CHROMOSOME INSTABILITY IN INTERGENERIC HYBRIDS *Triticum aestivum* x tritordeum (amphiploid *Hordeum chilense* (x) *Triticum turgidum*) WITH HIGH DOSAGE OF Ph_1 GENE OF WHEAT

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Key words: chromosome elimination, Ph_1 gene dosage; isochromosome 5BL; amphiploid *Triticum turgidum* x *Hordeum chilense* (: tritordeum)

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SUMMARY

In somatic cells of intergeneric hybrids Triticum aestivum (monoisosomic 5BL, 2n=6x=41) x tritordeum (2n=6x=42, amphiploid Hordeum chilense x 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 showed ~~have~~ 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 ~~in~~ on 5BL, regulates chromosome stability in the somatic cells of those hybrids.

INTRODUCTION

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 x Hordeum bulbosum and wheat x maize F$_1$ hybrids (Barcklay, 1975; Bennett et al., 1976; Kasha, 1974; Fedak, 1982; Laurie and Bennett, 1986)) and also in

young embryos and root tips of wheat x rye F_1 plants (Mello Sampayo et al., 1988 a 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). It has also been found 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). This was probably due to the centromeres of the eliminated chromosomes being less efficiently attached to the spindle in hybrid cells, since in wheat x 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 chromosomes 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 x 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 defficiency 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) or still to somatic chromosome association (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 x 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) carrying a single isochromosome 5BL instead of the normal homologous pair of 5B chromosomes and tritordeum plants (amphidiploid H. chilense x 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 growing cabinet and kept at 22ºC ±1ºC continuously lighted, before pollination of MI 5BL with tritordeum, took place. 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 emmerged and after that 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 ~~should~~ produce 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 *Ph1* 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 *Ph1* gene located in 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$_1$ 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$_1$ 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, 1.D), 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 phenotype confirmed: those plants lacking isochromosome 5BL developed normaly but the development of those with two extra doses of 5BL chromosome arm was substantially altered, showing only a deffective growth and no ear differentiation (Fig.1F).

The frequency distribution of chromosome number per cell in genotypes with 3 doses of 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 observed 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 5B long arm, and possibly the Ph1 wheat gene it carries, can affect the correct chromosome segregation in somatic cells. This is probably due to an incorrect chromosome alignement at the equatorial plate of hybrids cells further inducing some disturbed chromatid segregation in anaphase and nucleus recovery in telophase, which confirms previous studies in wheat x rye hybrids where mixoploidy was also observed (Mello-Sampayo et al., 1988a). Misalignement 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., in press). It must be noted, however, that no difference was found in the binding of antikinetochore antibodies to retained and segregant centromeres 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, 1984, 1987; Bennett, 1983, 1984; Finch et al., 1981; 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 rôle in this chromosome instability. We can speculate that Ph1 gene affects these attachments of telomeres on to the nuclear membrane, the strength of the attachements being directly dependent on Ph1 dosage. This regulatory effect of Ph1 dosage on the interaction of chromatin with the membrane would affect all mechanisms where these structures are involved. Therefore it would influence spindle sensitivity to c-mitosis, ~~c-mitotics~~,

