Chromosome-specific behaviour in wheat with alien genetic materials

Tsujimoto H, Garg M, Dou Q-W, Tanaka H

Laboratory of Plant Genetics and Breeding Science, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan

ABSTRACT

Although chromosomes belonging to a genome have similar characteristics and behave in concert with each other, some chromosomes act independently, out of harmony with the other chromosomes. Here we introduce three cases of such chromosome-specific behaviour. 1) Specific retention of chromosome 2D in hexaploid derivatives of octoploid Triticale, 2) Specific elimination of the chromosome 1D in homoeologous group-1 alien chromosome addition lines, 3) Specific amplification of chromosome 3B by interaction with chromosome 3C. These events indicate that seven chromosomes in a genome do not always behave together in evolution. Meiotic drive by these chromosome-specific behaviours must have had a significant role especially in evolution of polyploid species.

INTRODUCTION

Chromosomes belonging to a genome have similar characteristics and behave in concert with each other. The examples are seen in similar C-banding patterns of each chromosome in a genome (Gill et al. 1991), instability of a certain genome in interspecific hybrid (Subrahmanyam and Kasha 1973), occupation of specific spatial territory in the nuclei of interspecific hybrids (Schwarzacher et al. 1989, Kikuchi et al. 2007), and rapid evolution of genes on certain genome in interspecific hybrid (Ozkan et al. 2001). These phenomena are significant in consideration of evolution through interspecific hybrid and polyploidization as that in wheat and the related species. Here we present the cases of chromosome-specific behaviour that does not coincide with the idea of 'genome evolution' shown above from the results of our recent studies on wheat with alien genetic materials.

MATERIALS AND METHODS

Plant materials

Several types of wheat plants with alien materials were used. 1) Fourteen hexaploid derivatives from the progenies of 13 primary octoploid Triticales developed by crossing between Japanese or Korean common wheat cultivars and rye lines (Sasaki et al. 1985, Dou et al. 2006), 2) One hundred and seventy-seven alien chromosome addition lines of common wheat (Garg et al. 2007), 3) Disomic addition wheat line with chromosome 3C of *Aegilops triunicalis* carrying a gametocidal gene *Gc3-C1*, common wheat cultivar Norin 26 (N26) with *Igc1*, a suppressor for *Gc3-C1*, and ditelocentric 3BL and 3BS lines of Chinese Spring (CS) wheat (Tsujimoto 2005). These lines are available in genetic stocks of NBRP-wheat: http://www.shigen.nig.ac.jp/wheat/komugi/top/ top.jsp.

Cytological analysis

Mitotic chromosomes were prepared from root tip cells using the acetocarmine squash method and used for fluorescent *in situ* hybridization (FISH) analysis. Genomic DNAs of the species of chromosome donors were used as the probes in GISH. Clones pAs1 (Afa-family repeat of *Ae. tauschii*), pSc74 (350-bp repeat of rye), pTa71 (45S rDNA of wheat), and a synthesized 30-base length $(AAG)_{10}$ repetitive oligomer (C-band-like) were used as the probes in FISH. The procedures for FISH and GISH were reported in Kishii et al. (1999).

Seed storage proteins

The composition of glutenin subunits from the endosperm half of the seeds was determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE), using 10% acrylamide according to Smith and Payne (1984) with two modifications. The pH of the separating gel was changed from 8.8 to 8.6, and the volume of bisacrylamide was changed from 1.63 to 2.08 ml to increase the resolution (Garg et al. 2007).

RESULTS AND DISCUSSION

Specific retention of chromosome 2D in hexaploid Triticale

GISH analyses were carried out for the 14 hexaploid derivatives from 13 octoploid Triticales. The results showed that three lines carried 10 rye chromosomes, and the others carried 12 rye chromosomes. Sequential FISH using pAs1, pSc74, pTA71 and $(AAG)_{10}$ probes showed that these derivatives could be grouped into four types with respect to their chromosome constitutions: The majority of the lines (10 lines, 71% of the total) showed 12 rye chromosomes, complete A and B genome chromosomes, and a pair of chromosome 2D. Further reprobing with pSc74 revealed that chromosome 2R was not present. One line showed a pair of A-D translocation chromosomes in addition to chromosome 2D substituted for 2R, as mentioned above. From the FISH pattern the translocated chromosome is possibly 5DS-5AL. One line carried 10 rye chromosomes, complete A and B genome chromosomes, and pairs of chromosomes 2D and 3D. Two lines had 10 rye chromosomes, complete A and B genomes and pairs of chromosomes 1D and 2D. Reprobing with pSc74 and pTa71 revealed that rye chromosomes 1R and 2R were not included in this type. Although these lines were obtained independently in

different pedigrees, all of the lines carried chromosome 2D substituted for chromosome 2R.

