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R. A. Pickering · G. M. Timmerman · M. G. Cromey
G. Melz

# Characterisation of progeny from backcrosses of triploid hybrids between *Hordeum vulgare* L. (2x) and *H. bulbosum* L. (4x) to *H. vulgare*

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**Abstract** Interspecific hybridisations between *Hordeum vulgare* L. (cultivated barley) and *H. bulbosum* L. (bulbous barley grass) have been carried out to transfer desirable traits, such as disease resistance, from the wild species into barley. In this paper we report the results of an extensive backcrossing programme of triploid hybrids (*H. vulgare* 2x × *H. bulbosum* 4x) to two cultivars of *H. vulgare*. Progenies were characterised cytologically and by restriction fragment length polymorphism analysis and comprised (1) haploid and diploid *H. vulgare* plants, (2) hybrids and aneuploids, (3) single and double monosomic substitutions of *H. bulbosum* chromosomes into *H. vulgare* and (4) chromosomal rearrangements and recombinants. Five out of the seven possible single monosomic chromosome substitutions have now been identified amongst backcross progeny and will be valuable for directed gene introgression and genome homoeology studies. The presence amongst progeny of 1 plant with an *H. vulgare–H. bulbosum* translocated chromosome and one recombinant indicates the value of fertile triploid hybrids for interspecific gene introgression.

**Key words** *Hordeum vulgare* · *Hordeum bulbosum* · Fertile triploids · Gene introgression · Chromosome substitutions

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R. A. Pickering (✉) · G. M. Timmerman · M. G. Cromey
New Zealand Institute for Crop and Food Research Ltd, Private Bag 4704, Christchurch, New Zealand

G. Melz[1]
Bundesanstalt für Züchtungsforschung, Institutsplatz, D-18190, Groß Lüsewitz, Germany

*Present address:*
[1] Ringstraße 10, D-18276, Gülzow, Germany

## Introduction

Alien gene transfer in barley (*Hordeum vulgare* L.) has been difficult to achieve because of the scarcity of closely related species and because its diploid chromosome constitution precludes extensive chromosome manipulation. Furthermore, even in hybrids with a close relative, *H. bulbosum* L., genetic recombination appears to be a rare event (Pickering 1991a). Nevertheless, since *H. bulbosum* possesses desirable characteristics such as pest and disease resistance (Jones and Pickering 1978; Kindler and Springer 1991; Xu and Snape 1989), this species offers some scope for transferring resistance genes that may not be readily available within the existing barley gene pool.

Progress was made towards achieving gene introgression following the production of male-fertile triploid hybrids between *H. vulgare* 2x and *H. bulbosum* 4x (derived from colchicine-treated diploid genotypes; Pickering 1988). These fertile hybrids were subsequently backcrossed to barley, and progeny mostly comprised normal barley diploids and haploids. However, aneuploids with varying numbers of chromosomes from each species, monosomic *H. vulgare–H. bulbosum* chromosome substitutions and an *H. vulgare* translocation were also found (Pickering 1992). Initially, most plants were characterised cytologically by C-banding, but the identity of several substitution plants was subsequently confirmed by isozyme analysis (Thiele et al. 1992) and by restriction fragment length polymorphism (RFLP) analysis (Timmerman et al. 1993). Selfed seed was obtained from some of the substitution plants, but almost all progeny resembled normal barley. However, one putative *H. vulgare–H. bulbosum* recombinant was obtained that appeared morphologically abnormal but was indistinguishable from its barley parent in cytological or isozyme tests (Pickering 1992; Thiele et al. 1992). Previously, a recombinant between barley and *H. bulbosum* with mildew resistance transferred from the wild species was reported (Xu and Kasha 1992).

We have continued to use fertile triploid hybrids in backcrosses to barley, and in this paper we describe the development and characterisation of two more *H. vulgare* –*H. bulbosum* recombinants and 19 single monosomic substitutions. The latter plants, together with those previously identified (Pickering 1992), now comprise five out of the seven possible substitutions of single *H. vulgare* chromosomes by their *H. bulbosum* homoeologues.

