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Comparisons of the hybrids *Hordeum chilense* × *H. vulgare*, *H. chilense* × *H. bulbosum*, *H. chilense* × *Secale cereale* and the amphidiploid of *H. chilense* × *H. vulgare*

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Summary. Diploid hybrids between *Hordeum chilense* and three other species, namely *H. vulgare, H. bulbosum* and *Secale cereale,* are described together with the amphidiploid of *H. chilense* × *H. vulgare.* Both the diploid hybrid and the amphidiploid of *H. chilense* × *H. vulgare* were chromosomally unstable, *H. chilense* × *H. bulbosum* was less so, while *H. chilense* × *S. cereale* was stable. Differential amphiplasty was found in all combinations. No homoeologous pairing was found in the *Hordeum* hybrids but in *H. chilense* × *S. cereale* there was chromosome pairing both within the two genomes and between the genomes.

Key words: *Hordeum* – *Secale* – Interspecific, intergeneric hybrids

Introduction

Hordeum chilense has attracted a good deal of interest in recent years among cytologists and plant breeders. It forms fertile amphiploids with both *Triticum aestivum* (Martin and Chapman 1977) and *T. turgidum* conv. *durum* (Martin and Sanchez-Monge Laguna 1982), and this second amphiploid is considered by the authors as potentially useful breeding material. Finch and Bennett (1980) found that it hybridized easily with four *Secale* species, namely *S. cereale, S. africanum, S. montanum* and *S. vavilovii,* and felt that the high success rate could be an indication of the presence of crossability gene(s) in this species.

The hybrid between a synthetic autotetraploid of *H. chilense* and diploid *H. vulgare* was reported by Sanchez-Monge Laguna and Martin (1982) but they were unable to produce viable embryos when the two diploids were crossed. Recently a hybrid between diploid *H. chilense* and diploid *H. bulbosum* was described by Padilla and Martin (1983) and here we describe another diploid hybrid of this combination together with the diploid hybrids *H. chilense* × *H. vulgare* and *H. chilense* × *S. cereale* and the amphidiploid of *H. chilense* × *H. vulgare.*

Materials and methods

Four genotypes of *H. chilense* were obtained as seed from the USDA and three *H. vulgare* cultivars ('Emir', 'Tyra' and 'Vada') were selected on the basis of differences in their ability to set seed when pollinated with *H. bulbosum* (Pickering 1979). The one genotype of *H. bulbosum* used (Cb 2929/1) was chosen because we have found it can be crossed with a greater range of *H. vulgare* cultivars than other *H. bulbosum* genotypes. All genotypes used were diploid ($2n = 2x = 14$).

Crosses were carried out under the same environmental conditions, and using crossing and embryo culture techniques similar to those described by Thomas and Pickering (1983a).

The hybrid *H. chilense* × *S. cereale* (cv. 'Petkus') was kindly supplied by Drs M. D. Bennett and R. A. Finch, Plant Breeding Institute, Cambridge, and the details of the parents have been published (Finch and Bennett 1980).

For cytological studies, root-tips were pretreated in distilled water at 1 °C for 24 h, fixed in ethanol-acetic acid (3:1) and stained by the Feulgen method. Inflorescences were taken before emergence, fixed in Carnoy's solution (6:3:1) and anthers squashed in 1% aceto-carmine. Clonal parts of all the hybrids were treated with colchicine using the technique described by Thomas and Pickering (1983a) in an attempt to double their chromosome number.

Results

H. chilense × *H. vulgare*

Seed set and embryo differentiation for each parental combination are given in Table 1. As can be seen, all

Table 1. Seed sets, rates of embryo differentiation and plant regeneration

♀	♂	Florets pollinated	Seed set (%)	Differentiated embryos cultured	Embryos cultured as a % of seeds set	Hybrid plants
a) *H. chilense* × *H. vulgare*						
P.I. 255753	'Emir'	25	80.0	4	20.0	0
	'Tyra'	28	82.1	3	13.0	0
	'Vada'	98	75.5	46	62.2	7
P.I. 255754	'Emir'	219	43.8	39	40.6	1
	'Tyra'	59	47.5	12	42.9	0
	'Vada'	106	87.7	59	63.4	11[a]
P.I. 283374	'Emir'	17	52.9	5	55.6	0
	'Vada'	97	68.0	51	77.3	6
P.I. 283375	'Emir'	62	69.4	22	51.2	0
	'Tyra'	65	43.1	6	21.4	0
	'Vada'	111	58.6	44	67.7	6
b) *H. chilense* × *H. bulbosum*						
P.I. 255754	Cb 2929/1	88	61.4	25	46.3	2
P.I. 283375	Cb 2929/1	84	83.3	55	78.6	21[a]