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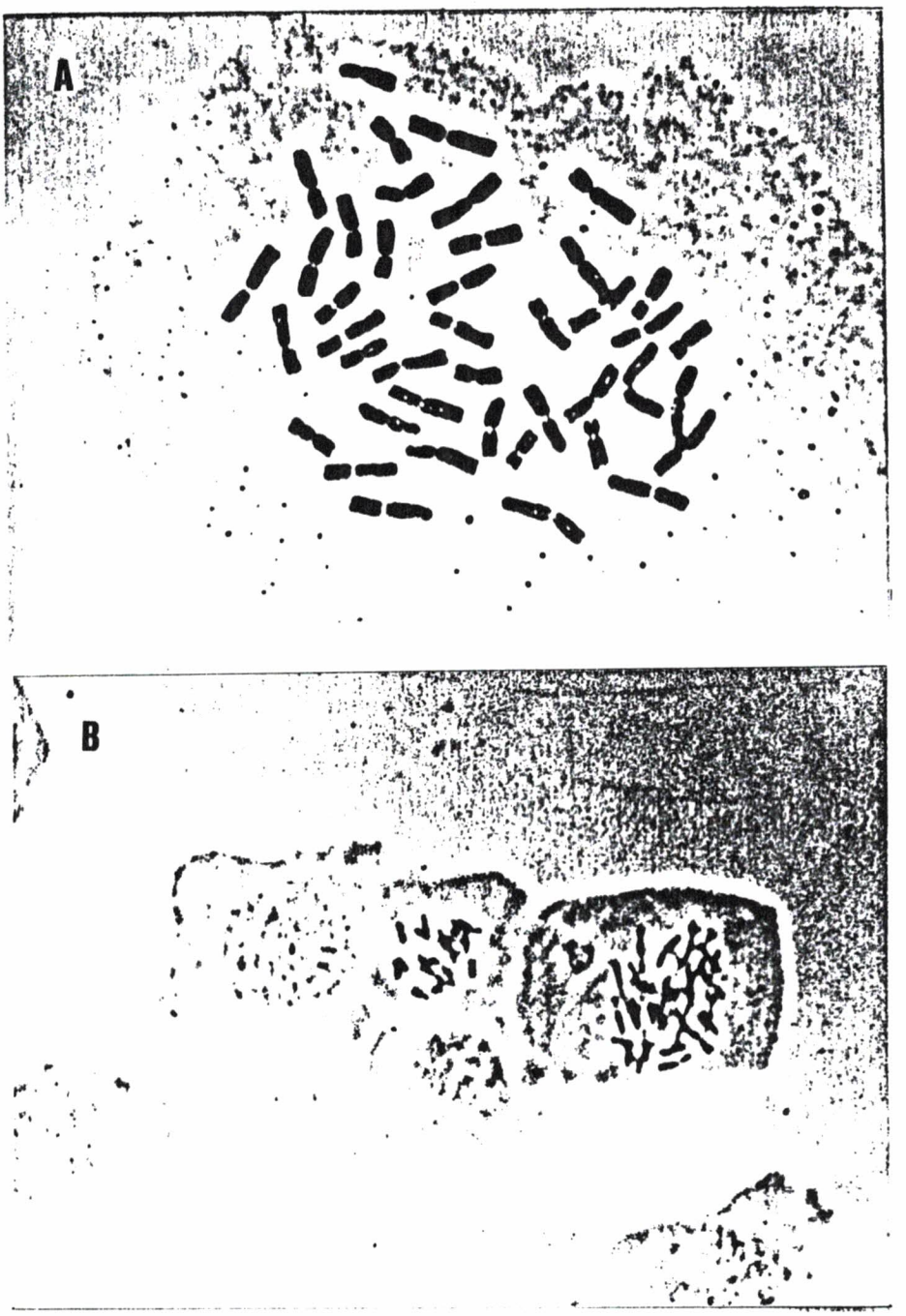
Table 1 - Somatic chromosome numbers of cells of an intergeneric hybrid between *Triticum aestivum* mono-isosomic 5BL (2n=41) and the amphiploid (2n=42) *Hordeum chilense* x *Triticum turgidum* var. *durum* (= tritordeum)