## Materials and methods

### Hybrid production and crossing

Fertile triploid hybrids (genome formula VBB) were produced from crosses between *H. vulgare* diploid cvs 'Emir' ('E') and 'Golden Promise' ('GP') and three *H. bulbosum* tetraploid genotypes obtained from colchicine-treated diploid clones, Cb2920/4, Cb2929/1 and HB2032 (Pickering 1988). The VBBs were backcrossed as pollinators to doubled haploid plants of 'E' and 'GP' in a heated glasshouse (22° ± 3 °C day/13 ± 2 °C night; 16-h daylength provided by natural daylight supplemented with sodium and mercury fluorescent lamps). Seeds possessed solid or watery endosperms, and embryos were either rescued onto nutrient medium (Thomas and Pickering 1983) or left to develop in situ within solid seeds and then germinated on moist filter paper. Seedlings were subsequently transferred to potting compost in the glasshouse. Plants were examined frequently throughout their development, and those with anomalous morphology or sterility were vegetatively propagated.

### Cytology

Somatic chromosome counts were undertaken on root-tip preparations from anomalous plants and a sample of morphologically normal *H. vulgare* plants using standard Feulgen staining procedures. Most of those plants with fewer than 16 chromosomes and several others with more than 16 chromosomes were also analysed with C-banding methods to identify individual chromosomes (Kakeda et al. 1991) using the traditional system for numbering *H. vulgare* chromosomes. Meiotic analyses were performed on anomalous 14 chromosome plants (Pickering 1992).

### RFLP analysis

A morphologically anomalous plant, 30X2, which was cytologically indistinguishable from barley, was examined using DNA hybridisation to determine whether *H. bulbosum* DNA had been incorporated into the *H. vulgare* genome. Total plant DNA isolations and RFLP analyses were carried out as described previously (Timmerman et al. 1993). The DNA probes used were two rye repetitive DNA sequences derived from plasmids pSC119.1 and pSC119.2 (McIntyre et al. 1990). Insert fragments were obtained by polymerase chain reaction amplification using primers with the pUC/M13 universal forward and reverse primer sequences. After the removal of unincorporated dNTPs and primers, probe DNA was [$^{32}$P]-labelled using the random priming method (Feinberg and Vogelstein 1983).

### Plant pathology

All parental genotypes of *H. vulgare* and *H. bulbosum*, triploid hybrids, *H. vulgare*–*H. bulbosum* chromosome substitutions and recombinants were screened for resistance to net blotch (*Drechslera teres* Sacc.), powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal) and scald (*Rhynchosporium secalis* (Oudem.) Davis) (Pickering 1991b). Briefly, reaction types (RT) of 0–4 (powdery mildew and scald) and 0–5 (net blotch) were used, with a high score indicating susceptibility.

## Results

### Hybridisations

Pollen from the VBB hybrids was used for hybridising 27 128 emasculated florets of 'GP' and 'E'. Overall, seed sets were 1465 (5.40%) and 980 (3.61%) for seeds with 'solid' and 'watery' endosperms, respectively. The female parent did not appear to influence seed set ('E' crosses = 9.86%; 'GP' crosses = 8.32%), but there were large differences attributable to the pollinator (ranging from 3.5% to 40.6%).

### Characterisation of progeny

Plant regeneration from embryos cultured from 103 'watery' seeds was very low (5.8%) compared with that from 'solid' seeds (26.5% after embryo culture from 102 seeds and 37.1% after germination of 434 ripe seeds). Only three haploid, two 14-chromosome hybrids (7 *H. vulgare* + 7 *H. bulbosum* chromosomes) and 1 single monosomic *H. vulgare*–*H. bulbosum* chromosome substitution plant (which subsequently died) were produced from 'watery' seeds. Details of the plants regenerated from 316 and 220 'solid' seeds of 'E' and 'GP' crosses, respectively, are presented in Table 1. Most of the plants resembled normal diploid *H. vulgare* (65.4% of total plants) with fewer numbers of haploid *H. vulgare* plants (2.1%). Sixty-one anomalous plants (54 from 'E' × VBB; 7 from 'GP' × VBB) comprising 32.4% of the total plant number were classified into the following groups:

