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Genes in Diploid Triticinae Compensating for the Low Temperature Regulating Gene *Ltp* in Chromosome 5D of *Triticum aestivum**

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Summary. Hybrids of *Triticum aestivum* (monosomic 5D or ditelosomic 5DL) x *T. speltoides* (= *Ae. speltoides*) showed that the genotype of *T. speltoides* carries gene(s) which can partially compensate for the expected decrease in chromosome association at low temperatures (10°C) in the absence of chromosome 5D. In hybrids of *T. aestivum* (normal, ditelosomic 3DL or ditelosomic 3DL-monosomic 5D) x *T. longissimum* (= *Ae. sharonensis*), this compensation was not observed.

In normal F_1 hybrids of *T. durum* x *T. longissimum* partial chromosome association occurred at 10°C and this stabilizer effect may be explained by the presence of a *Ltp*-like gene on chromosome 5A. When a line of *T. durum* carrying a homozygous translocated 5B-5D chromosome was used in the crosses an even higher chromosome association was observed.

These results suggest either the existence of a promoter gene for chromosome association in the 5D translocated segment or the loss of a weak suppressor gene in the removed segment of 5B. It was concluded that the translocated 5D segment did not carry the *Ltp* stabilizer gene.

Key words: Triticinae – Low temperature – Variation – Chromosome pairing

Introduction

Triticum aestivum is an allohexaploid species (2n = 6x = 42) with a genomic constitution AABBDD. The three genomes, each derived from a different diploid species, are genetically related. Sears (1958) showed that the corresponding chromosomes of those three genomes did indeed carry duplicate genes.

In meiosis of the *T. aestivum* chromosome pairing normally takes place only between fully homologous partners, a consequence of the balanced effect of several promoter and suppressor genes affecting the premeiotic alignment of chromosomes (Feldman 1966). The result is an entirely bivalent-forming meiotic constitution.

In addition to genetic components there are also environmental factors that influence the quantitative expression of chromosome pairing in wheat. Low and high temperatures have been found to generally diminish chromosome pairing in wheat. Temperature sensitivity is, however, under genetic control, with a major influencing factor in chromosome 5D (Riley 1966). The long arm of this chromosome (5DL) carries a gene (*Ltp*) that sustains such pairing (Hayter 1969). Bayliss and Riley (1972a) showed that the lowering of chiasma frequency at low temperatures in plants of *T. aestivum* deficient for chromosome 5D was correlated with failure of zygotene chromosome pairing. Moreover, they showed that the temperature-sensitive stage lies in the premeiotic interphase before premeiotic DNA synthesis (Bayliss and Riley 1972b). Chromosome 5A also exerts a weak stabilizing effect on chromosome association at low temperature (Riley et al. 1968). Hayter and Riley (1967) demonstrated the presence of another *Ltp* gene in tetraploid wheat (2n = 4x = 28; genomic constitution AABB) which is epistatic to the *Ltp* gene carried by the D genome, and which sustains chiasma formation at low temperatures. Vardi and Dover (1972) found a gene in *Triticum tripsacoides* (= *Aegilops mutica*) which was able to compensate for the absence of *Ltp* in F_1 hybrids *T. aestivum* (monosomic 5D) x *T. tripsacoides* lacking 5D. A genetic system which compensates for the absence of *Ltp* at different levels was also found in F_1 hybrids *T. aestivum* (monosomic 5D) x *T. speltoides* (= *Ae. speltoides*) (Attia et al. 1977).

This paper reports the variation in chromosome pairing

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at low temperatures in F_1 hybrids between *T. aestivum* x *T. longissimum* (= *Ae. sharonensis*), *T. durum* x *T. longissimum* and *T. aestivum* x *T. speltoides*, in the presence or absence of chromosomes 5D and 3D. Low temperature effects were also tested in F_1 hybrids between *T. longissimum* and a tetraploid variant of wheat in which a considerable segment of the long arm of chromosome 5B was substituted for a corresponding segment of the long arm of chromosome 5D. It was hoped that results obtained in this translocated 5B-5D line would show if the replaced 5D segment did carry the *Ltp* gene.

