Theor Appl Genet (1985) 71:437–442

The influence of parental genotype on the chromosome behaviour of *Hordeum vulgare* × *H. bulbosum* diploid hybrids

H. M. Thomas and R. A. Pickering *
Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Wales SY23 3EB, UK

Accepted June 25, 1985
Communicated by R. Riley

Summary. The PMCs of 74 diploid hybrids involving ten *H. vulgare* varieties and three *H. bulbosum* lines were analysed at metaphase I and chromosome number and chiasma frequency recorded. There were differences between parental combinations and between plants within those combinations for both chromosome and chiasma number. It is suggested that these characters are controlled by both parents and that differences between plants within families reflect the heterozygosity of the *H. bulbosum* parents. Chromosomally stable, high pairing lines have been identified for use in a backcrossing programme to introduce *H. bulbosum* characters to the *H. vulgare* germplasm.

Key words: *Hordeum vulgare* × *H. bulbosum* hybrids – Chromosome behaviour

Introduction

The cross between *Hordeum vulgare* and *H. bulbosum* is now widely used to produce doubled haploid barley plants in barley breeding programmes. The chromosomes of *H. bulbosum* are usually selectively eliminated during embryogenesis and the haploid *H. vulgare* embryos cultured on an artificial medium. The resultant haploid plants are then colchicine treated to produce homozygous diploid *H. vulgare* plants. However, in a proportion of embryos the *H. bulbosum* chromosomes are retained and hybrid plants are produced.

The production of hybrid versus haploid progeny from this cross has variously been reported to be influenced by environment (Pickering 1984) and by either the *H. vulgare* or *H. bulbosum* parent or by both (reviewed in Table 1, Pickering 1983). However, as the main interest in the cross has been high volume production of barley haploids, emphasis has been given to examining chromosome elimination in embryos and less attention has been paid to the chromosome behaviour of the hybrid plants.

Hordeum bulbosum possesses useful characters, such as disease resistance, winter hardiness and allogamy, which would be of interest to the plant breeder if introduced to the *H. vulgare* germplasm. Although Kasha and Sadasivaiah (1971) and Lange (1971a) found an encouragingly high level of allosyndetic chromosome pairing in their diploid hybrids, these were not chromosomally stable, and PMCs had variable chromosome numbers. The opportunities for obtaining recombinant chromosomes were thus greatly reduced.

Retention of the *H. bulbosum* chromosomes in hybrids is clearly vital to the transfer of *bulbosum* characters into the *H. vulgare* germplasm, as is a high level of chiasmate pairing between the chromosomes of the two species. To investigate the feasibility of selecting parental genotypes that will give stable, high pairing hybrids we examined the chromosome number and chromosome pairing in the PMCs of a range of *H. vulgare* × *H. bulbosum* diploid hybrids. Ten *H. vulgare* cultivars and three lines of *H. bulbosum* were used as parents.

Materials and methods

Experiment 1

The effect of the *H. vulgare* genotype on the chromosome behaviour of the hybrids was examined by crossing ten cultivars of *H. vulgare* with *H. bulbosum* line Cb 2929/1. Where possible four hybrid plants were analysed from each cross and two inflorescences from each plant.

* Present address: Crop Research Division, Lincoln, DSIR, Private Bag, Christchurch, New Zealand

Experiment 2

To examine the effect of the *H. bulbosum* parental genotype on the chromosome behaviour of the hybrids, four of the ten varieties were also crossed with *H. bulbosum* line Cb 2920/4.

Experiment 3

To examine further the effect of the *H. bulbosum* parent and provide greater precision, a second series of crosses was made between *H. vulgare* cv. 'Emir' and three *H. bulbosum* lines 'S1', Cb 2929/1 and Cb 2920/4. Only two 'Emir' plants were used, each pollinated by the three *H. bulbosum* lines, and to minimise any environmental or physiological effects, opposite sides of each spike were pollinated with different *H. bulbosum* genotypes. Five, eleven and six hybrids respectively were analysed from each of the three crosses 'Emir' × 'S1', Cb 2929/1 and Cb 2920/4. Again, two inflorescences were analysed from each hybrid plant.