1) Aneuploid *H. vulgare*; 2 plants. Code 34L5; 13 + 1 acrocentric *H. vulgare* chromosome number 1 (Fig. 1a). The plant was poorly productive and few spikes developed. Anthers were indehiscent. Code 49M7; 13 *H. vulgare* chromosomes (monosomic for chromosome 5). The plant was greatly stunted and died prematurely.

2) Hybrids and aneuploids comprising chromosomes from *H. vulgare* and *H. bulbosum*; 33 plants. The chromosome numbers of these plants ranged from 14 to 21. Several of the aneuploids possessed varying numbers of *H. bulbosum* chromosomes, but they were also monosomic for *H. vulgare* chromosomes [no. 1, 2 plants; no. 2, 1 plant; no. 3, 1 plant (which also had 1 normal and 1 acrocentric no. 1 chromosome), numbers 1 + 4, 1 plant; numbers 1 + 3, 1 plant).

3a) Single monosomic substitutions of *H. vulgare* chromosomes by an *H. bulbosum* chromosome; 21 plants. The following *H. vulgare* chromosomes were identified in the substitutions: chromosome 1 (14 plants), chromosome 2 (1 plant), chromosome 4 (2 plants) and chromosome 6 (4 plants).

3b) Double monosomic substitutions of *H. vulgare* chromosomes 1 and 4 by 2 *H. bulbosum* chromosomes; 3 plants.

Meiotic analyses performed on group 3 plants gave similar results to those reported previously (Pickering 1992).

**Table 1** Details of 188 plants obtained from 'solid' seeds derived from *H. vulgare* cvs 'Emir' ('E'-316 seeds) and 'Golden Promise' ('GP'-220 seeds) pollinated with triploid *H. vulgare* (2x) × *H. bulbosum* (4x) hybrids (*acro* Acrocentric chromosome, *mono* monosomic chromosome, *T* translocation)

| Progeny[a] | *H. vulgare* parent | Number (%) of plants | Code number | Number and identity of chromosomes | |
|---|---|---|---|---|---|
| | | | | *H. vulgare* | *H. bulbosum* |
| Haploid | E | 3 (1.6%) | | 7 | |
| | GP | 1 (0.5%) | | 7 | |
| Diploid | E | 69 (36.7%) | | 14 | |
| | GP | 54 (28.7%) | | 14 | |
| Group 1 | E | 1 } (1.1%) | 34L5 | 13 + *acro* 1 | |
| | E | 1 } | 49M7 | 13 (*mono* 5) | |
| Group 2 | E | 30 (16.0%) | | 14–21 } mixture of *H. vulgare* and | |
| | GP | 3 (1.6%) | | 18–21 } *H. bulbosum* chromosomes | |
| Group 3a | E | 19 (10.1%) | | 13 | 1 |
| | GP | 2 (1.1%) | | 13 | 1 |
| Group 3b | E | 2 (1.1%) | | 12 (*mono* 1 + 4) | 2 |
| | GP | 1 (0.5%) | | 12 (*mono* 1 + 4) | 2 |
| Group 4 | E | 1 (0.5%) | 16R4 | 12 (*mono* 1) + T4 – *Hb* | 2–3 |
| | GP | 1 (0.5%) | 30X2 | See text | |