Material and Methods

Plants of *T. aestivum* var. 'Chinese Spring' (2n = 42) and *T. durum* variety 'Ld 222' (2n = 28) were grown at the Biology Center, Oeiras, from seed stocks originally received from Dr. E.R. Sears, University of Missouri. The hexaploid seeds were either normal, monosomic 5D, ditelosomic 5DL or ditelosomic 3DL (= 3Dα).

A tetraploid wheat homozygous for a translocated 5B-5D chromosome, where a considerable segment of the long arm of chromosome 5D was substituted for the corresponding segment of the long arm of chromosome 5B, was isolated by Dr. Mello-Sampayo. The genetic background of the line is the same as *T. durum* variety 'LD 222' – several backcrosses were done using this variety. This line is usually denominated as 'Resende'.

Seeds of *T. longissimum* (2n = 14) and *T. speltoides* (2n = 14) were originally obtained from a stock kept at the National Institute of Genetics, Kyoto University.

Normal hybrids of *T. aestivum* X *T. longissimum* are nearly asynaptic. In order to detect any effect of low temperature on the frequency of chiasmata, it was necessary to increase the level of chromosome association in such hybrids. This was done through the use of a 3DL telocentric line which had lost the suppressor gene for chromosome association located on the short arm of chromosome 3D. Ditelosomic 3DL and monosomic 5D plants were crossed to each other, and the F_1 hybrids were used as female parents in crosses with *T. longissimum*. The resulting seeds were classified at mitosis in order to select the desired combinations (normal euploid, telosomic 3DL and telosomic 3DL-nullisomic 5D).

T. durum variety 'Ld 222' and tetraploid 'Resende' were crossed with *T. longissimum*. Due to the low number of germinated seeds, embryo cultures, using 'Difco' orchid agar, were tried and in many cases embryos were obtained by this method. Triploid seeds were made using the tetraploid as the female parent and main endosperm donor.

Crosses were also made between *T. aestivum* (normal, monosomic 5D, ditelosomic 5DL) X *T. speltoides* using hexaploid wheat as the female parent. F_1 hybrids carried either an entire gametic complement of common wheat or they were independently deficient for chromosome 5D or for the short arm of the same chromosome.

All the F_1 hybrids were grown in winter at normal greenhouse conditions (temperature 20°C ± 2°C). The fixation of the flowers in acetic acid-alcohol (1:3) was done in the greenhouse for each F_1 hybrid. Plants were then transferred to a continuous light condition at 10°C ± 2°C and left there for at least a week before sampling for meiotic stages began. This ensured that the sampled anthers had undergone their complete meiotic development at the controlled temperature (Bayliss and Riley 1972a, b). Meiocyte samples were taken from each plant at different intervals. Some of these plants were returned to the original greenhouse conditions and further meiocyte samples were taken in order to study chromosome pairing recovery.

Chromosome association was studied at the metaphase of the first division of meiosis (metaphase I) in pollen mother cells stained with acetocarmin. Frequency of chiasmata per cell, as considered here, refers specifically to the frequency of chromosome association, which is an expression of the intensity of homoeologous chromosome pairing in each plant.

Results

A gradual decrease in chromosome association was noticed in most F_1 hybrids studied over the period of treatment at low temperatures until a point was reached where stabilization occurred. Conversely, chromosome association gradually returned to normal levels when the plants were again exposed to greenhouse conditions.

F_1 hybrids *T. aestivum* x *T. longissimum* showed a very low degree of chromosome association, both at normal and low temperature (chiasma frequency per cell, 0.85). The absence of the short arm of chromosome 3D (= 3Dβ) resulted in an increase in chromosome association (chiasma frequency per cell, 7.18). At normal temperature the absence of the short arm of chromosome 3D and of the whole chromosome 5D resulted in levels of pairing similar to those observed in the absence of 3DS alone (Fig. 1). These hybrids showed, however, a very significant fall in the frequency of chiasmata (chiasma frequency per cell, 0.30) at 10°C. Some of these plants were returned to 20°C and a gradual recovery to their normal frequencies of chromosome association was observed.

