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Monosomic and double monosomic substitutions of *Hordeum bulbosum* L. chromosomes into *H. vulgare* L.

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Summary. One of the aims of the interspecific crossing programme between *Hordeum vulgare* L. and *H. bulbosum* L. has been to introgress desirable genes into barley from the wild species. However, despite their close taxonomic relationship there have been few reports of achieving this objective using amphidiploid hybrids. In order to broaden the range of available hybrids, partially fertile triploids between *H. vulgare* (2n = 2x = 14) and *H. bulbosum* (2n = 4x = 28) were developed and crossed with *H. vulgare* as female parent. From 580 progeny which were screened, eight putative single monosomic chromosome substitution lines and one double monosomic substitution were identified by cytological analysis. These involved the substitution of *H. vulgare* chromosome 1 (two lines), 6 (four lines), 6L (one line), 7 (one line) and 1 + 4 (one line) by their respective *H. bulbosum* homoeologues. The *H. bulbosum* chromosome was frequently eliminated during plant development, but it was observed regularly in pollen mother cells of two lines. However, pairing between the *H. bulbosum* chromosome and its *H. vulgare* homoeologue was low. Several of the lines were more resistant than their *H. vulgare* parents to powdery mildew (*Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal), net blotch (*Drechslera teres* Sacc.) and scald (*Rhynchosporium secalis* (Oudem.) Davis). Apart from their use in studying genome relationships, their value to plant breeders will depend on the ease of inducing translocations between the parental chromosomes.

Key words: *Hordeum vulgare* L. – *Hordeum bulbosum* L. – Interspecific hybrids – Chromosome substitutions – C-banding

Introduction

Hordeum bulbosum L. is considered to be the most closely related species to barley (*H. vulgare* L.) on the basis of electrophoretic (Jørgensen 1986), cytological (Bothmer et al. 1983) and molecular (Gupta et al. 1989) evidence. It has frequently been crossed with barley to introgress desirable genes into the cultivated species and to produce doubled haploids for use in barley breeding programmes. After *H. vulgare* (2n = 2x = 14) has been pollinated with *H. bulbosum* (2n = 2x = 14) fertilization takes place, but the *H. bulbosum* chromosomes are usually eliminated during embryo development. This results in the formation of haploid *H. vulgare* embryos that are rescued to a nutrient medium to regenerate haploid plants (Kasha and Kao 1970). Doubled haploids are produced after colchicine treatment. By the manipulation of genotype and environment (Pickering 1984) chromosome retention can be encouraged and sterile diploid hybrids obtained. The fertility of these diploid hybrids can be restored by doubling their chromosome number with colchicine, but despite the presence of homoeologous chromosome pairing at metaphase – I (MI) of meiosis recombinants have rarely been obtained. Possible reasons for this lack of success in transferring genes from *H. bulbosum* into barley are: chromosome instability in hybrids; low intergenomic chromosome pairing (Pickering 1991 a) and crossing-over (Pickering 1991 b); low viability and competitive ability of recombinant male gametes. Infertility is also frequently encountered in relatively stable triploid hybrids between *H. vulgare* (2n = 14) and *H. bulbosum* (2n = 28) (Kasha and Sadasivaiah 1971; Lange 1971), which has precluded their use in breeding programmes. These triploids (henceforth denoted 'VBB') might have been considered useful for gene introgression because multivalent formation regularly occurs between *H. vulgare* and *H. bulbosum* chromosomes at MI (Xu and Snape 1988). Thus, the development of partially fertile 'VBBs' (Pickering 1988) held some promise for overcoming some of the barriers to gene transfer mentioned above. A crossing programme was initiated between *H. vulgare* and several 'VBBs' to investigate the frequencies of recombinants in the progeny.

