the Gc chromosome; chromosomal rearrangements occur only in the latter one. Such Gc-induced chromosomal rearrangements are either lethal to gametes or semi-lethal, and in the latter case the gametes are fertilized to develop into viable zygotes carrying rearranged chromosomes. The Gc system proved to be effective in inducing structural rearrangements in barley chromosomes added to common wheat, as well as in common wheat chromosomes. Thus-induced rearranged chromosomes include deletions of barley chromosomes and translocations between the barley and wheat chromosomes. The present author termed common wheat lines carrying rearranged barley chromosomes ‘dissection lines’ of a barley chromosome. So far dissection lines for three barley chromosomes have been produced and used in the cytological mapping of the barley chromosomes. In this article the progress in the cytological dissection of the barley genome is described.

Key Words: barley, wheat, chromosome, gametocidal system, rearrangement.

Barley genome

Barley (Hordeum vulgare L., genome HH, 2n=2x=14) is an important cereal used for food and feed, and is ranked fourth in total production among world cereals after maize, rice, and wheat. Barley is cultivated worldwide and is more tolerant to cold, drought, saline and alkaline soils than other important cereals (Singh 2006). The barley genome is composed of ca. 5500 Mbp nucleotides (Bennett and Leitch 2005) that are organized in seven pairs of chromosomes. Based on the high level of synteny revealed by various studies, Linde-Laursen et al. (1997) recommended the present designation of the barley chromosomes; i.e., each of the seven barley chromosomes is designated by a numeral between 1 and 7 according to its homoeologous relationship with the chromosomes of the wheat genomes followed by the genomic symbol H (1H through 7H). Two pairs of SAT-chromosomes, 5H and 6H, are distinguishable from the others, but only experienced cytogeneticists can distinguish them from each other in a karyotype constructed from aceticarmine-stained mitotic metaphase chromosomes. The barley chromosomes have plenty of heterochromatin in pericentromeric regions as revealed by Giemsa C-banding and N-banding (Singh and Tsuchiya 1981, 1982), and their characteristic banding patterns allow the identification of all barley chromosomes (Fig. 1A). Another feature of the barley karyotype is the presence of massive centromere-specific repetitive sequences in each chromosome (Fig. 1B). Thus, the barley genome containing repetitive elements is so huge and complicated that it would almost impossible to assemble billions of sequenced pieces of the total genome in order, even with the help of next-generation sequencing technologies.

Dissection of the barley genome into individual chromosomes

Hexaploid wheat or common wheat (Triticum aestivum L., AABBDD, 2n=42) can tolerate aneuploidy to some extent and therefore monosomic, nullisomic and ditelosomic lines have been established in common wheat (Sears 1954, 1966,
Sears and Sears 1978). Also, the hexaploid nature of common wheat enabled many alien chromosome addition lines to be established in common wheat (Jiang et al. 1994). Islam et al. (1981) produced a series of barley chromosome addition lines of common wheat ‘Chinese Spring’ except for chromosome 1H, and Islam (1983) further produced telosome addition lines for each barley chromosome. Taketa and Takeda (2001) produced a complete set of wheat-wild barley (Hordeum vulgare ssp. spontaneum) chromosome addition lines. Fig. 2 shows the C-banded mitotic metaphase cell of disomic 6H addition line. The addition of chromosome 1H causes extreme cytological abnormalities at meiosis leading to sterility (Islam et al. 1981). Nevertheless, Islam and Shepherd (2000) isolated a self-fertile line carrying a pair of chromosome 6H and 1H/1HS (short arm) pair from a partly fertile double monosomic addition line in which chromosome 6H is present along with 1H. Taketa et al. (2002) found that the responsible factor for the sterility is located on the long arm of chromosome 1H. It may be said that these addition lines dissect the barley genome into its components, i.e. individual chromosomes or chromosome arms, in the genomic background of common wheat. We can predict the presence of barley chromosome-specific genes and DNA markers from the phenotypes and marker profiles of the addition lines, which is an effective approach to analyze the barley genome.

Suchánková et al. (2006) used the wheat-barley ditelosomic addition lines and successfully isolated the individual chromosome arms of barley chromosomes 2H–7H by chromosome flow-sorting. Since chromosome 1H is considerably smaller than chromosomes 2H–7H, Mayer et al. (2009) flow-sorted the 1H chromosomes of 95% purity from barley itself and conducted shotgun sequencing of chromosome 1H. This physical isolation of chromosomes or chromosome arms is definitely a powerful tool for the genome analysis in barley.

