Chromosome instability in intergeneric hybrids of *Triticum aestivum* × tritordeum (amphiploid *Hordeum chilense* × *Triticum turgidum*) with high dosage of Ph_1 gene of wheat

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VIEIRA, R., MELLO-SAMPAYO, T., QUEIROZ, A., MORAIS, L. and VIEGAS, W. S. 1991. Chromosome instability in intergeneric hybrids of *Triticum aestivum* × tritordeum (amphiploid *Hordeum chilense* × *Triticum turgidum*) with high dosage of Ph_1 gene of wheat. — *Hereditas 114*: 271-276. Lund, Sweden. ISSN 0018-0661. Received July 18, 1990. Accepted April 30, 1991

In somatic cells of intergeneric hybrids *Triticum aestivum* (mono-isosomic 5BL, 2n = 6x = 41) × tritordeum (2n = 6x = 42, amphiploid *Hordeum chilense* × *Triticum turgidum*) it was observed that high dosage of the long arm of 5B induced chromosome instability in hybrids 2n = 42. In hybrids 2n = 41 with only one dose of 5BL from the normal 5B genome of the tetraploid wheat, all cells have consistently 2n = 41 chromosomes and no morphological disturbance was detected in any phase of the cell cycle or during plant differentiation. In plants with 2n = 42 which carry three doses of 5BL (one isochromosome 5BL and one 5B chromosome) most of the metaphase cells had 2n = 42 chromosomes. However, other cells, in a reasonable frequency varying from 19 % to 40 % carried from 2n = 6 to 2n = 44, and showed marked disturbances in all phases of the cell cycle, leading to final failure in plant development. It is suggested that the Ph_1 gene of wheat, located on 5BL, regulates chromosome stability in the somatic cells of those hybrids.

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Chromosome instability is frequently detected both in somatic and in sexual intergeneric hybrids and is usually related to differential assembly of chromosomes at metaphase, lagging of chromosomes at anaphase and their subsequent exclusion from the daughter nuclei in telophase, forming micronuclei in interphase, which are commonly found in somatic cells in F_1 hybrids. This was detected in young embryos of wheat × *Hordeum bulbosum* and wheat × maize F_1 hybrids (KASHA 1974; BARCKLAY 1975; BENNETT et al. 1976; FEDAK 1982; LAURIE and BENNETT 1986) and also in young embryos and root tips of wheat × rye F_1 plants (MELLO SAMPAYO et al. 1988a and b). Several hypotheses have been formulated to explain the nature of these instabilities, and they are mainly ascribed to possible failures of a correct integration of chromosomes into the spindle of the hybrid cell (MIGEON 1968; HANDMAKER 1973) as the most plausible reason for the final chromosome segregation, although it has already been found that segregation was independent of the hybrid spindle constitution (ZELESCO and GRAVES 1987). A non-random positioning of the different genomic sets of chromosomes either in hamster-human (ZELESCO and GRAVES 1988) or in barley-rye hybrid cells (FINCH et al. 1981; FINCH 1983; SCHWARZACHER-ROBINSON et al. 1987), has also been found. This was probably due to the centromeres of the eliminated chromosomes being less efficiently attached to the spindle in hybrid cells; since in wheat × barley hybrids these excluded chromosomes had smaller centromeres than those retained, supporting the idea that the elimination could result from specific gene inactivation, through DNA methylation, of centromeres (FINCH 1983; FINCH and BENNETT 1983).

A correlation between chromosome instability and the wheat Ph_1 gene dosage has recently been detected in wheat × rye F_1 hybrids (MELLO-SAMPAYO et al. 1988a and b). The extra dosage of Ph_1 gene in the disomic 5B hybrid and its deficiency in that involving the High Pairing Mutant, resulted in a considerably increased frequency of micronuclei at interphase and of chromatin bridges and laggards at anaphase and telophase.

The mechanisms regulated by Ph_1 wheat gene have been extensively studied and the meiotic effects referring to either homoeologous chromosome pairing (OKAMOTO 1957; SEARS and OKAMOTO 1958; RILEY and CHAPMAN 1958; RILEY 1960; SEARS 1976), or to bivalent interlocking (YACOBI et al. 1982), or to synaptic and post-synaptic features involving crossing-over (HOBOLTH 1981; HOLM and WANG 1988), or to spindle sensitivity to antimicrotubules agents (AVIVI et al. 1970; AVIVI and FELDMAN 1973; CEOLONI et al. 1984; FELDMAN and AVIVI 1988) or still to somatic chromosome association (AVIVI and FELDMAN 1980; FELDMAN and AVIVI 1984) are all well documented.