Table 1. Mitotic chromosome number of *H. vulgare-H. bulbosum* substitution lines

Code	Pedigree	Chromosome number		Number of satellites		Awn/spike length rato
		Root tips	Ovaries	Large	Small	
894J11	Zephyr × (Emir × Cb 2920/4/Colch)	13/14+1 telocentric	13+1 telocentric	2	2	2.1
915E1	1024-23 × (Emir × Cb 2929/1/Colch)	14	13	2	2	1.7–2.1
916J2	Triumph × (Emir × 27/4)	14	–	1	2	2.3–2.6
917C2	907-12 × (Emir × Cb 2929/1/Colch)	14	13/14	2	1	2.5
919A3	Emir × (Golden Promise × Cb 2920/4/Colch)	14	12/13	2	2	2.9
919G2	Goldmarker × (Golden Promise × Cb 2920/4/Colch)	14	14	1	2	1.0–1.2
919Q4	Golden Promise × (Golden Promise × Cb 2920/4/Colch)	14	14	1	2	1.0–1.3
920C15	Golden Promise × (Golden Promise × Cb 2920/4/Colch)	14	13	2	2	2.3–2.7
926K2	907-12 × (Emir × Crete 6/2)	14	13	1	2	2.8–3.1
Emir		14	14	2	2	2.1
Golden Promise		14	14	2	2	1.4
H. bulbosum		14	14	2	0	0.3

– Denotes missing data (see text)

Materials and methods

Partially fertile triploid 'VBB' hybrids (Pickering 1988) were grown in a heated glasshouse (Pickering 1989). Several hybrids with high pairing between *H. vulgare* and *H. bulbosum* chromosomes were retained and used as pollen donors on two breeder's lines and eight *H. vulgare* cultivars (Table 1 + 'Fleet', 'Mata', 'Vada'). Gibberellic acid (GA_3) at 75 mg l^{-1} was sprayed onto florets 1 day after pollination, and seeds were either left *in situ* to develop to maturity (if endosperms were solid) or embryos were rescued from seeds with watery endosperms onto nutrient medium (Pickering and Morgan 1985).

Somatic chromosome counts (Pickering and Morgan 1985) were carried out on root tips of random samples of morphologically normal progeny and on root tips and ovaries of all aberrant plants. Mitotic C-banding preparations were also made from the anomalous plants and their parents. For this purpose, seeds of *H. vulgare* were germinated at 24 °C in darkness for 2 days, whereas shoots of the aberrant plants and *H. bulbosum* genotypes were grown in Jiffy 7 peat blocks in the glasshouse or in a controlled environment room maintained at 19°/16 °C (day/night), 18 h daylength. Root tips were pretreated in iced water (2 °C) for 14.5–16 h and fixed in 3:1 ethanol:acetic acid prior to storage in a refrigerator overnight and in a deep freeze for up to 2 weeks. Two techniques were attempted for chromosome banding: (1) Linde-Laursen (1975); (2) Kakeda et al. (1991) modified by (1) using a mixture of 4% cellulase R10 (Yakult) + 2% macerozyme R10 (Yakult) in buffer on the root tips for 45 min to 1 h prior to slide preparation, and (2) staining in 1–2.5% Giemsa improved R66 in 1/15 *M* phosphate buffer pH 6.8 for 2–6 h. C-banding (Kakeda et al. 1991) was also carried out on PMCs of 920C15, but the staining time was reduced to 1–2 h. Chromosome pairing was assessed at MI by fixing spikes in Carnoy's solution (6:3:1) for a minimum of 24 h prior to squashing anthers in 1% acetocarmine.

All plants with 14 chromosomes having cytological and morphological anomalies were screened pathologically for powdery mildew (*Erysiphe graminis* DC.f.sp. *hordei* Em. Marchal), net blotch (*Drechslera teres* Sacc.) and scald (*Rhynchosporium secalis* (Oudem.) Davis) using similar procedures to those described by Pickering (1991a). Selfed seeds from fertile plants were grown on in the glasshouse and examined at all stages of plant growth.