Crosses for experiments 1 and 2 were carried out in January 1983 in a heated glasshouse (15 °C ± 3 °C day, 10 °C ± 3 °C night) with 16 h daylength (natural daylight supplemented with high pressure mercury fluorescent bulbs). The crosses for experiment 3 were made in June 1983 with 25 °C ± 5 °C (day) and 15 °C ± 3 °C (night) with 16 h daylength. Crossing and embryo culture techniques used were similar to those described by Thomas and Pickering (1983a). All haploid plants, which were identified morphologically, were discarded at an early stage of plant development and only hybrid plants were retained.

For meiotic studies inflorescences were taken before emergence, fixed in Carnoy (6:3:1) solution and anthers squashed in 1% aceto-carmine. Twenty PMCs were analysed from each inflorescence and chromosome number and chiasma frequency recorded.

Where possible cells at late metaphase I were selected for analysis as at this stage it is easier to distinguish between genuine chiasmate bivalents and pseudo-bivalents i.e. univalents attached by end-to-end association. Where there may have been doubt we erred on the side of caution and therefore chiasma frequency recorded may sometimes be lower than in reality.

Results

1 Chromosome number

There was a strong negative correlation between inflorescence means and variances for chromosome number per PMC. Attempts at removing this correlation through transformation failed because the within-inflorescence chromosome numbers did not follow a normal distribution and most of the inflorescences with means of 14 had variances of 0. Valid statistical analysis were not therefore possible and genotypic differences can best be illustrated by the distribution of plant means in Table 1.

The *H. vulgare* varieties tested do not clearly fall into groups of those producing chromosomally stable hybrids and those that do not, as most varieties produced at least some stable hybrids. However, there were far greater differences between the hybrid plants in some crosses than others. For instance in the cross 'Zephyr' × Cb 2929/1 one plant had only one aneuploid cell (13 chromosomes) in 40 cells examined, all the others having 14 chromosomes, while another plant from the same cross had a mean chromosome number of 11.1 and a range of 8–16; only one cell had the euploid chromosome number. In contrast, in thirteen hybrids involving 'Vada' and 'Domen' with Cb 2929/1 and Cb 2920/4 of the 480 cells scored only 33 were aneuploid cells and these were mostly 13 and 15 chromosome cells.

Differences between hybrid plants involving the same parental genotypes are clearly seen in Experiment 3 particularly between the eleven plants from the cross 'Emir' × Cb 2929/1 (Table 1). There are no obvious differences between the three *H. bulbosum* genotypes.

2 Chromosome pairing

A reduction in chromosome number would obviously reduce the number of potential pairing sites and chiasma frequency was highly significantly correlated with chromosome number ($P < 0.001$). In order to examine differences in chiasma frequencies independently of chromosome stability, chiasmata were therefore related to the number of 'pairable' chromosomes i.e. chromosomes per cell with homologous or homoeologous partners. Thus, assuming that only *H. bulbosum* chromosomes are eliminated from diploid hybrid cells, a 13 chromosome cell would have six pairs of homoeologous chromosomes and a 12 chromosome cell five pairs of homoeologous chromosomes, i.e. equal to the number of *bulbosum* chromosomes present. In cells with more than 14 chromosomes, whatever the origin of the extra chromosome, it would clearly have a homologous partner present and all chromosomes in these cells would be capable of pairing. The number of chromosomes present considered as capable of pairing therefore was as follows:

Chromosomes per cell:	17	16	15	14	13	12	11	10	9	8	7
Pairable chromosomes:	17	16	15	14	12	10	8	6	4	2	0

In Experiment 1 a hierarchical analysis of variance on chiasmata per pairable chromosome revealed highly significant differences between inflorescences, between plants and between varieties ($P < 0.001$ in each case). The variety means for chiasmata per cell and chiasmata per pairable chromosome per cell are presented in Table 2 and grouped using Duncan's Multiple Range Test.