Specific elimination of the chromosome 1D in alien chromosome addition lines

Glutenin seed storage protein patterns of 177 addition lines available in NBPR-wheat were analysed using SDS-PAGE. Out of 177 lines, 24 seemed to carry the alien chromosome belonging to homoeologous group-1 because they carried additional alien high-molecular-weight glutenin subunits (HMW-GSs). SDS-PAGE patterns of five of these lines showed unexpected electrophoretic patterns, i.e., missing of the subunits encoded by the genes on chromosome 1D (Fig. 1). No absence of the subunits of chromosome 1A or 1B were recognized among the 177 lines. The lines missing chromosome 1D were the homoeologous-gourp-1 'addition lines' of *Agropyron intermedium* (chromosome 1?), *Ag. trichophorum* (1?), *Ag. elongatum* (1E), *Ae. peregrina* ($1S^v$), and *Ae. longissima* ($1S^l$). We conducted the cytological analysis for these lines.

In *Ag. intermedium* addition line, the chromosome 1D was obviously shorter in length than the regular 1D chromosome. In *Ag. trichophorum* line, numbers of chromosome 1D were segregated. Both in *Ag. elongatum* and *Ae. peregrina* lines, the pair of chromosome 1Ds did not appear. Because of spontaneous deletion or whole loss of chromosome 1D during maintenance of the lines, the HMW-GS of chromosome 1D did not appeared in the lines. It is unsurprising that a homoeologous wheat chromosome in addition lines is spontaneously substituted by the alien chromosome. However, it is significant that all of the substituted chromosomes in homoeologous group-1 addition lines are substitution of chromosome 1D and that no substitution of chromosome 1A and 1B appeared despite the chromosome $1S^v$ of *Ae. peregrina* and $1S^l$ of *Ae. longissima* must be more related to the chromosome 1B than 1D.

Specific amplification of chromosome 3B by interaction with chromosome 3C

Chromosome 3C of *Ae. triunicalis* carries a gametocidal gene, *Gc3-C1*. In the monosomic addition line of CS wheat, only the gametes carrying the alien chromosome function (Endo 1978). However, in the genetic background of cultivar N26, all gametes, irrespective of presence of chromosome 3C, function because N26 carries the suppressor, *Igc1*, for *Gc3-C1* (Tsujimoto and Tsunewaki 1985). In the crossed progeny for telocentric analysis to map *Igc1* on chromosome 3B, we found specific amplification of chromosome 3B caused by chromosome 3C (Table 1): First, N26 was crossed with ditelocentric 3BS (dt3BS) or 3BL (dt3BL) of CS, and then the F_1 was crossed with CS+3C3C. Both the chromosome constitution and the self-fertility of plants were observed (Table 1). In a cross with dt3BL, most of the plants carried the expected chromosomes, that is, 2n=42+3C and 41+3BL+3C. However, in a cross with dt3BS, 52 of 94 plants (55%) showed an unexpected chromosome constitution. Many of the unexpected plants carried one or two extra chromosome(s). C-banding analysis and meiotic chromosome configuration indicated that most of the extra chromosomes were chromosome 3B (Fig. 2). This strange event of chromosome amplification appeared in five independent crosses in which four different F_1 plants (CS x dt3BS) were used. This event also reappeared in plants crossed the next year. Because the hybrids using CS or CS+2C2C of *Ae. cylindrica* instead of CS+3C3C did not induce this phenomenon, combination between *Igc1* and chromosome 3C may cause this specific chromosome amplification.

Here we demonstrated the events on chromosome-specific behaviour found in our research using wheat lines with alien materials. The mechanisms are unknown but these events must have occurred in the early generations of interspecific hybrids and amphidiploids. Meiotic drive caused by chromosome-specific behaviour must have had a significant role especially in evolution of polyploid species that are tolerant to chromosome aberration.

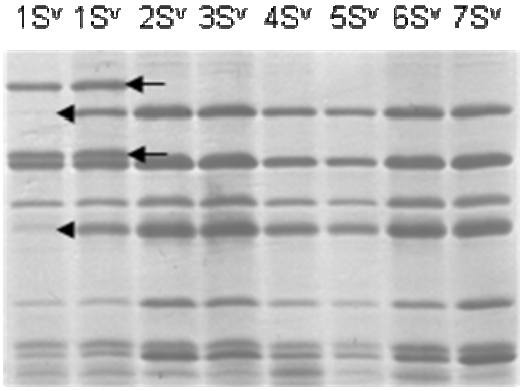

Fig. 1 SDS-PAGE of seed storage proteins in accessions registered as 'addition lines' of *Ae. peregrina* as an example of chromosome elimination. Bands of the alien chromosomes appeared in some plants in line $1S^v$ (arrows), but bands 2+12 coming from 1D were missing in some of the seeds (arrowheads in the first lane).