Results

After 2320 florets of *H. vulgare* had been pollinated with a range of 'VBBs', 580 seeds were obtained, 336 of which had solid endosperm and 244 had watery endosperm. Twenty *H. vulgare* haploids were regenerated from the latter after embryo culture, and six triploid or aneuploid plants were germinated from seed. One hundred and twenty-four plants resembling diploid *H. vulgare* were also raised from seeds with solid endosperm. A random sample of these plants contained 14 chromosomes in root-tip cells.

Eight progeny, all with 14 chromosomes in the root-tip cells, were germinated from seeds with solid endosperm, but they grew less vigorously than *H. vulgare* (Table 1). One of these plants (916J2) and one of the *H. bulbosum* parental genotypes (Crete 6/2) died prior to the completion of cytological and pathological analyses. A ninth plant (894J11) was regenerated by means of embryo culture from a seed with watery endosperm and contained

a telocentric chromosome together with 13 or 14 chromosomes in the root-tip cells. Spikes from these nine plants resembled *H. vulgare* but were frequently distorted with irregular floret distribution along the rachis. They possessed no obvious features of *H. bulbosum*, and awn/spike length ratios were closer to the *H. vulgare* parents than *H. bulbosum* (Table 1). Only one plant (919A3) exhibited any vegetative characteristics of *H. bulbosum* (densely pubescent leaf sheaths and sparsely pubescent leaves). Selfed seed set ranged from 0% to 10%, but seed inviability prevented progeny from being obtained from some of the 'fertile' lines.

Cytology

Root tip cells

Anomalous plants with 14 chromosomes. Five out of eight plants analysed had fewer than the 4 satellited (SAT) chromosomes expected in *H. vulgare* (Table 1). Four plants had 2 small and 1 large SAT-chromosome. 917C2 had 1 small and 2 large SAT-chromosomes, and a submetacentric chromosome was observed (Fig. 1 a) that was similar to the one found in *H. bulbosum* (Fig. 1 b). No other obvious differences in morphology were detected between chromosomes amongst the remaining plants.

849Jl1. Root tips were aneusomatic and cells contained a telocentric chromosome with large satellite and either 13 or 14 chromosomes. The telocentric appeared to be that of the short arm of *H. vulgare* chromosome number 6.

C-banding analysis

The technique of Kakeda et al. (1991) produced less distortion of chromosomes than the method of Linde-Laursen (1975), although bands were sometimes not as distinct, especially those associated with the nucleolar constrictions of *H. bulbosum*.

Parental genotypes. The C-banding patterns of *H. vulgare* (e.g. Fig. 1 d–f) were quite different from those of *H. bulbosum* and corresponded with what had been reported previously, (Linde-Laursen 1975; Kakeda et al. 1991). *H. bulbosum* chromosomes were distinguishable from *H. vulgare* as one or two pairs of the former were often unbanded depending on preparation. The remaining chromosomes were weakly banded at the centromere, but one pair was strongly banded at two points adjacent to the centromere (Fig. 1 c). The one pair of SAT-chromosomes possessed a band at the nucleolar constriction (see also Xu and Snape 1988; Linde-Laursen et al. 1990), but the intensity varied considerably with genotype and preparation.

Anomalous plants with 14 chromosomes. In the seven plants analysed, 13 *H. vulgare* chromosomes were recorded in six of them and 12 *H. vulgare* chromosomes in 919A3. The missing *H. vulgare* chromosomes were identified as number 1 (915E1, 920C15; Fig. 1 d); number 6 (919G2, 919Q4, 926K2 Fig. 1 e); number 7 (917C2); and numbers 1 and 4 (919A3; Fig. 1 f). All the lines possessed 1, or in the case of 919A3, 2 chromosomes similar to those of *H. bulbosum*.