Figure 2 shows the mean chromosome association at 20°C and 10°C in F_1 hybrids *T. durum* variety 'Ld 222' x

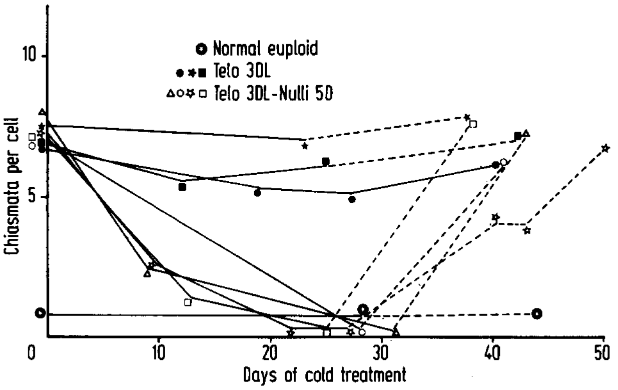

Fig. 1. Chiasmata per cell in F_1 hybrids *T. aestivum* X *T. longissimum* at metaphase I of meiosis. Straight lines: values obtained after different periods (0-40 days) at low temperature (10°C). Dotted lines: recovery to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's)

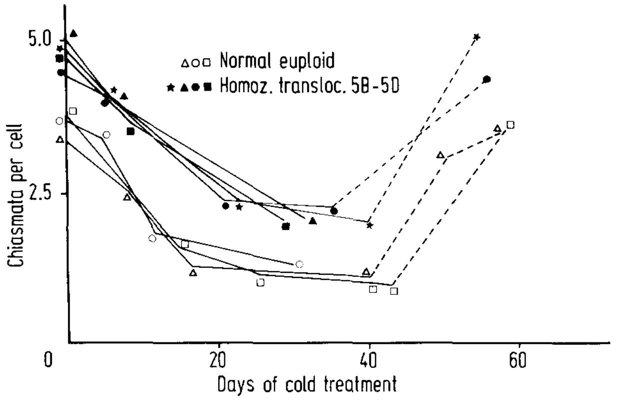

Fig. 2. Chiasmata per cell in F_1 hybrids *T. durum* × *T. longissimum* and in F_1 hybrids between a line of *T. durum* carrying a homozygous translocated 5B-5D chromosome × *T. longissimum* at metaphase I of meiosis. Straight lines: values obtained after different periods (0-43 days) at low temperatures (10°C). Dotted lines: recovery to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's)

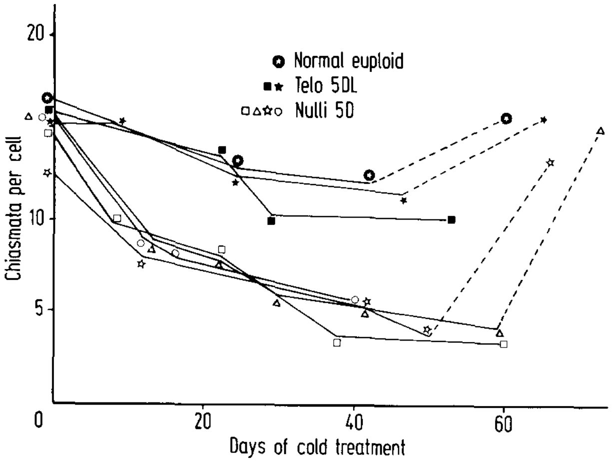

Fig. 3. Chiasmata per cell in F_1 hybrids of *T. aestivum* × *T. speltoides* at metaphase I of meiosis. Straight lines: values obtained after different periods (0-59 days) at low temperature (10°C). Dotted lines: recovery to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's)

T. longissimum (chiasma frequency per cell, 3.50) to be higher than that observed in euploid hybrids *T. aestivum* × *T. longissimum*. The presence of a translocated 5B-5D segment in 'Resende' hybrids significantly increased chromosome association (chiasma frequency per cell, 4.72) in relation to that of normal triploid hybrids. At 10°C, hybrids of *T. longissimum* with 'Ld 222' and those with 'Resende' showed a similar significant decrease in the frequency of chiasmata.