[a] See text for description of groups

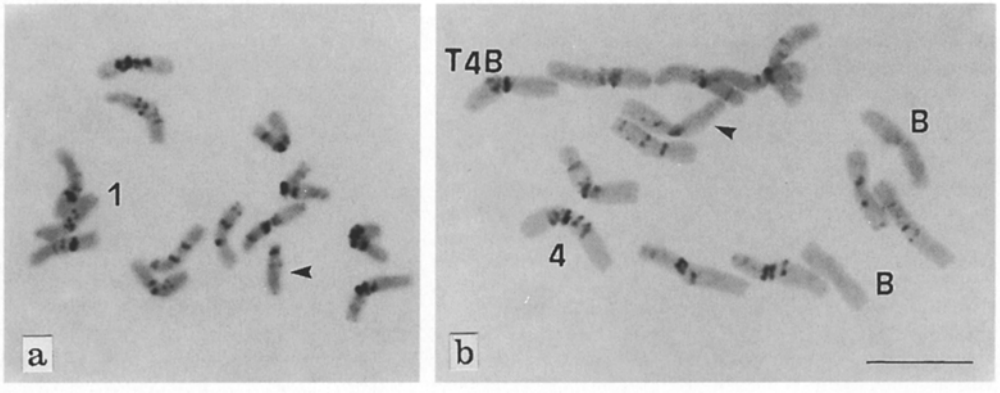


**Fig. 1a, b** C-banded meta-phase preparations from root tip cells of anomalous plants derived from backcrosses of VBB hybrids to *H. vulgare*. **a** 34L5; 13 *H. vulgare* chromosomes including normal chromosome 1 (*1*) + acrocentric chromosome 1 (*arrow*). **b** 16R4; 12 *H. vulgare* chromosomes including monosome number 1 (*arrow*) + 2 *H. bulbosum* chromosomes (*B*) + translocated *H. vulgare–H. bulbosum* chromosome (*T4B*). Normal *H. vulgare* chromosome 4 is labelled (*4*). *Bar*: 10 μm

4) *H. vulgare–H. bulbosum* recombinants; 2 plants. 16R4 was monosomic for *H. vulgare* chromosome number 1, with a rearrangement between *H. vulgare* chromosome 4 and an unknown *H. bulbosum* chromosome (Fig. 1b). There were also 2 (occasionally 3) *H. bulbosum* chromosomes present. Code 30X2 was indistinguishable from *H. vulgare* on its C-banding pattern and formed seven bivalents at metaphase I, but it possessed pubescent leaf sheaths, a characteristic of *H. bulbosum* but not of 'E' or 'GP'. The presence of *H. bulbosum* DNA was confirmed by probing genomic Southern blots with rye repeat sequence probes pSC119.1 and pSC119.2 (Fig. 2). These probes hybridise strongly to *H. bulbosum* DNA sequences but only weakly (this paper) or not at all to *H. vulgare* (Xu et al. 1990). Probe pSC119.1 produced a weak signal when hybridised with 30X2 DNA, however the single faint band, which is not present in the 'GP' background, comigrates with a band from Cb 2920/4 (Fig. 2). In situ hybridisations have shown that pSC119.1 labels *H. bulbosum* centromeres and some interstitial regions (Xu et al. 1990). In contrast, probe pSC119.2 produced a strong pattern when hybridised with DNA from 30X2, indicating the presence of DNA from *H. bulbosum* parent Cb 2920/4. Xu et al. (1990) found that pSC119.2 homologous sequences are found predominantly in *H. bulbosum* telomeric regions, but not all telomeres are labelled.

### Morphology of the substitutions and recombinants

There was little consistency in plant characteristics within each series of substitution plants. However, among group 3 plants pubescent leaf sheaths were only observed when *H. vulgare* chromosomes 1 and 4 were substituted. Even within these two series, some plants were glabrous. Plants with chromosome 1 and 6 substitutions tended to be more normal in appearance and of higher fertility than other monosomic substitutions.