In lines 919G2, 919Q4 and 926K2, which were monosomic for *H. vulgare* chromosome 6, the putative replacement for the missing chromosome was the *H. bulbosum* SAT-chromosome that has a band associated with the nucleolar constriction. This was particularly noticeable in 926K2, which had Crete 6/2 as the donor (Fig. 1 e). The satellite itself was not visible because of the absence of the nucleolar constriction, a phenomenon previously observed in *H. vulgare* × *H. bulbosum* hybrids (Kasha and Sadasivaiah 1971). In 917C2 the submetacentric chromosome possessed a centromeric band (not illustrated) and provided further evidence that it originated from *H. bulbosum*.

The proposed *H. bulbosum* replacement for *H. vulgare* chromosome 1 in 915E1 and 920C15 (Fig. 1 d) could not be positively associated with a particular *H. bulbosum* chromosome. However, it was dissimilar from the satellited and submetacentric chromosomes and the *H. bulbosum* chromosome with two relatively strong centrometric bands.

Double monosomic substitution line 919A3 contained 2 unidentified *H. bulbosum* chromosomes (Fig. 1 f), one of which was usually unbanded and the other possessed as single centromeric band.

894Jl1. The presence of the satellited *H. bulbosum* chromosome (Fig. 1 g) probably compensated for the absence of the long arm of *H. vulgare* chromosome 6. The long arm of 1 *H. vulgare* number 7 chromosome was reduced in length, whereas the short arm of 1 number 5 chromosome was increased in length (Fig. 1 g).

Meiotic pairing analysis (Table 2)

Anomalous plants with 14 chromosomes. Fewer than 14 chromosomes in PMCs were commonly observed in all lines except 919G2 and 919Q4, and confirmatory data were obtained from ovary-wall mitotic chromosome counts (Table 1). 919G2 and 919Q4 were therefore the most stable lines but the proportions of PMCs containing six bivalents and two univalents or one pseudobivalent were 82.7% (919G2) and 77.8% (919Q4; Fig. 2 a). Thus, there was no regular pairing between the *H. vulgare* and *H. bulbosum* monosomes. In the lines involving single chromosome substitutions, mean bivalent formation ranged from 5.94 (920C15) to 6.27 (916J2) and mean univalent formation, from 0.86 (916J2) to 1.72 (919G2). Pseudobivalents, or secondary associations, were observed in 919Q4 and 926K2 (Table 2). In 919A3 (double mono-

