

# ***Hordeum bulbosum* is an Exploitable Source of Disease Resistance Genes for Barley Breeders**

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## **Introduction and Aim**

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world but New Zealand's contribution to world production is minor (ca. 80 000 ha). However, its cultivation in New Zealand is important for domestic malting and animal feed industries. Like most intensively cultivated crops, barley suffers from many pests and diseases that limit yield and quality. To control these, farmers rely on the application and sometimes overuse of pesticides and fungicides, whereas breeders aim to introduce genetic sources of resistance into adapted breeding material. Until recently, sexual transfer of genetic material from wild barley species into cultivated barley has been restricted to *H. vulgare* ssp. *spontaneum* (C. Koch) Thell. (Lehmann, 1991). Within the secondary gene pool, one species (*H. bulbosum* L.) has been used extensively to obtain doubled haploids (DHs) from early generation hybrid material (Kasha and Kao, 1970). Despite their close relationship, *H. bulbosum* differs from *H. vulgare* as it is an outcrossing perennial. It occurs naturally in the Mediterranean and the Fertile Crescent and exists as two cytotypes, diploid ( $2n = 2x = 14$ ) and autotetraploid ( $2n = 4x = 28$ ). Its importance for barley improvement is its resistance to many fungal and viral pathogens and to insects such as Russian wheat aphid (Zeller, 1998). Our aim, therefore, is to develop new cultivars of *H. vulgare* with resistance genes transferred from *H. bulbosum* and subsequently to assess the durability of these resistance genes.

## **Problems and Solutions**

To introgress desirable genes from *H. bulbosum* into *H. vulgare*, several barriers must be overcome. These include:

- pre- and post-fertilisation barriers such as pollen tube-stylar incompatibility resulting in low seed setting (Pickering and Hayes, 1976);
- endosperm degeneration in the developing hybrid seeds;
- chromosome instability because of the elimination of the *H. bulbosum* genome (Kasha and Kao, 1970);
- low chromosome pairing and crossing-over between the homoeologues of the two species (Pickering, 1991; Zhang, Pickering and Murray, 1999);
- hybrid infertility;
- certation effects (gametophytic selection) leading to distortion of the ratios of parental and

recombinant progenies.

By careful selection of parental genotype and the environment in which to carry out crosses, we have solved the problems of pre-fertilisation incompatibility (Pickering and Rennie, 1990), chromosome instability and low chromosome pairing (Thomas and Pickering, 1985). Endosperm degeneration can be overcome through conventional embryo rescue and we have developed partially fertile triploid hybrids for backcrossing to *H. vulgare* (Pickering, 1988). These triploid hybrids were developed from colchicine-treated diploid *H. bulbosum* genotypes to obtain tetraploid *H. bulbosum*, which were crossed as female parent to *H. vulgare*. All triploid hybrids, henceforth denoted as VBBs, from this cross had dehiscent anthers and shed viable pollen, unlike the sterile triploid VBB hybrids reported previously. Selfed seeds are rarely observed. We have also carried out androgenesis (anther culture) to circumvent the normal fertilisation process and avoid certation effects, but with only limited success (Pickering and Fautrier, 1993; Gilpin et al. 1997). The most difficult problem, low crossing-over between the two species' chromosomes must be accepted as being inherent in many wide hybrids. With crossover frequencies of only about 8% of that expected in *H. vulgare* intraspecific hybridisations (Pickering, 1991; Zhang, Pickering and Murray, 1999), reduced recombination necessitates screening many backcross progenies to obtain the desired genotype.

Despite the problems outlined above, we have succeeded in producing agronomically useful plants by backcrossing the VBB hybrids to *H. vulgare* and selecting among the progenies. The backcross (BC) *H. vulgare* parents we use as females in hybridisations with the VBBs are the cultivars Emir, Golden Promise and Morex. These are susceptible to many major fungal and viral pathogens and pests and the transfer of resistances from *H. bulbosum* into these backgrounds will generally not be masked. In addition, Emir has been well-characterised in crosses with *H. bulbosum* and hybrids are easy to obtain, Golden Promise is one of the most responsive *H. vulgare* cultivars in tissue culture and Morex has been used in the North American Barley Genome Mapping Project. Seed setting after pollinating the backcross parent with the VBBs is around 10% (6.6% and 3.6% of seeds with solid and watery seeds, respectively). The average germination from seeds with solid endosperm is 42% and progenies comprise haploid (mainly from seeds with watery endosperm) and diploid *H. vulgare* plants, chromosome substitution lines (SLs) (Pickering, 1992) and recombinants or introgression lines (ILs) (Pickering, Hill and Kynast, 1997).

Tetraploid hybrids (VVBBs) comprising 14 chromosomes from each species, have been used successfully in Germany (Michel et al. 1994), but apart from selecting one IL (code number 81882; Pickering et al. 1995) with powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Em Marchal) and leaf rust (*Puccinia hordei* Otth) resistance, we have not achieved good results until the last two years. Most of the progenies either resembled diploid *H. vulgare* or retained their hybridity. However, we recently selected a high-pairing sterile diploid (VB) hybrid derived from a cross between diploid Emir and diploid *H. bulbosum* genotype HB2032. The mean chromosome pairing was 6.7 bivalents per pollen mother cell (Zhang, Pickering and Murray, 1999) and its fertility was restored after colchicine treatment to produce a tetraploid VVBB hybrid. Seed setting was 36% after selfing and 26% of the 159 progeny that were screened appeared to be normal diploid *H. vulgare* whereas 74% showed some *H. bulbosum* characteristics. Tests are continuing on these progenies, but eight ILs have been positively identified after performing molecular analyses and genomic in situ hybridisations (GISH) and show partial resistance to powdery mildew and/or leaf rust or some morphological trait from *H. bulbosum* (eg hairy leaf sheath). The advantage of VVBB hybrids is that no backcrossing

is necessary since selfed seeds are readily formed. We are producing more VVBs from crosses of HB2032 with Golden Promise and Morex.