[a] Meiotic analyses on hybrids from these combinations

but one of the hybrid plants came from crosses involving the cultivar 'Vada'. *H. chilense* × 'Vada' crosses produced larger and better differentiated embryos than crosses involving other *H. vulgare* cultivars. Following vernalization, only nine hybrid plants flowered and all were from the combination P.I. 255754 × 'Vada' indicating possible effects of the *H. chilense* parent. The other hybrids were high tillering and remained in the vegetative state. The reciprocal cross was attempted but no seed set was obtained.

A total of 20 cells from the root-tips of three plants were chromosome counted. The mean chromosome number was 14.2; five cells had 15 chromosomes, one had 13 chromosomes and the remainder were euploid. Differential amphiplasty was found, and as the two large satellite pairs of *H. chilense* can be easily distinguished from the smaller satellites of *H. vulgare*, it was evident that only the *H. vulgare* secondary constrictions were expressed.

Of the PMCs of the hybrid analysed, only one of 50 cells had 14 chromosomes, others ranged from 7 to 16 chromosomes (Table 2). The chromosomes were mostly unpaired and chromosome fragments and degraded chromosomes were found in 38% of the cells. Achiasmate secondary associations between univalents were particularly common. These comprised end-to-end, end-to-side and side-to-side associations and a mixture of these in multiple associations of up to five chromosomes. Fold-back associations were found in 10% of cells.

Following colchicine treatment a tetraploid inflorescence was found but the PMCs were difficult to analyse. Although complete analysis was impossible, all the associations observed were bivalents together with a

Table 2. Chromosome number and chromosome pairing at metaphase I in three hybrids involving *H. chilense* (♀)

Male parent	Chromosome no.		% Cells with extra chromatin	Chromosome pairing				Univalents involved in secondary associations	
	Mean	Range		Trivalents	Ring bivalents	Rod bivalents	Xta	Mean	Range
H. vulgare	11.38	7–16	52	–	0.04	0.02	0.1	5.54	0–13[a]
H. bulbosum	13.78	13–15	0	–	0.04	–	0.08	0.68	0–6[b]
S. cereale	14	–	0.5	0.02	–	0.62	0.66	0.9	0–4[b]

[a] Including fold-back associations

[b] Predominantly end-to-end

number of univalents. The chromosome number varied between 14 and 24.

H. chilense × *H. bulbosum*

Of the two *H. chilense* lines used, P.I. 283375 was the more successful with 21 hybrids produced from that cross (Table 1). However, the hybrids from both crosses were vigorous and all flowered following vernalization. The cross was only attempted in the direction shown.

A total of forty cells from the root-tips of five plants were chromosome counted. The mean chromosome number was 13.9; only 15% of cells were aneuploid. Again differential amphiplasty was found. In this case the *H. chilense* secondary constrictions were expressed while the single *H. bulbosum* secondary constriction was not.

At meiosis there was again virtually no pairing, just two ring bivalents were scored in 50 cells. Only 28% of the PMCs were aneuploid and these had either 13 or 15 chromosomes.

Following colchicine treatment tetraploid sectors were identified at the root-tip level but no tetraploid inflorescences have been found.

H. chilense × *S. cereale*

Details of the crossability of the two species have been published by Finch and Bennett (1980). The chromosome number in the root-tips was stable and differential amphiplasty was again found. In this case only the *H. chilense* secondary constrictions were expressed. At first metaphase of meiosis most chromosomes remained unpaired though there were bivalents and also one trivalent in the 50 PMCs scored (Table 2). Because of the size difference of the two sets of chromosomes, autosyndetic and allosyndetic pairing are distinguishable. The larger homomorphic bivalents were interpreted as rye-rye bivalents, the smaller homomorphic bivalents as *chilense-chilense* bivalents and the very heteromorphic bivalents (Fig. 1) as rye-*chilense*. Of the 31 bivalents recorded in 50 PMCs, four were the large rye-rye bivalents, seven were *chilense-chilense* bivalents and the remaining 20 bivalents were between rye and *H. chilense* chromosomes. The trivalent was interpreted as being between two *H. chilense* chromosomes and one of rye, with the rye chromosome in the middle.