In Experiment 2 using a two-way analysis of variance on chiasmata/pairable chromosome of hybrids between *H. vulgare* cultivars 'Domen', 'Vada', 'Lion' and 'Emir' and the two *H. bulbosum* lines Cb 2929/1 and Cb 2920/4, significant differences were found between the *H. vulgare* parents ($P < 0.01$) and between plants within hybrid combinations ($P < 0.001$). There

Table 1. Distribution of plant mean chromosome numbers in *H. vulgare* × *H. bulbosum* diploid hybrids (varieties and *H. bulbosum* lines in order of mean chromosome number of cross)

Parents		No. of plants with mean chromosome number																		Total
H. vulgare ♀	*H. bulbosum* ♂	>14	14	13.9	13.8	13.7	13.6	13.5	13.4	13.3	13.2	13.1	13.0	12.9	12.8	12.5	12.2	11.5	11.1	
Expt. 1																				
'Vada'	Cb 2929/1		2																	2
'Domen'	Cb 2929/1		4																	4
'Lion'	Cb 2929/1		1	1	1															3
'Emir'	Cb 2929/1		1	1		1	1													4
'Edelmut'	Cb 2929/1		2				1			1[a]										4
'Midas'	Cb 2929/1		2		1									1[a]						4
'Golden Promise'	Cb 2929/1		1				1		1[a]		1[a]									4
'Zephyr'	Cb 2929/1		1	1	1														1[b]	4
'Sabarlis'	Cb 2929/1			1						1			1[a]				1[a]			4
'Br 76R23-1'	Cb 2929/1			1[a]	1[b]						1[b]					1[b]				4
Expt. 2																				
'Domen'	Cb 2920/4	1	2																	3
'Vada'	Cb 2920/4	1	3																	4
'Lion'	Cb 2920/4		2	1	1															4
'Emir'	Cb 2929/4		1		1	1				1[a]										4
Expt. 3																				
'Emir'	'S1'		1	1				1	1	1										5
'Emir'	Cb 2929/1		4		3					1		1		1[b]				1[b]		11
'Emir'	Cb 2920/4		1	2			1					1			1[b]					6

[a] 25%–49% PMCs containing degraded chromosomes
[b] 50% or more PMCs containing degraded chromosomes

Table 2. Chromosome pairing in the hybrids from 10 varieties of *H. vulgare* crossed with *H. bulbosum* Cb 2929/1, means connected by the same line are not significantly different at the 5% level according to Duncan's Multiple Range Test. Each mean represents up to 4 hybrid plants (see right hand column Table 1)

Variety	'Edel-mut'	'Emir'	'Vada'	'Zephyr'	'Domen'	'Lion'	'Sabar-lis'	'Golden Promise'	'Midas'	'Br 76R23-1'
Mean Xta/cell	5.244	4.912	4.712	3.882	3.244	2.775	2.162	1.906	1.281	0.800
Mean Xta/pairable chromosome	0.387	0.365	0.321	0.298	0.233	0.205	0.174	0.143	0.094	0.063

Table 3. Individual plant means for chiasmata per pairable chromosome of crosses between *H. vulgare* cv. 'Emir' (♀) and three *H. bulbosum* lines (♂). Means connected by the same line are not significantly different at the 5% level according to Duncan's Multiple Range Test

H. bulbosum	Plant means of Xta/pairable chromosome										
Cb 2929/1	0.447	0.348	0.324	0.317	0.316	0.299	0.243	0.229	0.216	0.214	0.171
Cb 2920/4	0.477	0.439	0.375	0.306	0.201	0.139					
'S1'	0.302	0.137	0.130	0.126	0.116						

was no significant difference between the two *H. bulbosum* lines and no interaction was found between the two sets of parents.