The gametocidal system

It is undoubtedly more useful for genome analysis if each barley chromosome is broken up into sub-arm pieces. Breaks in the interstitial regions of chromosome arms result in the generation of deletions and translocations. Diploid plants would not endure most of chromosomal deletions and non-reciprocal translocations, but hexaploid common wheat would endure them as assumed from the establishment of the aneuploids in common wheat. Although the use of mutagens such as ionizing radiation and certain chemical compounds is a common way to induce chromosomal breakage, extreme caution should be exercised when using such mutagens. In wheat, chromosome 5B has a gene (Ph) responsible for the suppression of homoeologous pairing (Sears 1976). Removing chromosome 5B, Taketa et al. (2005) succeeded in inducing homoeologous recombination between wheat chromosome 5D and barley chromosome 5H. Thus, the use of the Ph gene has been demonstrated to be an effective genetical method to dissect alien chromosomes introduced into common wheat. As a safe genetical mutagen, Endo (1988) found an alien chromosome that induces chromosomal structural changes with high frequency in common wheat. It is one of the alien chromosomes known as ‘game-tocidal’ chromosomes (Endo 2007). The following is a brief description of the Gc chromosomes.

Alien chromosomes added to common wheat are not
stable in general, so that disomic alien additions often become monosomic additions due to irregular meiotic pairing, and the monosomic state rapidly returns into the euploid state. This is because euploid gametes, especially pollen, have an advantage over aneuploid gametes carrying an alien chromosome. Maan (1975) and Endo and Tsunewaki (1975) first reported exceptional cases in wheat that certain alien chromosomes introduced from wild species of the genus Aegilops into wheat are preferentially transmitted to the next generation, namely gametes carrying the alien chromosome had a definite advantage over euploid gametes. This preferential transmission of the Aegilops chromosomes was explained to be due to the gametocidal action of the Aegilops chromosome in a sporophyte on euploid gametes because the monosomic of the alien chromosome severely reduces the fertility (Fig. 3). Therefore, such Aegilops chromosomes with the gametocidal action are called gametocidal (abbreviated to Gc) chromosomes (Endo 1978, 1982) or ‘cuckoo’ chromosomes because of their selfish behavior in common wheat (Miller et al. 1982). Finch et al. (1984) observed frequent chromosome breaks in meiospores of a monosomic addition line of common wheat carrying one of such alien chromosomes and suggested that the chromosome breaks ensure the transmission of the alien chromosome. Since gametes with the Gc chromosome develop normally and preferentially took part in fertilization, most of the progeny of a monosomic Gc addition plant become disomic Gc addition plants in which chromosomal rearrangements would no longer occur. The Gc chromosomes belong to three homoeologous groups 2, 3 and 4, and Gc chromosomes of different homoeologous groups have different types of gametocidal action (Endo 1990, 2007). Three Gc chromosomes derived from Ae. cylindrica (2C) and Ae. triuncialis (3C and 3CSAT) have semi-lethal Gc action, allowing the gametes carrying chromosomal changes to be fertilized successfully, and therefore have been used as so-called genetic mutants to induce breakage in common wheat chromosomes and in alien chromosomes added to common wheat. The 2C and 3CSAT chromosomes induce chromosomal rearrangements in a common wheat cultivar ‘Chinese Spring’ and the 3C chromosome in some Japanese common wheat cultivars including ‘Norin 26’ (Endo 1990, 2007, Tsujimoto and Tsunewaki 1985). The present author developed a genetic system (termed gametocidal (Gc) system) involving these Gc chromosomes for the induction of chromosomal rearrangements in common wheat. Using the Gc system, Endo and Gill (1996) induced deletions in ‘Chinese Spring’ wheat, identified them by chromosome banding or C-banding, and established 436 deletion stocks in common wheat. The deletions occurred in all 21 wheat chromosomes, but the frequencies of the occurrence were various among the chromosomes. The Gc chromosomes and most of the deletion stocks are available from National BioResource Project (http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpLgku.jsp).