### *Characterisation of Progeny*

The response to powdery mildew and the plant morphology of selfed and BC progenies are recorded in the glasshouse. Seeds multiplied in this environment are subsequently grown for two generations in the field to evaluate their response to natural infections of pests and diseases. In New Zealand, the main fungal diseases are leaf rust, net blotch (*Drechslera teres* (Sacc.) Shoem.) and scald (*Rhynchosporium secalis* (Oud.) J. J. Davis). Any interesting lines are then analysed in more detail. The methods of analysis comprise:

- cytological examination (mitotic chromosome counts and meiotic chromosome pairing);
- molecular analyses with single or low-copy restriction fragment length polymorphism (RFLP) probes and amplified fragment length polymorphism (AFLP);
- in situ hybridisation.

RFLP analyses have confirmed the presence of substituted chromosomes and introgressions of *H. bulbosum* chromatin into the *H. vulgare* genome (Pickering, Hill and Kynast, 1997), whereas AFLPs have been used as a complementary tool to identify ILs at early stages in the backcross programme. We are also developing *H. bulbosum* chromosome-specific markers by excising amplified fragments from AFLP gels that are present in *H. bulbosum* and ILs or SLs but absent from the *H. vulgare* parent. After cloning, the fragments are sequenced and primers developed for PCR analysis with a tester set of *H. vulgare* – *H. bulbosum* ILs to assess their specificity. So far, two markers specific for the *H. bulbosum* homoeologue of *H. vulgare* chromosome 6H have been developed.

A repetitive sequence (pSc119.2) derived from rye (McIntyre et al. 1990) has some value as a probe in RFLP analyses to identify SLs and ILs (Pickering et al. 1994). It hybridises strongly to subtelomeric sites on several chromosome arms of *H. bulbosum* (e.g. six sites in Cb2920/4 and D5, seven in Cb2929/1 and eight in HB2032; Pickering, unpublished; see also de Bustos et al. 1996; Xu, Procnier and Kasha, 1990). Since only some of the *H. bulbosum* chromosomes have complementary sequences to pSc119.2 it is only partially diagnostic for the presence of *H. bulbosum* chromatin in the *H. vulgare* background. However, this is an advantage when pSc119.2 is used in fluorescence in situ hybridisation (FISH) experiments to assign landmarks to the *H. bulbosum* genome. By recording the presence of signals in well-characterised SLs and ILs we have established hybridisation sites on several chromosome arms of HB2032 namely, 6H<sup>b</sup>S, 4H<sup>b</sup>L, 7H<sup>b</sup>(L?) and possibly 1H<sup>b</sup> by deduction (Pickering, unpublished). By subsequent probing with the oligonucleotide sequence (CTT)<sub>10</sub> an incomplete karyotype, revealing chromosome homoeology to *H. vulgare*, was developed on the basis of quantitative and qualitative differences among the *H. bulbosum* chromosomes. By the sequential use of GISH and FISH with the (CTT)<sub>10</sub> probe we can also locate introgressions of *H. bulbosum* chromatin on particular chromosome arms of *H. vulgare*. This is achieved by first carrying out genomic in situ hybridisation (GISH) using labelled genomic *H. bulbosum* DNA onto chromosome preparations of the putative IL. Once the sites of the introgressed DNA are recorded, the chromosome preparation is washed and re-probed with (CTT)<sub>10</sub>, which produces signals on *H. vulgare* that are similar to N-banding patterns and allows us to determine which *H. vulgare* chromosome contains the introgression (Pickering et al. 1999).

## Results and Prospects

Using the above techniques, we have identified 43 single, double and triple monosomic chromosome SLs involving the substitution of all the *H. vulgare* chromosomes (except 1H) by their *H. bulbosum* homoeologues (Pickering 1992). The most frequent are 7H (49%) and 6H (28%), and these are the only SLs to show partial fertility. The SLs have been used to characterise the *H. bulbosum* genome with FISH as described above and for gene mapping. Among the BC progenies of VBBs we have also obtained 37 ILs with introgressions located on 11 out of the possible 14 chromosome arms of *H. vulgare*. Up to three different introgressions in homozygous form have been detected in a single plant. Most of the introgressions are distally located but occasional double crossovers result in interstitial introgressions. The most frequent introgressions occur on barley chromosomes 2HS, 2HL, 4HL, 6HS and 7HS. This may reflect their actual gametic frequency, but is more likely to be a combination of this and the relative abilities of recombinant gametes to effect fertilisation (certation effects). Hence, it is impossible at the moment to determine whether or not there are interspecific recombination hotspots. Several of the ILs show improved resistance to pathogens virulent on barley, for example powdery mildew and leaf rust (Pickering et al. 1995; Pickering et al. 1999). Before distributing the ILs to breeders, we try to obtain DH lines from them to induce homozygosity, although this is not always possible since in some cases the presence of a homozygous introgression results in lethality or reduced vigour of the homozygote. These newly transferred genes from *H. bulbosum* may or may not be more durable than existing resistance genes in the primary genepool, but they offer breeders the chance to expand their armoury in the fight against pests and pathogens. We are also looking more closely at *H. bulbosum* itself to see whether it is a potential source of other useful traits that will improve the performance and quality of *H. vulgare*.

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