In this study we evaluate the influence of different doses of the long arm of chromosome 5B (5BL) on the degree of chromosome instability in somatic cells of hybrids between *T. aestivum* (2n = 6x = 42, genomes AABBDD) and the amphiploid (2n = 6x = 42, genomes AABBHchHch) *Hordeum chilense* × *T. turgidum* produced by MARTIN and SANCHEZ-MONGE LAGUNA (1982) and designated as tritordeum.

Materials and methods

Mono-isosomic 5BL (MI 5BL) plants of *T. aestivum* cv. Chinese Spring originally from a stock sent by Prof. E. R. Sears (University of Missouri, USA) and carrying a single isochromosome 5BL instead of the normal homologous pair of 5B chromosomes, and tritordeum plants (amphidiploid *H. chilense* × *T. turgidum* var. *durum*, kindly supplied by Dr. A. Martin (ETSEA, Cordoba, Spain), were grown in the field until reaching the boothing stage. They were then placed in a continuously lighted growing cabinet and kept at 22°C ± 1°C. MI 5BL was pollinated with tritordeum. Further development until seed maturation in the cabinet followed. Seeds from these crosses were set to germinate in Petri dishes during 2–3 days until 1 cm long root tips could be excised and fixed in a 3:1 ethanol-acetic acid solution. Some of the root-tips were treated during 4 hours with a saturated solution of 1-bromonaphthalene before fixation in order that mitotic indexes could be maximized through the arresting of metaphase cells (c-metaphase) and chromosomes could be observed and counted. All root-tips were Feulgen stained and squashes were performed in 45 % acetic acid. Cells with aberrant mitotic configurations were identified and studied in untreated root tips and the chromosome number in metaphase cells of treated material was recorded. After removal of root tips from different seeds these were further let to develop in Petri dishes in growing cabinets until one or two new root tips emerged when they were transferred to Jiffy pots where seedlings developed until final transfer to normal pots in normal greenhouse conditions.

Results and discussion

The crosses performed produced two kinds of hybrid plants: those carrying the isochromosome 5BL from Chinese Spring and therefore with a chromosome number of 2n = 42 (3 doses of Ph_l gene per cell, two from iso 5BL and one from normal 5B chromosome, which came from the tritordeum) and those lacking that isochromosome, with 2n = 41 (with only one dose of Ph_1 gene located on the 5B chromosome). The records of the chromosome numbers in cells of treated root tips in different seeds showed that when the isochromosome 5BL was absent metaphase cells consistently scored 2n = 41 in contrast with root-tips from 2n = 42 plants, where most cells had a chromosome number 2n = 42 (Fig. 1A) but which also included a significant number of cells (from 19 to 40 %) with chromosome numbers varying between 2n = 6 and 2n = 44 (Fig. 1B) as presented in Table 1.

These results clearly show that in hybrids with a single dose of the Ph_l gene (in plants with 2n = 41), chromosome stability is complete and that it is substantially disrupted in the presence of the isochromosome 5BL, as the number of Ph_l genes increased to three. Careful analysis of untreated root tip meristems from the same seeds previously analysed, in different phases of the cell cycle, also show a higher proportion of aberrant cells in hybrids in which the isochromosome 5BL is present than in those lacking it. Aberrant cells include interphase cells with micronuclei and mitotic cells showing anomalies such as two independent prophase nuclei, metaphases with regions of not well aligned chromosomes in the equatorial plate (Fig. 1C), metaphases with two groups of chromosomes and laggards in between (Fig. 1D), anaphases with lagging chromosomes and multipolar anaphases (Fig. 1E). None of these aberrations was detected in any phase of the cell cycle in root tips of plants lacking the isochromosome 5BL.

The anomalies observed will easily preclude the elimination of entire chromosomes or chromosome fragments which should drastically affect plant development as further analysis of both types of phe-