Dissection of the barley chromosomes

Endo et al. (1994) demonstrated that the Gc system is also effective in inducing rearrangements of a rye chromosome in a common wheat cultivar. Stimulated by this fact and the successful establishment of the common wheat deletion stocks (Endo and Gill 1996), Shi and Endo (1997) started to introduce a gametocidal chromosome 2C into the wheat-barley addition lines except for 1H and produced six alien addition lines of ‘Chinese Spring’ wheat that are disomic for each barley chromosome and monosomic for 2C (20''+1''1'H+1''2C). Later, Endo (unpublished) introduced another gametocidal chromosome 3CSAT into the same wheat-barley addition lines. Shi and Endo (1999, 2000) demonstrated that chromosome 2C is capable of inducing structural changes in every barley chromosome in ‘Chinese Spring’. Shi and Endo (2000), Ashida et al. (2007) and Sakai et al. (2008) employed the Gc system with chromosome 2C or 3CSAT to induce structural changes in barley chromosomes 3H, 5H and 7H, and establish so-called ‘barley dissection lines’ of common wheat. These works showed that chromosome 3CSAT is as effective as chromosome 2C in inducing structural rearrangements in barley chromosomes. The Gc-induced rearrangements of barley chromosomes are straight terminal deletions and translocations between barley and wheat chromosomes. As shown in Fig. 4, in situ hybridization techniques, GISH (genomic in situ hybridization) and FISH (fluorescence in situ hybridization), are indispensable for the identification of rearranged barley chromosomes. The present author and co-workers are continuing the production of dissection lines for the rest of barley chromosomes.
Fig. 4. Identification of rearranged barley chromosomes by FISH/GISH combined with C-banding. In the black and white FISH/GISH photographs (dark backgrounds), the brightest parts show the FISH signals of the subterminal HvT01 repeats, the second brightest parts are the GISH signals of barley chromatin, and the darkest chromosomal regions are those of wheat stained with DAPI. The boundaries between the second brightest and the darkest regions are the translocation points. (A) FISH/GISH images of normal chromosome 5H (left) and a translocation chromosome between 5H and wheat chromosomes. The presence of the secondary constriction and the much stronger HvT01 FISH signal in the short arm indicate that the translocation point is in the long arm. (B) C-banding and FISH/GISH images of a deletion of chromosome 7H. The C-banding image indicates that the breakpoint is in the short arm. The FISH/GISH image does not show in which arm the breakpoint is located because chromosome 7H is metacentric and the HvT01 FISH signals at the short- and long-arm ends are indistinguishable in intensity.

Fig. 5. Comparison of the physical and genetic maps of barley chromosome 3H. The 36 EST markers are divided into 20 chromosomal bins flanked by the breakpoints of the rearranged 3H chromosomes, and the order of the bins is consistent with the order of the markers in the genetic map. (From Sakai et al. 2009. With permission)
Cytological mapping of barley chromosomes

The barley dissection lines carrying single rearranged barley chromosomes are useful in locating genes and DNA markers on specific chromosomal regions. Serizawa et al. (2001) conducted deletion mapping of AFLP and STS markers that had been used in a genetic mapping and translocation mapping (Künzel et al. 2000) and found that the marker orders matched previous maps. Using the barley dissection lines and barley-specific EST markers (Nasuda et al. 2005a), Nasuda et al. (2005a), Ashida et al. (2007) and Sakai et al. (2009) conducted deletion mapping of barley chromosomes 7H, 5H and 3H, respectively. All these studies revealed that the distal regions of the barley chromosomes are richer in EST markers than the proximal regions. Sakai et al. (2009) compared the cytological map with a genetic map constructed with the map data for the same EST markers reported by Nejad et al. (2004) and found that the marker orders matched previous maps. Using the barley dissection lines and barley-specific EST markers (Nasuda et al. 2005a), Nasuda et al. (2005a), Ashida et al. (2007) and Sakai et al. (2009) conducted deletion mapping of barley chromosomes 7H, 5H and 3H, respectively. All these studies revealed that the distal regions of the barley chromosomes are richer in EST markers than the proximal regions. Sakai et al. (2009) compared the cytological map with a genetic map constructed with the map data for the same EST markers reported by Sato (2009) to find that the order of all EST markers was consistent between the two maps (Fig. 5). Qi et al. (2004) reported the same facts for wheat chromosomes. Masoudi-Nejad et al. (2005) employed the method of radiation hybrid mapping to analyze 90 7H dissection lines with PCR-based markers. The analytical method of radiation hybrid might become an effective way to construct cytological maps when large numbers of markers and dissection lines become available. The previous studies showed that the Gc system induces frequent breakage in the centromeric region of the barley chromosomes, generating barley telocentric chromosomes and whole-arm translocations between barley and wheat chromosomes. Nasuda et al. (2005b) found two telosomes of the 7H short arm that lacked the whole barley-specific centromere repetitive sequences. They analyzed the telosomes to reveal that the barley centromeric repeats are not indispensable for the function of the centromere or kinetochore.

Literature Cited