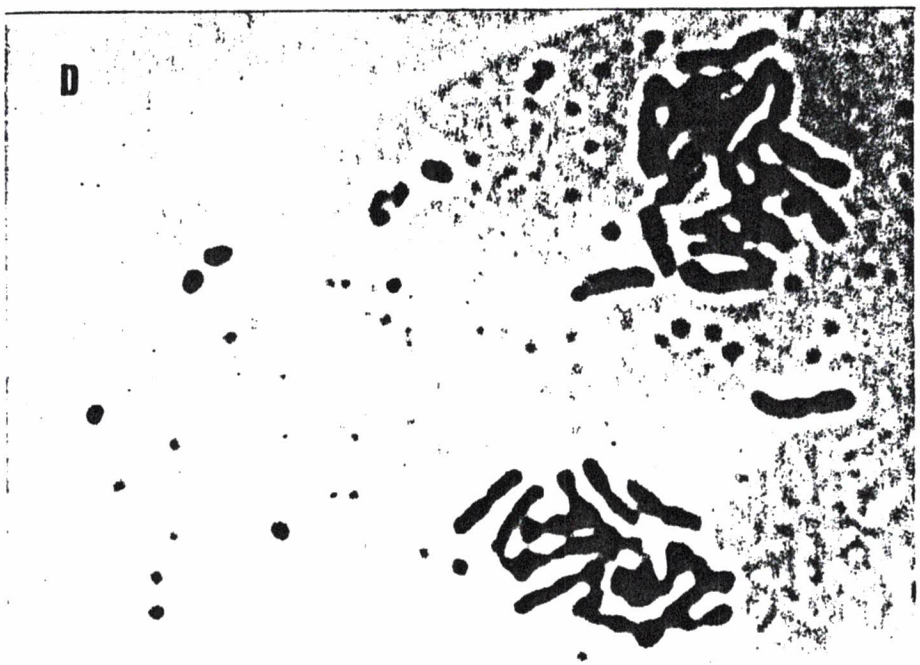
Plant number	Isochromosome 5BL in the hybrids	Frequency of cells with different chromosome numbers (%)				Number of C-metaphase cells scored
		40(*)	41	42	42(**)	
3	absent	--	100	--	--	38
4	absent	--	100	--	--	43
10	absent	--	100	--	--	18
6	absent	--	100	--	--	18
1	present	19	--	81	--	21
2	present	26	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

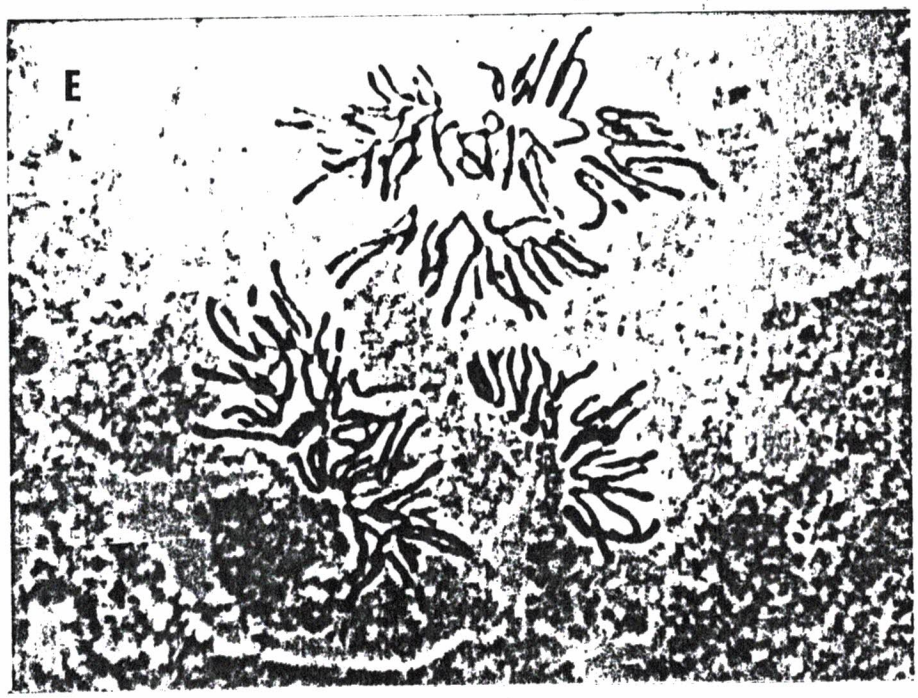
(*) Include all cells with chromosome numbers from 2n=6 to 2n=40

(**) Include cells with chromosome numbers of 2n=43 and 2n=44

Fig. 1 - Intergeneric hybrids *T. aestivum* (mono-isosomic 5BL) x tritordeum (amphiploid *H. chilense* x *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- mulitpolar 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 deffective growth.