Table 1 Chromosome amplification appeared in telocentric mapping of *Igc1*

Cross combination	Chromosome constitution	No. of fertile plants	No. of semi-sterile plants	Total
(dt3BL x N26)	42+3C	38	2	40
x CS+3C3C	41+3BL+3C	2	31	33
	Others	3	3	6
	Total	43	36	79
(dt3BS x N26)	42+3C	20	8	28
x CS+3C3C	41+3BL+3C	0	14	14
	43+3C	30	0	30
	42+3BL+3C	5	11	16
	44+3C	1	0	1
	43+3BS+3C	3	0	3
	Others	1	1	2
	Total	60	34	94

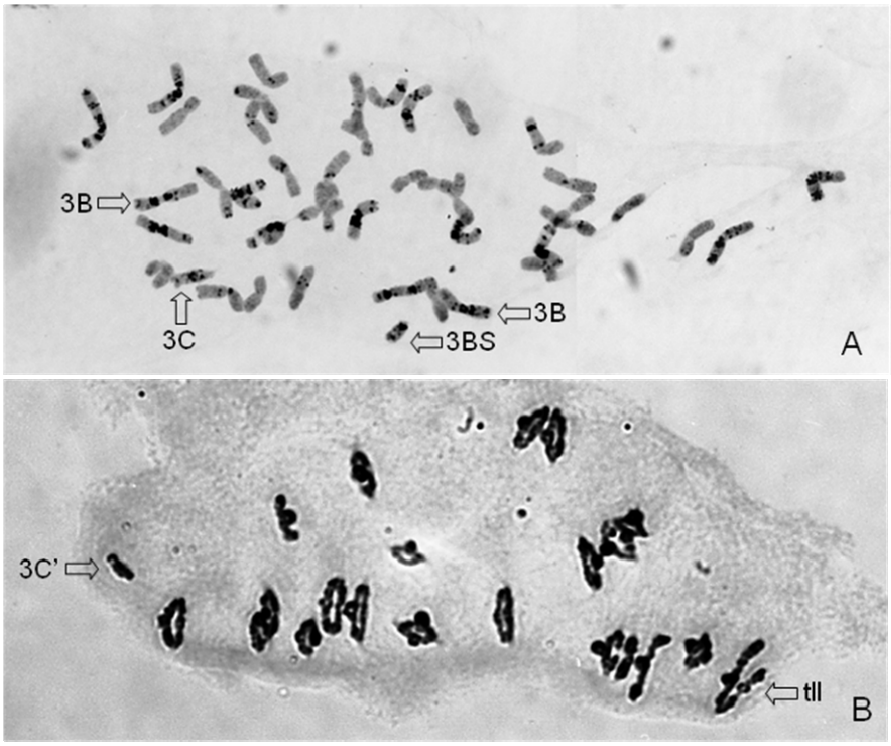

Fig. 2 Plants with an unexpected extra chromosome appeared in the cross, (ditelo3BS x N26) x CS+3C3C. A: C-banded mitotic chromosomes of a plant having 2n=42+3BS+3C. B: Meiotic metaphase cell of the same plant showing 1'+20"+tll. tll indicates a heteromorphic trivalent.

REFERENCES

Endo TR (1978) On the Aegilops chromosomes having gametocidal action on common wheat. In: Proc. 5th Int. Wheat Genet. Symp., New Delhi 1:306-314.

Dou QW, Tanaka H, Nakata N, Tsujimoto H (2006) Molecular cytogenetic analyses of hexaploid lines spontaneously appearing in octoploid Triticale. Theor. and Appl. Genet. 114:41-47.

Garg M, Elamein HMM, Tanaka H, Tsujimoto H (2007) Preferential elimination of chromosome 1D from homoeologous group-1 alien addition lines in hexaploid wheat. Genes and Genet. Syst. 82:403-408.

Kikuchi S, Tanaka H, Wako T, Tsujimoto H. (2007) Centromere separation and association in the nuclei of an interspecific hybrid between *Torenia fournieri* and *T. baillonii* (Scrophulariaceae) during mitosis and meiosis. Genes Genet. Syst. 5:369-375

Kishii M, Nagaki K, Tsujimoto H, and Sasakuma T (1999) Exclusive localization of tandem repetitive sequences in subtelomeric heterochromatin regions of *Leymus racemosus* (Poaceae, Triticeae). Chromosome Res. 7:519-529.

Garg M., Dhaliwal HS, Chhuneja P, Kumar D, Dou Q-W, Elamein HMM, Tanaka H, and Tsujimoto H (2007) Negative effect of chromosome 1A on dough strength shown by modification of 1D addition in durum wheat (*Triticum durum*). Theor. Appl. Genet. 114: 1141–1150.

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34: 830-839.

Ozkan H, Levy AA, Feldman M (2001) Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. Pl. Cell 13:1735-1747.

Sasaki M, Yasumuro Y, Nakata N (1985) Development of chromosome substitution Triticale lines and their utility value as feed. In: Proc 15th Int. Grassland Cong., Kyoto, Japan, pp253-255.

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ localization of parental genomes in a wide hybrid. Ann. Bot. 64:315-324.

Smith DB, and Payne PI (1984) A procedure for the routine determination of electrophoretic band patterns of barley and malt endosperm proteins. J. Nat. Ins. Agri. Bot. 16: 487–498.

Subrahmanyam NC, Kasha KJ (1973) Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma 42: 111-125.

Tsujimoto H (2005) Gametocidal genes in wheat as the inducer of chromosome breakage. Wheat Inf. Serv. 100:33-48.

Tsujimoto H, Tsunewaki K (1985) Gametocidal gene in wheat and its relatives. II. Suppressor of the chromosome 3C gametocidal gene of *Aegilops triunicalis*. Can. J. Genet. Cytol. 27:178-185.