Examining chiasmata per pairable chromosome in Experiment 3, highly significant differences were found between inflorescences and between plants ($P < 0.001$) and the *H. bulbosum* lines also differed ($P < 0.05$). This was due to a significant difference between 'S1' and the other two *H. bulbosum* lines. The hybrids involving Cb 2929/1 and Cb 2920/4 did not differ from each other, and therefore the results of Experiments 2 and 3 are not contradictory. Differences between plants are continuous and they do not fall clearly into high and low pairing groups (Table 3).

3 Chiasma frequencies of chromosomally stable plants

A total of eleven plants were found where all 20 PMCs in each inflorescence had 14 chromosomes and the chiasma frequencies of these were examined separately.

From Experiment 1 there were five such plants one from each of the *H. vulgare* cultivars 'Edelmut', 'Emir', 'Golden Promise', 'Midas' and 'Vada' × *H. bulbosum* Cb 2929/1 and they differed significantly ($P < 0.001$). From Experiment 2 there were four stable plants from the crosses between cultivars 'Domen', 'Emir' and 'Lion' with Cb 2920/4. Two of the hybrids involved Lion and their chiasma frequencies did not differ from each other but there were highly significant differences between these two and the 'Emir' and 'Domen' hybrids ($P < 0.001$). In Experiment 3 there were two chromosomally stable plants, both 'Emir' × Cb 2929/1 and their chiasma frequencies differed significantly ($P < 0.01$).

With this small number of plants we cannot determine whether differences between plants are due to the different *H. vulgare* parents or to the heterozygosity of *H. bulbosum*. The two plants from Experiment 3 suggests the latter.

Discussion

In crosses between *H. vulgare* and *H. bulbosum* at the diploid level the progeny most commonly consist of *H. vulgare* haploids, and usually only a small proportion of the progeny are interspecific hybrid plants. In contrast, when diploid *H. vulgare* (2n=2x=14) is crossed with tetraploid *H. bulbosum* (2n=4x=28) the progeny are invariably triploid with the genomic constitution 1 *vulgare* : 2 *bulbosum* (Lange 1971b; Kasha and Sadasiviah 1971). Kasha et al. (1970) proposed a hypothesis that the genomic balance in the hybrids determined their chromosome stability and Ho and Kasha (1975) described genes in *H. bulbosum* as being able to neutralize the effects of the *H. vulgare* elimination genes, or confer some form of protection on the *H. bulbosum* chromosomes. Usually twice the number of *H. bulbosum* to *H. vulgare* genomes is required to produce chromosomally stable combinations.

The proportion of hybrid to haploid progeny in crosses between diploid parents varies with the *H. vulgare* cultivars used (Jensen 1976; Pickering 1983), indicating that the elimination genes are less effective in some varieties than in others. Although the elimination mechanism is less effective beyond a critical stage in embryo development (Pickering 1984) it seems likely that the same elimination genes in combination with the neutralizing genes from *H. bulbosum* determines the degree of chromosome instability in the hybrid plants produced. With some varieties described here, e.g. 'Domen' or 'Vada', where the elimination genes seem less effective, all the hybrid progeny are chromosomally stable irrespective of the *H. bulbosum* gamete involved. However, with those varieties more efficient at chromosome elimination, e.g. 'Zephyr' or 'Sabarlis' we may be seeing the effect of the neutralizing genes from the *H. bulbosum*. As an outbreeder *H. bulbosum* is probably heterozygous for these neutralizing genes. Therefore, in crosses with cultivars with the strongly eliminating genes (e.g. 'Zephyr' or 'Sabarlis') *H. bulbosum* will produce stable and unstable hybrids.

The different levels of effectiveness of the elimination genes in *H. vulgare* and the neutralizing genes in *H. bulbosum* is probably illustrated at its extreme level in VVB triploid hybrids. The rare occurrence of these hybrids with two *H. vulgare* genomes and one *H. bulbosum* genome (Pohler and Szigat 1982; Thomas and Pickering 1983b) must be a combination of *H. vulgare* genomes that are less effective at eliminating *H. bulbosum* chromosomes and a *H. bulbosum* genome that is particularly effective in neutralizing the effect of the *H. vulgare* eliminating genes.