A
B
C


D


E

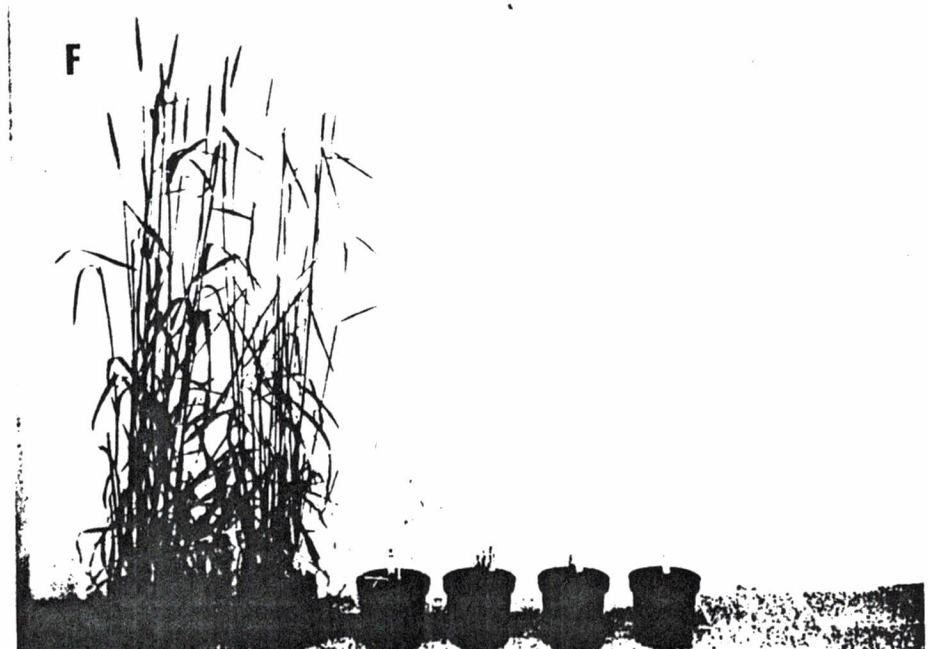
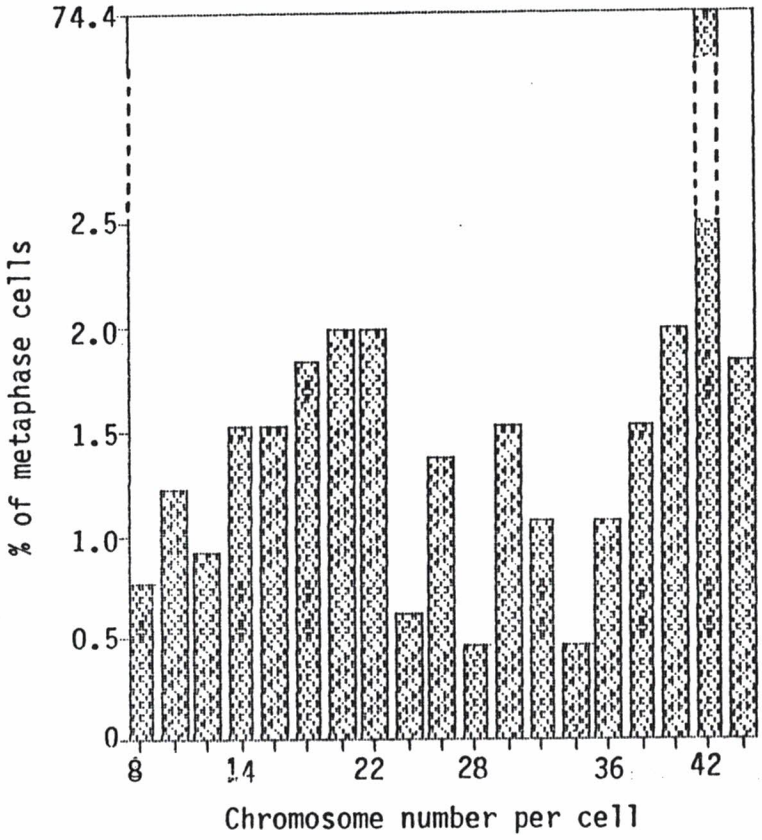

F

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.


74.4
2.5
2.0
1.5
1.0
0.5
0
% of metaphase cells
8
14
22
28
36
42
Chromosome number per cell