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Cytology and morphology of the amphiploid *Hordeum chilense* (4x) × *Aegilops squarrosa* (4x)

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Summary. The intergeneric amphiploid *Hordeum chilense* × *Aegilops squarrosa* has been synthesized. The amphiploid plants have the expected chromosome number of 28. The average meiotic chromosome pairing was 12.48 bivalents + 3.04 univalents. The morphology of the amphiploid resembles that of the *Aegilops* parent. Nucleoli from both *H. chilense* and *A. squarrosa* are expressed in the amphiploid. Neither chromosome instability nor homoeologous pairing was found. The amphiploid is fertile and vigorous.

Key words: Intergeneric amphiploid *Hordeum chilense* × *Aegilops squarrosa* – Tetraploid tritordeum – Morphology – Cytology

Introduction

Wide crosses have played an important role in the study of phylogenetic relationship among species and in the process of widening the genetic basis of crops. In addition, in a few cases such hybrids, after chromosome doubling, have given rise to plants not established in nature, which present characteristics of possible new crops. In the tribe Triticeae, wide crossing has been used successfully for the former purpose. Nevertheless, two of the genera of this tribe having the highest agronomical interest, barley and wheat, are among the most difficult to cross.

Beginning with the first success achieved by Kruse (1973) crossing cultivated barley and wheat (*Hordeum vulgare* × *Triticum aestivum*, *T. turgidum*, and *T. monococcum*), several hybrids between the two genera have been produced. They include crosses of cultivated barley with the tetraploid wheats *T. dicoccum* (Thomas et al. 1977) and *T. timopheevi* (Cauderon et al. 1978) and with the hexaploid species *T. crassum* (Fedak and Nakamura 1981).

The diploid wild species of barley *H. pusillum* (Finch and Bennett 1980), *H. publiflorum* (Fedak 1983), and *H. californicum* (Gupta and Fedak 1985) and the tetraploid wild species *H. jubatum* (Comeau et al. 1988) and *H. bulbosum* (Wang et al. 1982) have been crossed with common wheat, whereby viable hybrids were obtained but not fertile amphiploids. Hybrids between *Hordeum* and diploid related species of cultivated wheat are rare. Hybrids have been obtained between *H. vulgare* and *T. monococcum* (Kruse 1973), *Aegilops squarrosa* × *H. bulbosum* (Fedak 1985), and *H. chilense* × *A. squarrosa* (Martín 1983).

Fertile amphiploids between both genera have been achieved by chromosome doubling with colchicine of the hybrids *T. timopheevi* × *H. bogdanii* (Kimber and Sallee 1979), *H. chilense* × *T. aestivum* (Martín and Chapman 1977), and *H. chilense* × *T. turgidum* (Martín and Sánchez-Monge Laguna 1979), the last two called octoploid and hexaploid tritordeums, respectively (Martín and Cubero 1981). However, no fertile amphiploids between *Hordeum* and the diploid progenitors of cultivated wheat have thus far been obtained.

In this paper we present tetraploid tritordeum: the first fertile intergeneric amphiploid between *Hordeum* and *Aegilops*.

Materials and methods

Tetraploid *H. chilense* (2n = 4x = 28) was obtained after colchicine treatment of a diploid line originally obtained from Dr. Lange, Wageningen, The Netherlands. Tetraploid *A. squarrosa* (syn. *T. tauschii*) (2n = 4x = 28) was obtained from the former P.B.I., Cambridge, UK, collection.

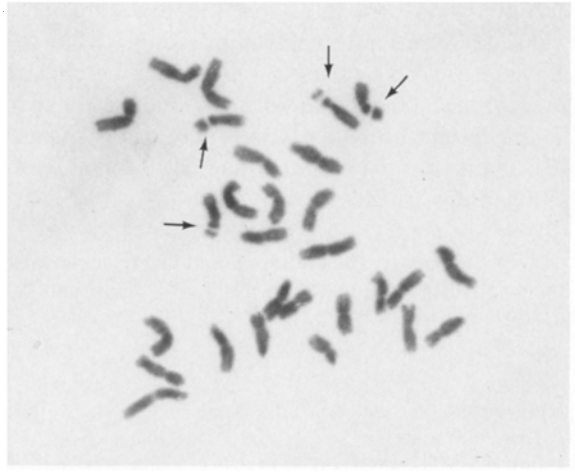

Fig. 1. Mitotic metaphase of the amphiploid *H. chilense* × *A. squarrosa* (2n = 28). Satellited chromosomes indicated by *arrows*

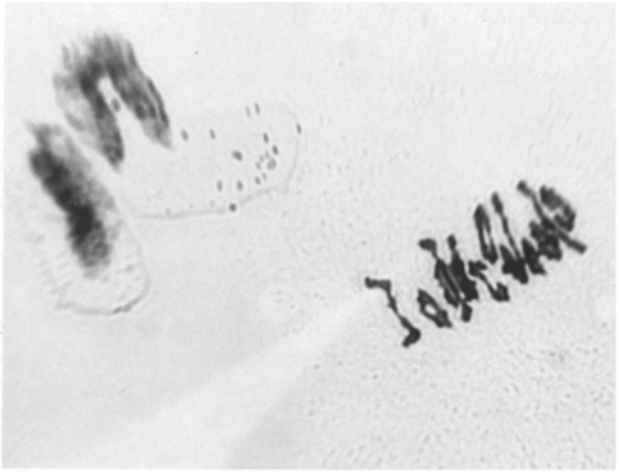

Fig. 2. Meiotic metaphase I of the amphiploid *H. chilense* × *A. squarrosa* showing 14 bivalents

One or two days after emasculation, spikes of the tetraploid *H. chilense* were pollinated with tetraploid *A. squarrosa*. Gibberellic acid, at a concentration of 75 ppm, was applied to pollinated florets 24 h after pollination by means of a syringe. Between 3 and 4 weeks later, the embryos were excised under sterile aseptic conditions and cultured in orchid agar. The cultured embryos were kept in the dark at 10 °C until differentiation occurred. Then they were placed in a growth cabinet at 20 °C under continuous light. When cultured embryos reached the three-leaf stage, they were transplanted into pots.