F_1 hybrids of *T. aestivum* × *T. speltoides* at 20°C had very high levels of chromosome association (chiasma frequency per cell 15.20). The absence of the short arm of chromosome 5D or the entire chromosome 5D in these hybrids did not modify the pattern of chromosome association at 20°C. At low temperature, however, a significant fall in chromosome association was observed in nullisomic 5D hybrids, with a subsequent recovery after a few days at 20°C (Fig. 3).

Discussion

The chromosomes of species belonging to the Sitopsis section of *Aegilops* are closely related to those of the B genome of *Triticum* (Sears 1956; Riley et al. 1958; Feldman 1978). This close relationship reveals itself in the relatively consistent chromosome pairing shown in F_1 hybrids *T. durum* × *T. longissimum* (chiasma frequency per cell, 3.50). The presence of a D genome in F_1 hybrids *T. aestivum* × *T. longissimum* induces asynapsis (chiasma frequency per cell, 0.85). The most plausible explanation is derived from the presence of a chromosome pairing suppressor gene located on the short arm of chromosome 3D (Mello-Sampayo 1971). Evidence for this hypothesisis primarily based on the higher chromosome association shown by telosomic 3DL plants (chiasma frequency per cell, 7.18). A very significant drop in chromosome association (chiasma frequency per cell, 0.30) was found in F_1 hybrids *T. aestivum* × *T. longissimum* at low temperature when chromosome 5D and 3DS were simultaneously absent. This result agrees with what was expected since the *Ltp* gene was missing.

A very different pattern was seen in hybrids with tetraploid wheat. The hybrids 'Ld 222' × *T. longissimum* and 'Resende' × *T. longissimum* showed similarly shaped curves of chromosome association (expressed in frequency of chiasmata per cell) versus number of days at low temperature. This indicates that a '*Ltp*-like' gene is acting in both hybrids. Hayter (1969) suggested that *T. durum* carries a *Ltp* gene allele since the frequency of chiasmata remains at a constant level with lower temperatures. A single dosage of this gene in F_1 hybrids should be enough to stabilize chromosome association to some degree, even if lower than that of *T. durum* in the same situation.

The results obtained in 'Resende' × *T. longissimum* show a constant higher value of chromosome association with increasing number of days at low temperature, as compared with 'Ld 222' × *T. longissimum*. It is possible that the translocated 5B-5D chromosome of 'Resende' is responsible for this different level which is maintained either at normal or at low temperatures.

It has been suggested (Mello-Sampayo 1972; Mello-Sampayo and Viegas 1973) that the intermediate type of pairing shown in hybrids carrying such translocated 5B-5D chromosome is due either to the addition of a promoter gene carried by the segment of 5DL or to loss of a weak suppressor gene carried in the distal segment of 5BL. The pattern obtained for chromosome association in both hybrids (parallel curves) indicates that no *Ltp* gene was carried by the translocated segment of 5D. Moreover, the observed pattern (a constant difference for chromosome association between both hybrids over days of cold treatment) suggests that the effect of the stabilizer gene for low temperature is the same in both curves. It seems, then, that their difference may be explainable simply by the different starting levels of chromosome association.

Hybrids of *T. aestivum* with *T. speltoides* yielded two different types of curves: when hybrids had a normal 5D or telosomic 5DL, a high chromosome association at low temperature was observed; when the hybrids were deficient for chromosome 5D a drastic reduction in chromosome association occurred, at 10°C. Both results confirm that the *Ltp* gene is located in the long arm of chromosome 5D. This is seen in Figure 3, which also shows that chromosome pairing stabilizes at a low but significant level. This indicates that *T. speltoides* carries a low temperature stabilizer gene which appears to have a weaker effect than the one carried by chromosome 5D (Attia et al. 1977).

The availability and the interrelationship of pairing stabilizer genes for low temperature in Triticinae species must be related to their geographical distribution. Chromosome engineering designed to increase their content in the genome of useful varieties would enhance stability for a wider temperature range.

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