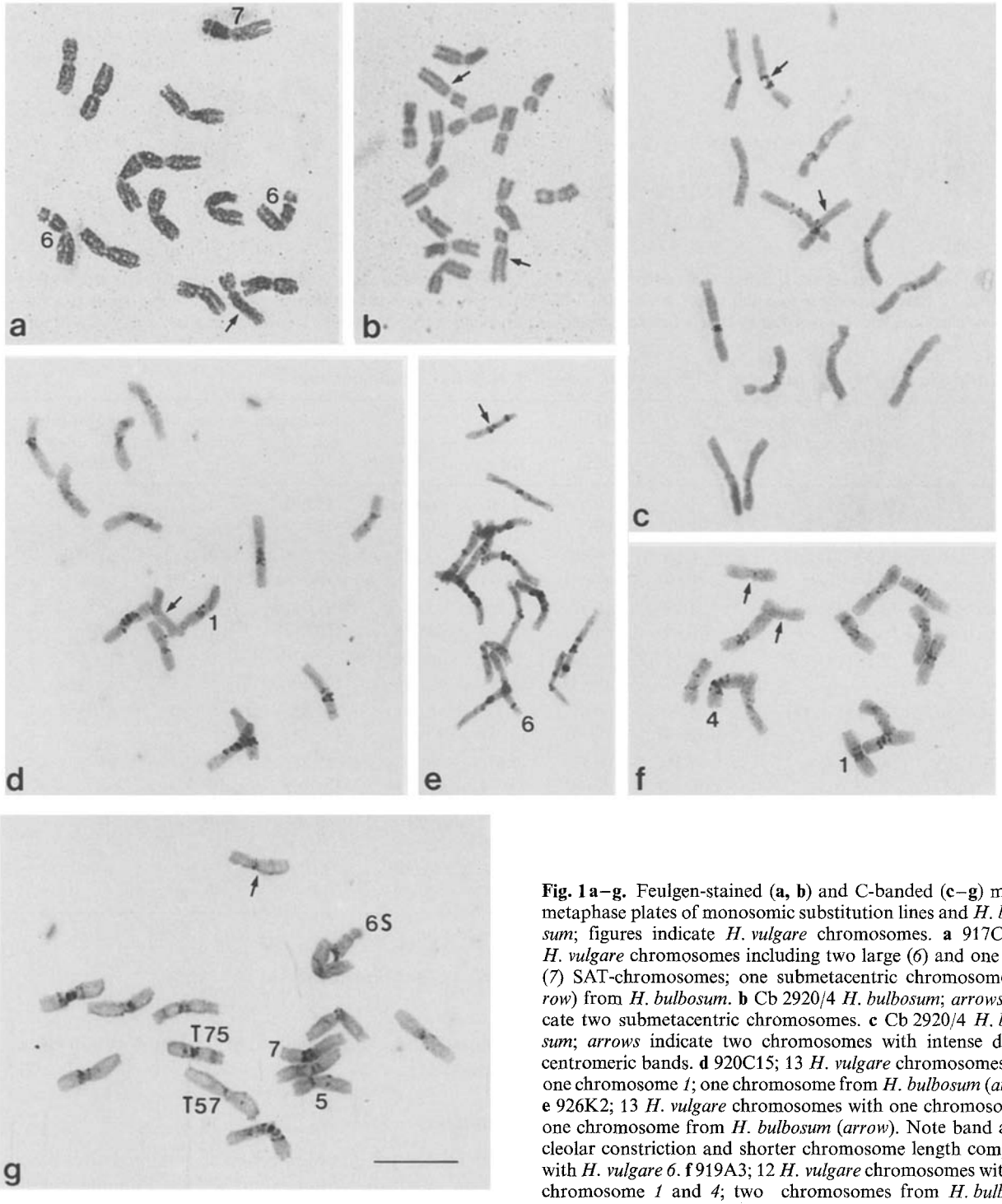

Fig. 1 a–g. Feulgen-stained (**a, b**) and C-banded (**c–g**) mitotic metaphase plates of monosomic substitution lines and *H. bulbosum*; figures indicate *H. vulgare* chromosomes. **a** 917C2; 13 *H. vulgare* chromosomes including two large (*6*) and one small (*7*) SAT-chromosomes; one submetacentric chromosome (*arrow*) from *H. bulbosum*. **b** Cb 2920/4 *H. bulbosum*; *arrows* indicate two submetacentric chromosomes. **c** Cb 2920/4 *H. bulbosum*; *arrows* indicate two chromosomes with intense double centromeric bands. **d** 920C15; 13 *H. vulgare* chromosomes with one chromosome *1*; one chromosome from *H. bulbosum* (*arrow*). **e** 926K2; 13 *H. vulgare* chromosomes with one chromosome *6*; one chromosome from *H. bulbosum* (*arrow*). Note band at nucleolar constriction and shorter chromosome length compared with *H. vulgare 6*. **f** 919A3; 12 *H. vulgare* chromosomes with one chromosome *1* and *4*; two chromosomes from *H. bulbosum* (*arrows*). **g** 894J11; 13 chromosomes + one telocentric (*6S*) from *H. vulgare*. Compare arm lengths of normal chromosomes *5* and *7* with the interchanged chromosomes *5* (*T57*) and *7* (*T75*). *Arrow* indicates *H. bulbosum* chromosome with weak bands at the centromere and nucleolar constriction. *Bar* = 10 μm

somic substitution for *H. vulgare* chromosomes 1 and 4), chromosome pairing was further reduced with a mean univalent and bivalent formation of 3.91 and 4.23, respectively. Fragments, probably the *H. bulbosum* chromosome undergoing elimination (Thomas and Pickering 1985), were observed in 10.5% of the PMCs in 916J2. A chromosome was eliminated during meiosis in 920C15 since 10% of cells at diakinesis contained 14 chromosomes (Fig. 2b) while all of the PMCs at MI had 13 chromosomes.