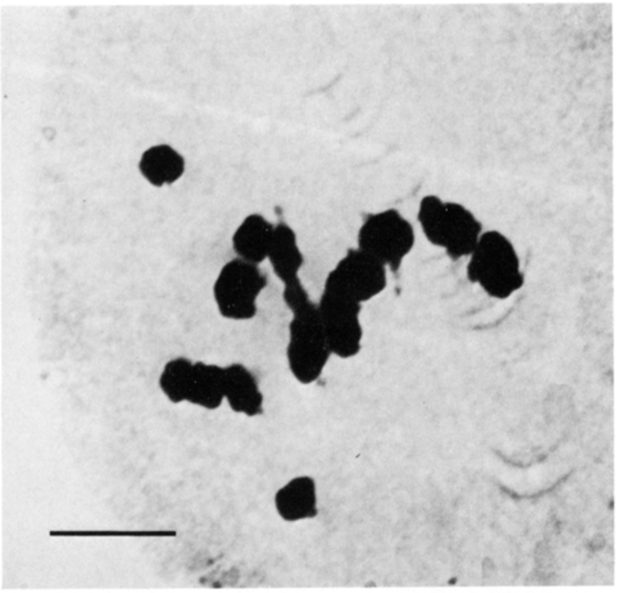

Fig. 1. *H. chilense* × *S. cereale*. One *chilense*-rye II + six *chilense* I + six rye I. Bar represents 10 μm

Although tetraploid root-tip cells have been found in this hybrid following colchicine treatment, no tetraploid inflorescences have yet been found.

Discussion

'Vada' was the most successful of the *H. vulgare* parents when crossed with *H. chilense*, though it is known to be highly incompatible with *H. bulbosum* (Pickering and Hayes 1976). A seed set of 87.7% was obtained when 'Vada' was used to pollinate P.I. 255754 and in all 'Vada' crosses the embryos were bigger and better differentiated. In the *H. chilense* × *H. bulbosum* cross the genotype of *H. chilense* used had a significant effect on seed set and the eventual production of hybrid plants (Table 1).

Differential amphiplasty has been widely reported in *Hordeum* interspecific hybrids and Subrahmanyam and Azad (1978) proposed a hierarchy of dominance in suppressing the secondary constrictions in the order procerum or *parodii* > *vulgare* > *bulbosum*. As the *H. chilense* satellite chromosomes are easily distinguished from the two pairs in *H. vulgare* and from the single pair in *H. bulbosum* we were able to identify with certainty the satellite chromosomes in the hybrids. From our observations *H. chilense* fits into the hierarchical order between *H. vulgare* and *H. bulbosum*, its nucleolar organizers being suppressed by *H. vulgare* and it in turn suppressing the nucleolar organizers of *H. bulbosum*.

In the *H. chilense* × *H. bulbosum* hybrid 28% of PMCs were aneuploid while in *H. chilense* × *H. vulgare* aneusomy at metaphase I of meiosis was significantly greater ($P < 0.001$). Variation in the chromosome number in the PMCs of *H. chilense* × *H. vulgare* is a reflection of the unstable nature of these hybrids. Moreover, in the *H. chilense* × *H. vulgare* hybrid 52% of PMCs contained degraded or fragmented chromosomes (Table 2) and this is a phenomenon closely associated with chromosome elimination in *H. vulgare* × *H. bulbosum* hybrids (Thomas and Pickering 1983 a, b). However, there was no evidence of complete chromosome elimination in embryonic tissue in any of the hybrid combinations since no haploids were found.