In common with earlier reports of *Hordeum* interspecific hybrids (Thomas and Pickering 1983a, b; Thomas and Pickering 1985) a high incidence of degraded or fragmented chromosomes in the PMCs was always associated with chromosome elimination in that inflorescence. As can be seen in Table 1, in chromosomally unstable plants more than 25% and in some instances more than 50% of PMCs contained degraded chromosomes.

The chromosome pairing data from Experiment 1 (Table 2) shows that the variety of *H. vulgare* used has an influence on chiasma frequency independently of chromosome number. Differences between plants within varieties over and above differences between inflorescences, suggests that *H. bulbosum* may also influence chiasma frequency and that Cb 2929/1 is heterozygous for genes influencing this character. This is also clearly shown in Experiments 2 and 3 with Cb 2920/4 and 'S1' where there were again differences between plants within crosses. In Experiment 2 there was no significant difference between the two *H. bulbosum* parents Cb 2929/1 and Cb 2920/4 but in Experiment 3 although these two *H. bulbosum* parents again did not differ, pairing in the hybrids of the third *H. bulbosum* parent 'S1' was significantly lower ($P<0.05$). It appears therefore that the *H. bulbosum* genotype can influence chiasma frequency.

Chromosome pairing is genotypically controlled independently of chromosome number as is further demonstrated by the highly significant differences between the chiasma frequencies of the hybrids that were completely chromosomally stable.

The chances of obtaining recombinant chromosomes is dependent on the total number of chiasmata, which is influenced also by the number of chromosomes present. Szigat and Pohler (1982) have shown it is possible to introduce *H. bulbosum* characters into the *H. vulgare* germplasm by exploiting chromosomally stable tetraploid combinations of the two species. From our data we believe it is possible to select *H. vulgare* parents that will achieve similar results using stable, high pairing diploid hybrids in a backcrossing programme.

Acknowledgement. We are grateful to Mr. P. W. Morgan for his valuable technical assistance.

References

Ho KM, Kasha KJ (1975) Genetic control of chromosome elimination during haploid formation in barley. Genetics 81:263–275

Jensen CJ (1976) Barley monoploids and doubled monoploids: techniques and experience. In: Barley genetics, vol III. Proc 3rd Int Barley Genet Symp, pp 316–345

Kasha KJ, Kao KN, Reinbergs E (1970) Genetic control over chromosome stability in hybrids from interspecific *Hordeum* crosses. Genetics Suppl 64:33

Kasha KJ, Sadasivaiah RS (1971) Genome relationships between *H. vulgare* L. and *H. bulbosum* L. Chromosoma 35: 264–287

Lange W (1971a) Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. 2. Elimination of chromosomes in hybrid tissues. Euphytica 20:181–194

Lange W (1971b) Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. 1. Production, morphology and meiosis of hybrids, haploids and dihaploids. Euphytica 20:14–29

Pickering RA (1983) The influence of genotype on doubled haploid barley production. Euphytica 32:863–876

Pickering RA (1984) The influence of genotype and environment on chromosome elimination in crosses between *Hordeum vulgare* L. × *Hordeum bulbosum* L. Plant Sci Lett 34:153–164

Pohler W, Szigat G (1982) Versuche zur rekombinativen Genübertragung von der Wildgerste *Hordeum bulbosum* auf die Kulturgerste *H. vulgare*. 1. Mitt. Die Rückkreuzung VV × BBVV. Arch Züchtungsforsch Berlin 12:87–100

Szigat G, Pohler W (1982) *Hordeum bulbosum* × *H. vulgare* hybrids and their backcrosses with cultivated barley. Cereal Res Commun 10:73–78

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

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

Thomas HM, Pickering RA (1985) Comparisons of the hybrids *Hordeum chilense* × *H. vulgare*, *H. chilense* × *H. bulbosum*, *H. chilense* × *Secale cereale* and the amphidiploid of *H. chilense* × *H. vulgare*. Theor Appl Genet 69:519–522