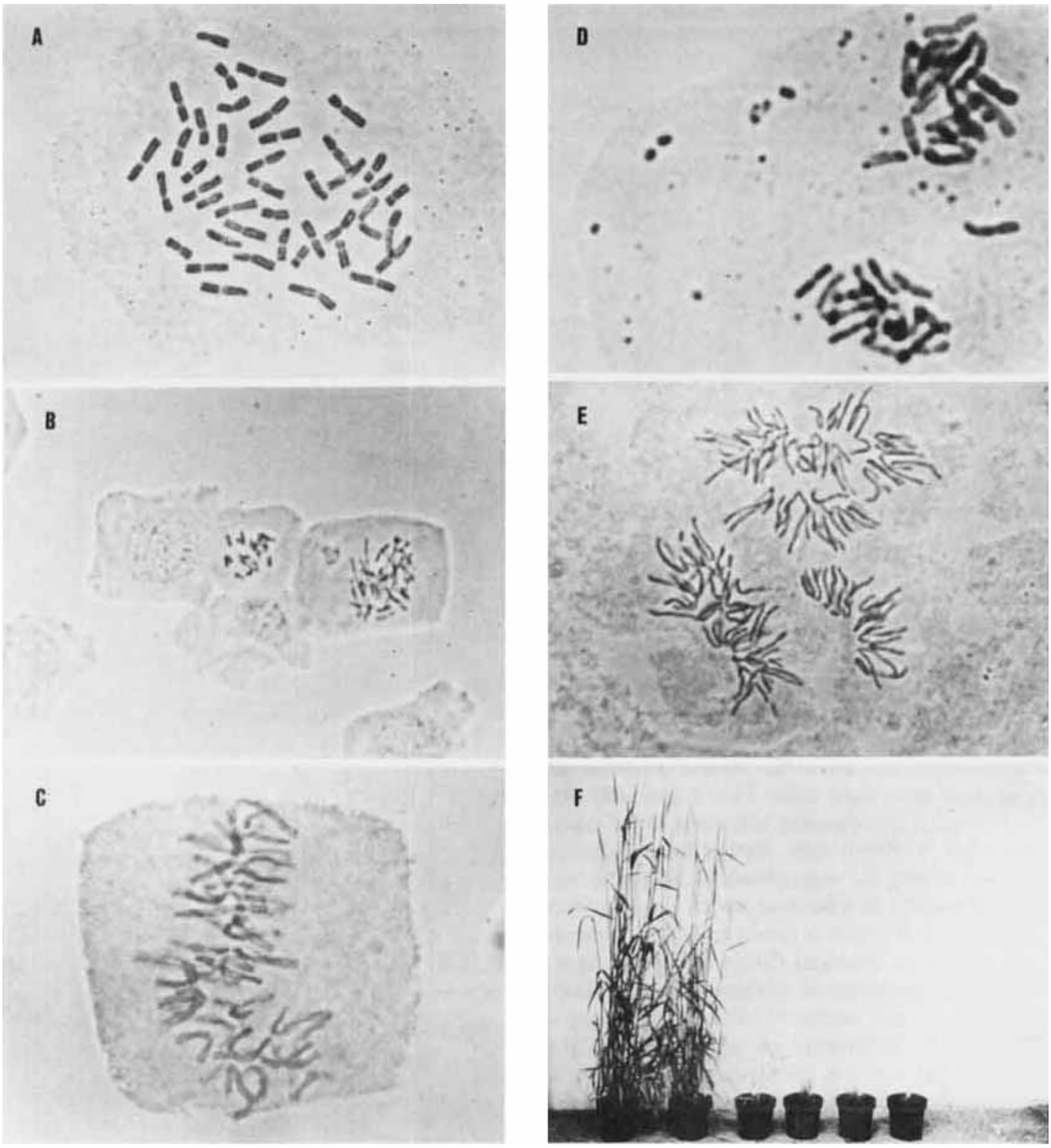

Fig. 1A–F. Intergeneric hybrids *T. aestivum* (mono-isosomic 5BL) × tritordeum (amphiploid *H. chilense* × *T. turgidum*). Cells from seeds with isochromosome 5BL. **A** A c-metaphase cell with 2n = 42. **B** Cells with distinct chromosome numbers. **C** Equatorial plate with regions of not well aligned chromosomes. **D** Metaphase with two groups of chromosomes and laggards in between. **E** Multipolar anaphases. **F** At the left hand two normal plants without isochromosome 5BL (2n = 41) and at the right hand four plants with isochromosome 5BL (2n = 42) and a defective growth.

notype confirmed: those plants lacking isochromosome 5BL developed normally but the development of those with two extra doses of the 5BL chromosome arm was substantially altered, showing only a defective growth and no ear differentiation (Fig. 1F).

