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INTRODUCTION

Hybridizations between *Hordeum vulgare* L. (cultivated barley) and *H. bulbosum* L. have been performed over many years with two aims. First, the production of doubled haploid barley cultivars (Kasha and Kao 1970); second, the transfer of desirable traits such as resistance to fungal and viral pathogens from the wild species into barley. Apart from the report of an occasional recombinant (Xu and Kasha 1992; Pickering *et al* 1994), successful gene introgression has been hampered by several barriers. In this report we will describe recent progress in overcoming these barriers.

BREEDING

Incompatibility

Incompatibility that occurs in crosses between *H. vulgare* and *H. bulbosum* before fertilization (pollen tube inhibition, Pickering and Hayes 1976) or after fertilization (endosperm breakdown) has been resolved, respectively, by selecting particular parental genotypes in the crossing program (Pickering 1980) and using conventional embryo rescue techniques.

Chromosome instability

After fertilization, *H. bulbosum* chromosomes are usually eliminated from the immature embryo (Kasha and Kao 1970), especially when genome ratios of 1V : 1B occur (where V and B are the *H. vulgare* and *H. bulbosum* genomes, respectively). *H. bulbosum* chromosome retention can be promoted by manipulating the genotype and by allowing embryos to develop below 17.5°C (Pickering 1985). This has simplified diploid VB hybrid production, and even enabled triploid VVB (2V : 1B) hybrids to be obtained directly from crosses of *H. vulgare* (4x) x *H. bulbosum* (2x) (Pickering 1991a). Conversely, VBB (1V : 2B) hybrids from *H. vulgare* (2x) x *H. bulbosum* (4x) crosses can, as expected, frequently be regenerated.

Chromosome pairing

Pairing between *H. vulgare* and *H. bulbosum* homoeologues was inexplicably absent in VVB hybrids described by Pickering (1991a), compared with those VVB hybrids reported by Pohler and Szagat (1982). In VB and VBB hybrids allosyndesis is variable and influenced by parental genotype (Thomas and Pickering 1985; Xu and Snape 1988; Pickering 1992) making it possible to identify high-pairing hybrids. Since environmental conditions affect seed development (Thörn 1992) and chromosome stability in *H. vulgare* x *H. bulbosum* crosses (Pickering 1985), the influence of temperature on chromosome pairing in VB and VBB hybrids was studied. To promote allosyndesis, a temperature of 21°C was more suitable than 15°C during meiosis (Pickering 1990) but, as temperatures greater than 17.5°C also induce *H. bulbosum* chromosome elimination (Pickering 1985), optimum temperatures should be determined for particular hybrid combinations to combine maximum chromosome retention and chromosome pairing.

Hybrid infertility

A pre-requisite for using *H. vulgare* x *H. bulbosum* hybrids in conventional breeding programs is fertility. VB hybrids possess indehiscent anthers and have not proved

useful even after obtaining rare backcross seed. Fertility can be induced by doubling the chromosome number to create tetraploid VVBB hybrids. Selfed progeny from the VVBB hybrids usually resemble their *H. vulgare* parents or maintain their hybridity, but occasional recombinants or 'modified' barley plants have been obtained (see final section). Although VVB hybrids are moderately fertile, their value for gene introgression is limited because of low allosyndetic pairing (Pickering 1991a). Until recently, only indehiscent anthers were found in VBB hybrids, but when *H. vulgare* (2x) was pollinated with *H. bulbosum* (4x) that had been derived from colchicine-treated diploid *H. bulbosum* genotypes, partially fertile VBB hybrids were obtained (Pickering 1988). It is possible that the colchicine treatment induced anther dehiscence in the VBB hybrids, since colchicine can cause heritable disturbances to plant development (Hague and Jones 1987) and gametogenesis (Hassan and Jones 1994). The VBB hybrids have been successfully backcrossed to barley and chromosomally engineered plants and recombinants identified (see final section).