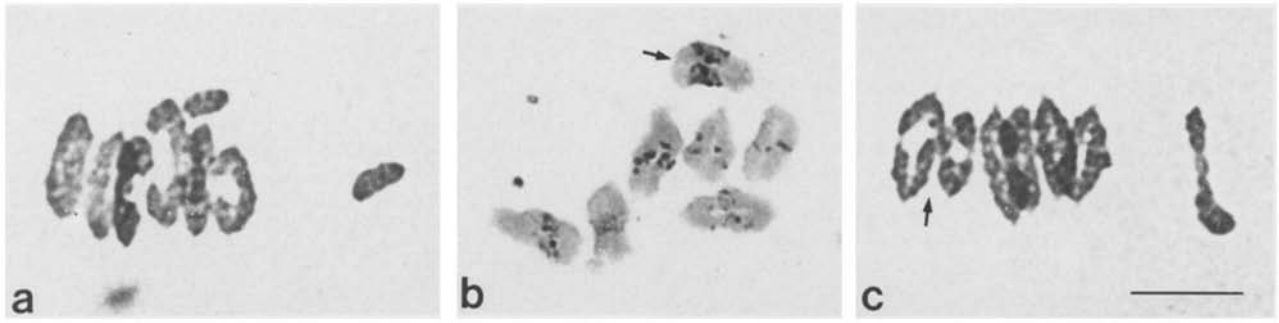

Fig. 2a–c. Meiotic MI (**a, c**) and diakinesis (**b**) of three monosomic substitution lines; **a, c** acetocarmine stained; **b** C-banded. **a** 919Q4; 6 bivalents + 2 univalents (note unequal sizes). **b** 920C15; 7 bivalents. Heavily banded bivalent is probably homologous pairing of *H. vulgare* chromosome 4 (*arrow*). **c** 894J11; 1 heteromorphic rod bivalent, 4 ring bivalents, 1 quadrivalent (*arrow*). *Bar* = 10 μm

Table 2. Meiotic chromosome pairing at MI in seven *H. vulgare-H. bulbosum* substitution lines

Code	*n*	Mean chromosome number per PMC	I	II			Chiasmata per cell	Pseudo-bivalents	Substituted *H. vulgare* chromosome
				Rod	Ring	Total			
915E1	29	13.00 (13)	1 (1)	0 (0)	6.00 (6)	6.00 (6)	12.00 (12)	0	1[a]
916J2	57	13.39 (13–14)	0.86 (0–2)	0.46 (0–3)	5.81 (4–7)	6.27 (5–7)	12.08 (10–14)	0	6[b]
917C2	8	13.28 (13–14)	1.28 (0–3)	0.17 (0–1)	5.78 (5–6)	5.95 (5–7)	11.73 (10–13)	0	7[a,b]
919A3	22	12.36 (11–13)	3.91 (2–7)	1.59 (0–3)	2.64 (1–5)	4.23 (3–5)	6.87 (4–10)	0	1+4[a]
919G2	76	13.99 (13–14)	1.72 (0–4)	0.41 (0–3)	5.72 (3–7)	6.13 (5–7)	11.85 (9–14)	0	6[a,b]
919Q4	45	14.00 (14)	1.16 (0–2)	0.53 (0–3)	5.67 (3–7)	6.20 (5–7)	11.87 (9–14)	0.22 (0–1)	6[a,b]
920C15	53	13.00 (13)	1.11 (1–3)	0.17 (1–2)	5.77 (4–6)	5.94 (5–6)	11.72 (9–12)	0	1[a]
926K2	17	13.53 (13–14)	0.94 (0–2)	0.29 (0–2)	5.71 (5–6)	6.00 (5–6)	11.71 (10–12)	0.29 (0–1)	6[a,b]

n, Number of PMCs analysed; values in parentheses are the range; see text for 894J11 data
[a] Substituted *H. vulgare* chromosome identified by C-banding
[b] Substituted *H. vulgare* chromosome identified by number and size of satellited chromosomes