The two ring bivalents observed were in 15 chromosome cells and were probably associations between the homologous pair arising in these 2n + 1 PMCs. There was therefore no evidence of interspecific chromosome pairing in the *H. chilense* × *H. bulbosum* hybrid. In *H. chilense* × *H. vulgare* a total of three bivalents were found; two were in 13 chromosome cells and the third in a 15 chromosome cell. But these bivalents could also have been homologues as, with this degree of aneusomy there may have been homologous pairs present even in 13 chromosome cells.

The *H. chilense* × *S. cereale* analysed by Finch and Bennett (1980) had no chromosome pairing in contrast to the one reported here which had a mean of 0.66 chiasmata per cell. They were sister plants but some rye genotypes are known to have genes which promote pairing in wheat and rye hybrids (Dvořák 1977). It is possible that the rye parent used was heterozygous for at least some of these genes and that they have the same effect in combination with *H. chilense* as with wheat.

There was some intragenomic pairing amongst the *H. chilense* chromosomes and among rye chromosomes. However, the rye-rye pairing falls within the range published for *S. cereale* haploids (Levan 1942). The interspecific chromosome pairing, especially when including the trivalent, is the highest recorded between *Secale* and *Hordeum* chromosomes.

The low or non-existent pairing between *H. chilense* and the other two *Hordeum* species in disappointing in terms of achieving the transfer of desirable genes such as its resistance to the root-knot nematode (*Meloidogyne naasi*) (Cook and York 1981), but interesting phylogenetically as there appears to be greater homology between *H. chilense* and rye than between it and the other two *Hordeum* species. The tetraploid inflorescence analysed from the *H. chilense* × *H. vulgare* hybrid was unstable and unless more chromosomally stable combinations of these species are found the amphiploid will be of little economic use. On the evidence presented here it is difficult to visualize an amphiploid arising from this combination as promising as the *H. chilense* × *T. turgidum* amphiploid produced by Martin and Sanchez-Monge Laguna (1982).

References

Cook R, York PA (1981) Genetics of resistance to *Heterodera avenae* and *Meloidogyne naasi.* In: Proc 4th Int Barley Genet Symp, Edinburgh 1981, pp 418–424

Dvořák J (1977) Effect of rye on homoeologous chromosome pairing in wheat × rye hybrids. Can J Genet Cytol 19:549–556

Finch RA, Bennett MD (1980) Mitotic and meiotic chromosome behaviour in new hybrids of *Hordeum* with *Triticum* and *Secale.* Heredity 44:201–209

Levan A (1942) Studies on the meiotic mechanism of haploid rye. Hereditas 28:177–211

Martin A, Chapman V (1977) A hybrid between *Hordeum chilense* and *Triticum aestivum.* Cereal Res Commun 5: 365–368

Martin A, Sanchez-Monge Laguna E (1982) Cytology and morphology of the amphiploid *Hordeum chilense* × *Triticum turgidum* conv. *durum.* Euphytica 31:261–267

Padilla JA, Martin A (1983) Morphology and cytology of *Hordeum chilense* × *H. bulbosum* hybrids. Theor Appl Genet 65:353–355

Pickering RA (1979) Further investigations on partial incompatibility in crosses between *Hordeum vulgare* L. and *H. bulbosum* L. In: Proc Conf Broadening Genet Base Crops. Pudoc, Wageningen, pp 319–325

Pickering RA, Hayes JD (1976) Partial incompatibility in crosses between *Hordeum vulgare* L. and *H. bulbosum* L. Euphytica 25:671–678

Sanchez-Monge Laguna E, Martin A (1982) *Hordeum chilense* × *Hordeum vulgare* hybrids. Z Pflanzenzücht 89: 115–120

Subrahmanyam NC, Azad AA (1978) Nucleoli and ribosomal RNA cistron numbers in *Hordeum* species and interspecific hybrids exhibiting suppression of secondary constriction. Chromosoma 69:265–273

Thomas HM, Pickering RA (1983 a) Chromosome elimination in *Hordeum vulgare* × *H. bulbosum* hybrids. 1. Comparisons of stable and unstable amphidiploids. Theor Appl Genet 66:135–140

Thomas HM, Pickering RA (1983 b) Chromosome elimination in *Hordeum vulgare* × *H. bulbosum* hybrids. 2. Chromosome behaviour in secondary hybrids. Theor Appl Genet 66:141–146