For somatic chromosome counts, root tips of adult plants were fixed in alcohol:acetic acid (3:1) and stained by the Feulgen procedure. For meiotic studies, immature inflorescences with pollen mother cells (PMC) at meiotic stages were fixed and stained by the same procedure.

A silver-staining method (Fernández-Gomez et al. 1969) was used to count the number of nucleoli.

Results

A total of five embryos was obtained by pollinating *H. chilense* (4x) with *A. squarrosa* (4x), and two adult plants were established. The somatic chromosome number in the amphiploid was 2n = 4x = 28, as expected (Fig. 1). In the somatic metaphase cells examined, neither variation in chromosome number was observed, nor were abnormalities in chromosome structure found. A high frequency (ca. 90%) of the plants in the progenies of the amphiploids was euploid.

Cytological analysis of 100 PMCs of the $H^{ch}H^{ch}DD$ amphiploids revealed an average of 12.48 bivalents and 3.04 univalents per cell. The frequency of PMCs showing 14 bivalents was 15%. Other chromosome associations were 13 bivalents (36%), 12 bivalents (34%), 11 bivalents (12%), and 10 bivalents (3%). No cells with less than ten bivalents were observed. Figure 2 shows a metaphase I stage of the amphiploid with 14 bivalents. With the silver-staining procedure, a maximum of six nucleoli per nucleus was counted in the amphiploid. The general appearance of the *H. chilense* × *A. squarrosa* amphiploid is that of the *Aegilops* (Fig. 3), with some modifications that make it difficult to identify *A. squarrosa* as the *Aegilops* parent, since the amphiploid is more similar in appearance to other *Aegilops* ssp., e.g., *A. speltoides*.

Fig. 3. From *left* to *right*, spikes of *H. chilense*, amphiploid *H. chilense* × *A. squarrosa*, and *A. squarrosa*

The amphiploid is fertile and vigorous. Analysis of 26 spikes from the two plants revealed an average of 32, 69 spikelets/spike and 22.65 seeds/spike.

Discussion

It has been shown (Martín 1983) that homoeologous pairing is absent in *H. chilense* × *A. squarrosa* hybrids. The results obtained by the meiotic analysis of the $H^{ch}H^{ch}DD$ amphiploids are in agreement with that finding. No trivalents or quadrivalents were observed in PMCs. This lack of pairing between *H. chilense* and *A. squarrosa* chromosomes will be a major hurdle to the interchange of genetic information between these species. However, a high frequency of bivalent formation has been found. Such meiotic regularity in the amphiploids could be one of the reasons for the good fertility observed.

H. chilense possesses two satellited chromosome pairs: chromosomes $5H^{ch}$ and $6H^{ch}$ (Fernández 1989). The D genome of *A. squarrosa* has only one pair of satellited chromosomes: chromosome *5D* (Crosby 1957). As expected, both autotetraploids, *H. chilense* and *A. squarrosa*, showed correspondence between the maximum number of nucleoli per nucleus and the observed number of satellites (eight and four, respectively). On the other hand, nucleolar analysis of root tips from the *H. chilense* (4x) × *A. squarrosa* (4x) amphiploids did not exhibit such a correspondence. The amphiploid $H^{ch}H^{ch}DD$ showed interphase nuclei with more than four nucleoli (from one to six), with a mean value of nucleoli per nucleus of 2.3, which is intermediate to the mean values of both parent (3.1 and 1.7, respectively). This result revealed that *H. chilense* nucleolar organizing regions (NORs) are active in the amphiploid and that the additional nucleolar activity detected was due to chromosome pair *5D*. However, no secondary constriction corresponding to chromosome paris *5D* was observed in these plants.

As in the case of the *H. chilense* × *A. squarrosa* hybrid (Martín 1983), the plant morphology of the $H^{ch}H^{ch}DD$ amphiploid resembled that of the *Aegilops* parent. This is in agreement with the general plant morphology determined to date of the hybrids and amphiploids of diploid forms of *Hordeum* with either tetraploid or hexaploid *Triticum* species (Fedak 1985). The only exception, as far as we know, is the hybrid between *H. jubatum* (4x) and *T. aestivum* reported by Comeau et al. (1988), which showed a plant morphology intermediate between both parents. Finally, we found in this amphiploid characters, such as tough rachis, that were absent in both parents. This was also found by Martín (1983) in the $H^{ch}D$ hybrid. In both cases, complementation exists in the system controlling brittle rachises in *Hordeum* and *Aegilops*.

In spite of some favorable characteristics, such as the lack of pairing between the wild barley and *Aegilops* chromosomes, the presence of tough rachis, and good fertility, we do not think that this new amphiploid has value for direct use in plant breeding due to its poor biomass yield. However, as additional material of general cytogenetic interest, this new combination involving *Hordeum* and *Aegilops* genomes may also be of particular use, mainly for the production of new genomic combinations in the Triticeae.

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