894J11. In 50 PMCs from one spike, six ring bivalents and one heteromorphic rod were formed, presumably *H. vulgare* chromosome 6 pairing with 6S. In a total of 55 PMCs from two other spikes one quadrivalent and five bivalents (four rings + 1 heteromorphic rod) were the commonest configurations observed (Fig. 2c). The reason for the inter-spike differences remains unclear unless the regenerated plant was initially chimaerical. The presence of a quadrivalent indicated that 894J11 was heterozygous for a translocation possibly involving chromosomes 5 and 7 (see C-banding data). To confirm this, crosses were made between progeny of 894J11 homozygous for the suspected interchange and five translocation stocks. Seven bivalents were observed at MI in PMCs of the F_1-894J11 × T5-7b, indicative that chromosomes 5 and 7 were involved in the translocation (Burnham 1962).

Pathology

Inconsistent results were generally obtained after inoculation with three pathogens. 917C2 was immune to scald, but the responses of all the other lines ranged from moderately resistant to susceptible. Immunity of 915E1 and 919G2 to powdery mildew was conferred by the female *H. vulgare* parent.

Progeny from the lines

Small quantities of selfed seed were obtained and germinated from 894J11, 916J2, 919G2 and 926K2 (all in-

volved the substitution of *H. vulgare* chromosome 6). Apart from the semi-sterility associated with translocation heterozygosity in 894Jl1, only 2 plants (both from 916J2) out of 63 examined from the four lines showed any anomalies. Both had 14 chromosomes in root-tip cells, and one appeared relatively normal vegetatively although seed set was low (<20%). The plant died prior to further cytological analysis. The second plant was narrow leaved and bushy during vegetative development with a seed set of only approximately 10%. At MI seven bivalents were regularly formed of which 38% were rods in comparison, with less than 20% normally observed in the PMCs of a range of *H. vulgare* cultivars and F_1 hybrids grown in similar conditions.

Discussion

Substitution lines have been produced in wheat by crossing monosomic series with appropriate donor species addition lines (Riley and Kimber 1966; Sears 1968). The missing wheat chromosome is substituted by the donor homoeologue, which compensates to a greater or lesser degree for the deficiency. This technique has not been applied to barley since suitable addition lines are not available and also because its diploid constitution does not tolerate the loss of a chromosome for the production of a monosomic series. For example, an *H. vulgare* plant with 13 chromosomes plus one telocentric was obtained by Finch (1983) but died after setting only two seeds. Despite the reported production of disomic additions and substitutions of *H. bulbosum* chromosomes into wheat (Wang et al. 1987), crosses between *H. vulgare* and *H. bulbosum* usually result in the genome of the latter species being totally eliminated, leading to haploid *H. vulgare* plants, or retained with the formation of interspecific hybrids. Several monosomic substitution lines have now been produced by pollinating *H. vulgare* plants with partially fertile 'VBBs'. These must have arisen following post-meiotic or post-fertilization elimination of all but 1 or 2 chromosomes from *H. bulbosum*, which compensated for the deficiencies in the *H. vulgare* genome.

Although it should now be possible to produce a complete monosomic substitution series that would be useful in investigating genome homologies, their use for gene introgression may be limited for several reasons.

1) No disomic substitutions have been obtained following selfing of those monosomic substitutions retaining 14 chromosomes in PMCs.

2) Spike degeneration and infertility was frequently observed, but doubling the chromosome number may improve fertility.