The frequency distribution of chromosome number per cell in genotypes with 3 doses of the 5BL chromosome arm is shown in Fig. 2. Although this distribution is centered around 21 chromosomes our results do not allow the assumption of a preferential genome segregation similar to that ob-

Table 1. Somatic chromosome numbers of cells of an intergeneric hybrid between *Triticum aestivum* mono-isosomic 5BL (2n = 41) and the amphiploid (2n = 42) *Hordeum chilense* × *Triticum turgidum* var. *durum* (= tritordeum)

Plant number	Isochromosome 5BL in the hybrids	Frequency of cells with different chromosome numbers (%)				Number of C-metaphase cells scored
		40 (*)	41	42	42 (**)	
3	absent	–	100	–	–	38
4	absent	–	100	–	–	43
10	absent	–	100	.	–	18
6	absent	–	100	–	–	18
1	present	19	–	81	–	21
2	present	26	3	68	3	31
5	present	23	–	75	2	100
7	present	15	2	81	2	61
11	present	28	2	69	1	161
12	present	30	10	60	–	61
15	present	16	4	77	3	108
16	present	30	–	68	2	113

(*) Include all cells with chromosome numbers from 2n = 6 to 2n = 40
(**) Include cells with chromosome numbers of 2n = 43 and 2n = 44

served in wheat-barley hybrids and in hybrids between barley and rye (FINCH et al. 1981; FINCH and BENNETT 1983; FINCH 1983; SCHWARZACHER-ROBINSON et al. 1987) or in hamster-human cells (ZELESCO and GRAVES 1988). The results obtained, therefore, suggest that the 5B long arm, and possibly the *Ph₁* gene it carries, can affect the correct chromosome segregation in somatic cells. This is probably due to an incorrect chromosome alignment at the equatorial plate of hybrid cells, further inducing some disturbed chromatid segregation in anaphase and nucleus recovery in telophase, which confirms previous studies in wheat × rye hybrids where mixoploidy was also observed (MELLO-SAMPAYO et al. 1988a). Misalignment of chromosomes has been reported in several instances and could be due to a failure in the attachment of centromeres to the spindle as the result of the specific suppression of genes involved in centromere function, perhaps by DNA methylation, as suggested by FINCH and BENNETT (1983) and FINCH (1983), in a process similar to the suppression of specific nucleolar organizers in some intergeneric hybrids (FLAVELL et al. 1983; VIEIRA et al. 1990). It must be noted, however, that no difference was found in the binding of antikinetochore antibodies to retained and segregant centromers in Chinese hamster-human hybrid cells (ZELESCO and GRAVES 1989).

The spatial distribution of chromosomes, which has been found in hybrids to be non-random both in metaphase cells and in interphase cells (AVIVI and FELDMAN 1980; FELDMAN and AVIVI 1984, 1988; FINCH et al. 1981; BENNETT 1988; SCHWARZACHER et al. 1989) and which is probably maintained by the initial anchoring of chromosome ends on to the nuclear membrane at telophase—interphase—prophase stages (ASHLEY and POCOCK 1981) could play a role in this chromosome instabil-

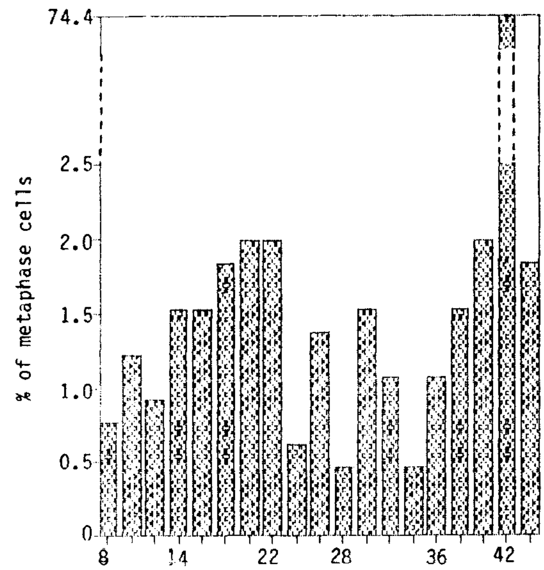

Fig. 2. Frequencies of metaphase cells with distinct number of chromosomes observed in monoisosomic 5BL hybrids. Each class shown represents the sum of the frequencies of cells with contiguous number of chromosomes. For example, class 22 represents the sum of the frequencies of cells with 21 and with 22 chromosomes.

ity. Moreover, in callus tissues from tetrasomic 5B plants a higher percent of aneuploid cells was detected, when compared with tetrasomic 5A or 5D, and some of those cells seemed to have chromosomes fused end-to-end (OKAMOTO et al. 1973). We can therefore speculate that Ph_l gene affects the attachments of telomeres on to the nuclear membrane, the strength of the attachements being directly dependent on Ph_l dosage. This regulatory effect of Ph_l dosage on the interaction of chromatin with the membrane could affect all mechanisms where these structures are involved: it could influence spindle sensitivity, leading to c-mitosis, homoeologous chromosome pairing, somatic chromosome association and would also lead to chromosome instability as a result of irregular congression of chromosomes on the equatorial plate during mitosis.

Acknowledgements. — This work was supported by a grant of INIC, Portugal.

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