Crossing over

Because of the relative scarcity of *H. vulgare* - *H. bulbosum* recombinants among progeny from fertile hybrids (Lange and Jochemsen 1976), crossing over between paired homoeologues may be very low. To investigate this possibility, a paracentric inversion in barley was crossed with a diploid *H. bulbosum* genotype and several VB hybrids were obtained. These hybrids were cytologically analysed and compared with a barley inversion heterozygote (Pickering 1991b). In the latter, an inversion loop is formed, and when crossing over occurs within the loop, several anomalies can be observed at meiotic anaphase I and II comprising bridges and/or fragments. The proportions of bridges and fragments vary according to the frequency of crossovers and the length and location of the inversion. Aberrations in the VB hybrids occurred less frequently (1.0%) than in the barley inversion heterozygote (12.3%), but their presence indicated that crossovers between the parental chromosomes occasionally took place. However, the rarity of this event constitutes a considerable barrier to obtaining recombinants, and no satisfactory method of inducing crossing over has yet been found in *H. vulgare* x *H. bulbosum* hybrids.

Certation (Pollen tube competition)

In backcrosses of hybrids to *H. vulgare*, competition between pollen grains of different chromosomal constitution derived from hybrids remains a problem. In VBB hybrids, gametes with seven *H. bulbosum* chromosomes should predominate but different combinations of *H. vulgare* and *H. bulbosum* chromosomes are also likely to arise, according to the observed meiotic configurations (Lange 1971; Xu and Snape 1988). Fertilization of *H. vulgare* eggs by gametes with seven *H. bulbosum* chromosomes would lead to haploid *H. vulgare* plant formation after *H. bulbosum* chromosome elimination. However, *H. vulgare* haploids were less commonly observed than diploid *H. vulgare* plants among progeny from *H. vulgare* x VBB crosses (Pickering 1992), and fertilization must have been effected preferentially by gametes containing seven *H. vulgare* chromosomes. To overcome this problem, reciprocal crosses (VBB x *H. vulgare*) were attempted, but success has been limited. To avoid conventional hybridizations altogether, androgenesis was carried out by culturing anthers from various hybrid combinations, but only those hybrids that possessed dehiscent anthers yielded positive results (Pickering and Fautrier 1993). Seven viable green plants were regenerated that included (i) an aneuploid comprising 14 *H. vulgare* chromosomes + 1 acrocentric *H. vulgare* chromosome 4(4I) and one *H. bulbosum* chromosome; (ii) a 14-chromosome plant similar to a VB morphologically but containing 6 *H. vulgare* + 8 *H. bulbosum* chromosomes. This plant was backcrossed to barley and two plants involving double and triple monosomic substitutions of *H. vulgare* chromosomes by their *H. bulbosum* homoeologues were obtained.

Progress and future prospects

Despite the obstacles preventing consistent gene introgression between *H. vulgare* and *H. bulbosum*, 40 plants from backcrosses of VBB hybrids to barley have been produced that involve the single, double and triple monosomic substitutions of barley chromosomes 1(7I), 2(2I), 3(3I), 4(4I), 6(6I) and 7(5I) by their *H. bulbosum* homoeologues (Pickering *et al* 1994). Retention of the *H. bulbosum* chromosome in the substitution plants can be promoted by growing plants in a suitable environment (15°C; Pickering 1994). The most fertile and frequent plants to arise are those having barley chromosomes 1(7I) or 6(6I) substituted, and recombinants have been identified among selfed progeny. Transfer of single genes from *H. bulbosum* has also been reported following backcrosses of VBB hybrids to barley (Xu and Kasha 1992; Timmerman *et al* 1993). The use of tetraploid hybrids has not been as fruitful. Szigat and Pohler (1982) selected 'modified' barley plants from BBVV backcross progeny, and introgression of *H. bulbosum* DNA into a plant with pubescent leaf sheaths was confirmed using a repetitive sequence molecular probe - pSc119.2 (Pickering, Smart and Melz, unpubl). Michel *et al* (1994) described a plant with a single mildew resistance gene and a plant with barley mild mosaic virus (BaMMV) resistance, following screening of selfed progeny from a VVBB hybrid. Based on electrophoretic evidence, the transferred *H. bulbosum* DNA of the BaMMV resistant plant is located on barley chromosome 6(6I).

CONCLUSION

From the results presented above, we conclude that interspecific gene transfer from *H. bulbosum* into *H. vulgare* is possible, but difficult. The techniques are labour intensive and time consuming, but the introduction of novel traits and disease resistances into barley has made this program worthwhile.

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