3) Although most lines retained all 14 chromosomes in root-tip cells, some aneusomaty was observed in 894Jl1, and in several lines the *H. bulbosum* chromosome(s) was eliminated during plant development. Chromosome retention may be promoted by growing plants at temperatures lower than 17.5 °C (Pickering 1984).

4) Low or irregular homoeologous chromosome pairing at MI was present in PMCs containing 14 chromosomes and may have been influenced by parental genotype (Thomas and Pickering 1985). The *H. vulgare* genome itself also has an adverse effect on allosyndetic pairing in other interspecific hybrids (Bothmer et al. 1983; Gupta and Fedak 1985) and in triploid hybrids involving *H. bulbosum* (Pickering 1991 a).

Of the nine lines which survived long enough for preliminary cytological analysis, five involved either the substitution of *H. vulgare* chromosome 6 (916J2, 919G2, 919Q4 and 926K2) or 6L (894Jl1) by a satellited *H. bulbosum* chromosome, which confirms previous reports of its homoeology with *H. vulgare* chromosome 6 (Xu and Snape 1988; Linde-Laursen et al. 1990). These substitutions also comprised the most stable, fertile and vigorous plants, suggesting that the satellited *H. bulbosum* chromosome compensates more adequately for its missing *H. vulgare* homoeologue than the other *H. bulbosum* chromosomes.

H. vulgare chromosome 7 was substituted in 917C2 by the submetacentric chromosome of *H. bulbosum*, and confirms their homoeology (Kasha and Sadasivaiah 1971). *H. vulgare* chromosomes 1 and 4 were substituted in 919A3, which possessed pubescent leaf sheaths. It seems likely that genes controlling this character are located on the *H. bulbosum* homoeologue of *H. vulgare* chromosome 4 as 915E1 and 920C15 (*H. vulgare* chromosome 1 substitutions) were both glabrous.

Further cytological anomalies were observed in 894Jl1, namely a telocentric (*H. vulgare* chromosome 6S) and a translocation between chromosomes 5 and 7. The telocentric probably arose by univalent misdivision and the translocation by spontaneous breakage and reunion of two *H. vulgare* chromosomes during meiosis in the 'VBB'. It may therefore be possible to obtain similar exchanges between *H. vulgare* and *H. bulbosum* chromosomes, but their frequency would probably be very low.

Apart from the scald resistance of 917C2 associated with the *H. bulbosum* homoeologue of *H. vulgare* chromosome 7, there were inconsistencies in the reaction of the remaining lines to all three pathogens. Possible explanations are: (1) incomplete expression of *H. bulbosum* resistance genes in a *H. vulgare* background; (2) *H. vulgare* × *H. bulbosum* genotype interaction affecting response; (3) heterozygosity of resistance genes in the *H. bulbosum* parents.

All but two of the selfed progeny from four substitution lines resembled barley, and there has been no sexual transmission of the *H. bulbosum* homoeologue because it is usually eliminated before meiosis in PMCs. In 919G2, although the *H. bulbosum* chromosome was retained throughout the life of the plant, no conclusions were drawn about its sexual transmission as the number of screened selfed progeny (six) was too small. The 2 anomalous 14 chromosome plants from 916J2 were both semi-sterile, and in 1 of them, the number of rod bivalents observed was greater than normal. This may be due to an insertion or transfer of a small segment of *H. bulbosum* chromosome, perhaps terminally, into the *H. vulgare* genome.

In conclusion, addition and substitution lines have been of great value in the Triticeae for investigating chromosome homoeologies and species relationships. Their agronomic value is usually limited until translocations are effected to transfer desirable characters such as disease resistance. In barley, progress in this direction will be slow because its diploid constitution will not tolerate chromosome engineering to the same extent as polyploids such as wheat. However, a complete chromosome substitution series between *H. vulgare* and *H. bulbosum* should be relatively easy to obtain using techniques described herein and will prove useful in determining the chromosome homoeologies of these two species.

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