Chromosome instability in intergeneric hybrids of *Triticum aestivum* × *tritordeum* (amphiploid *Hordeum chilense* × *Triticum turgidum*) with high dosage of Ph1 gene of wheat

R. VIEIRA1, T. MELLO-SAMPAYO2, A. QUEIROZ, L. MORAIS and W. S. VIEGAS3

1 Universidade de Coimbra, Coimbra, Portugal
2 Estação Nacional de Melhoramento de Plantas, Elvas, Portugal
3 Departamento de Botânica, Instituto Superior de Agronomia, Lisboa, Portugal

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 F1 hybrids. This was detected in young embryos of wheat × *Hordeum bulbosum* and wheat × maize F1 hybrids (KASHA 1974; BARKLEY 1975; BENNETT et al. 1976; PEDAK 1982; LAURIE and BENNETT 1986) and also in young embryos and root tips of wheat × rye F1 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; HANDEMAKER 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 (ZELESKO and GRAVES 1987). A non-random positioning of the different genomic sets of chromosomes either in hamster-human (ZELESKO 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 Ph1 gene dosage has recently been detected in wheat × rye F1 hybrids (MELLO-SAMPAYO et al. 1988a and b). The extra dosage of Ph1 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 Ph1 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 (Yacob 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 AABBBHchHch) *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 booting 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 *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 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 *Ph1* 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 *Ph1* 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-
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-
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 x 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 somatic 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; Schwar-
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 Ph1 gene affects the attachments of telomeres on to the nuclear membrane, the strength of the attachments being directly dependent on Ph1 dosage. This regulatory effect of Ph1 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.

References


Okamoto, M. 1957. A synaptic effect of chromosome V. — Wheat Inf. Serv. 5: 6