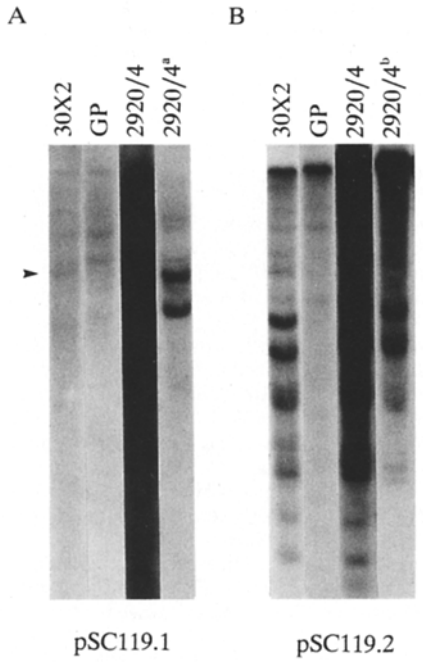


**Fig. 2A, B** DNA Hybridisation analysis of *H. vulgare–H. bulbosum* recombinant 30X2 using rye repetitive sequence probes pSC119.1 and pSC119.2. Total plant DNA was digested with *Bam*H1, and Southern blots were prepared. The DNA samples loaded are indicated at the *top* of the figure. So that the *H. bulbosum* DNA banding patterns could be visualized and compared with the 30X2 banding pattern, autoradiograms were exposed for different times. **A** For probe pSC119.1, lanes labelled 30X2, 'GP' and 2920/4 were exposed for 4 days at −80 °C, and lane 2920/4[a] was exposed for four h at −80 °C. **B** For probe pSC119.2, lanes labelled 30X2, 'GP' and 2920/4 were exposed for 4 days at 20 °C, while lane 2920/4[b] was exposed for 1.5 h at −80 °C. The *arrow* (▶) indicates the faint band labelled in 30X2 by probe pSC119.1 that comigrates with a *H. bulbosum* 2920/4 band

The two recombinants (16R4 and 30X2) also had pubescent leaf sheaths. 16R4 was poorly productive, and few normal spikes developed. Although anthers were dehiscent, no selfed seeds were obtained. Spikes of 30X2 were later to emerge than those of 'GP', but fertility was normal. Selfed progeny segregated 132 pubescent: 41 glabrous (not significantly different from a 3:1 ratio; $\chi^2 = 0.094$), indicating that pubescence was governed by a single dominant gene that had been transferred from *H. bulbosum*.

### Pathology of the substitutions and recombinants

*H. bulbosum* parents were immune (RT 0) to scald and powdery mildew and highly resistant to net blotch (RT 1), whereas both *H. vulgare* cultivars were susceptible. *H. bulbosum* resistance to powdery mildew and scald was expressed in the VBB hybrids, but RTs of 1 (spot) to 4 (netting) were observed in VBBs inoculated with net blotch. Among the substitution plants (groups 3a and 3b), only 1 plant involving a chromosome 2 substitution was immune to powdery mildew. There was no consistency in the RTs to scald or net blotch, and none of the plants was as resistant as *H. bulbosum*. Of the two *H. vulgare – H. bulbosum* recombinants, 16R4 was susceptible to powdery mildew and scald but showed an intermediate response (RT 3) to net blotch. 30X2 was fully susceptible to all three pathogens.

## Discussion

### Seed setting

After backcrossing several VBBs to *H. vulgare*, mean seed sets of 9% were obtained, considerably less than the 25% recorded previously (Pickering 1992). These inconsistencies may be attributable to genotype differences between pollinators and/or changes in personnel during the investigation. The proportions of 'solid' and 'watery' seeds were very similar to those reported earlier (Pickering 1992; Xu and Kasha 1992).

### Plant regeneration

Haploid *H. vulgare* plants comprised 2% of progeny obtained and arose from the fusion of an *H. vulgare* egg with a male gamete containing 7 *H. bulbosum* chromosomes. Diploid *H. vulgare* plants were more frequently obtained (65%) and resulted from fertilisation by a male gamete having 7 *H. vulgare* chromosomes with or without *H. bulbosum* chromosomes. In both cases, *H. bulbosum* chromosomes would have been eliminated during embryonic development (Kasha and Kao 1970).

Regarding the anomalous plants, there was a marked genotypic influence on their formation, since very few (7 out of 61 plants) were derived from 'GP' crosses. Amongst these anomalous plants, 2 *H. vulgare* aneuploids were obtained (group 1). In 34L5, the *H. vulgare* complement derived from the VBB comprised 6 normal chromosomes and an acrocentric number 1 chromosome formed through univalent breakage at meiosis and a loss of most of the short arm. The plant survived to maturity despite this deletion, but development was poor. However, the loss of a complete chromosome in 49M7 was not tolerated, since the plant failed to survive to maturity. Both these plants probably arose after the fusion of an *H. vulgare* egg with a male gamete comprising fewer than 7 *H. vulgare* chromosomes plus 1 or more *H. bulbosum* chromosomes. The latter would have been eliminated after fertilisation.

Hybrids and aneuploids combining chromosomes from each species (group 2) were the most frequent category and were generally fairly productive plants.

Single and double monosomic *H. vulgare–H. bulbosum* chromosome substitutions were the next most frequent class of anomalous plants (group 3) formed from the fertilisation of an *H. vulgare* egg by a male gamete having 6 *H. vulgare* chromosomes and at least 1 *H. bulbosum* chromosome. Surplus *H. bulbosum* chromosomes would have been eliminated after fertilisation. Five out of the seven possible single monosomic substitutions of *H. vulgare* chromosomes have now been obtained (Pickering 1992; present results). Including the plants described previously (Pickering 1992), single monosomics involving the substitution of *H. vulgare* chromosomes number 1 (16 plants) or number 6 (9 plants) were the most common, vigorous and fertile plants obtained. Fertile substitution plants could be valuable for gene introgression resulting from pairing and recombination between the *H. vulgare* and *H. bulbosum* homoeologues. For example, after carrying out molecular analyses of progeny from a substitution plant (916J2; Pickering 1992), we have identified 1 plant that has *H. bulbosum* DNA inserted into chromosome 6 of *H. vulgare* (paper in preparation). In double monosomic

substitutions, *H. vulgare* chromosomes 1 + 4 were those most frequently replaced by 2 *H. bulbosum* chromosomes. Thus, *H. bulbosum* homoeologues of *H. vulgare* chromosomes 1, 4 and 6 seem best able to compensate for deficiencies in the *H. vulgare* chromosome complement. The absence of a plant involving the monosomic substitution of *H. vulgare* chromosome 5 suggests that this chromosome is least compensated for by its *H. bulbosum* homoeologue. Islam et al. (1981) also initially reported the absence of barley chromosome 5 amongst wheat-barley addition lines.

The two recombinants obtained (group 4) were characterised further, 16R4 cytologically and 30X2 by RFLP analysis. Neither will be useful for breeding programmes because of sterility (16R4) and the unimportance of the transferred character (30X2). However, the development of a mildew-resistant recombinant from similar interspecific crosses (Xu and Kasha 1992) demonstrates that it is possible to extract valuable breeding material from these hybridisations.

Disease resistance and phenotypes of substitution plants

The morphological heterogeneity and inconsistencies in the responses to three pathogens of the substitution plants involving the same chromosome may be a reflection either of the heterozygous nature of *H. bulbosum*, and/or that particular resistance genes in *H. bulbosum* may not always confer resistance in an *H. vulgare* background. For example, alien gene introgression in the Triticeae is not always associated with complete expression (Baum et al. 1992). The 1 chromosome 2 substitution plant was immune to mildew and must contain the *H. bulbosum* chromosome carrying the gene for resistance.

## Conclusion

In summary, we have shown that gene transfer from *H. bulbosum* to *H. vulgare* is possible although directed introgression may be difficult to achieve. However, by backcrossing VBB hybrids to *H. vulgare*, we have obtained recombinants, chromosome substitutions and rearrangements and have demonstrated the potential for introgressing *H. bulbosum* genes into cultivated barley using appropriate procedures. Apart from their use for gene introgression, the substitution plants also provide valuable information on genome homoeologies and the location of desirable genes.

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