Chapter 17
The Secondary Gene Pool of Barley (*Hordeum bulbosum*): Gene Introgression and Homoeologous Recombination

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17.1 Introduction

When applying the gene pool concept (Harlan and de Wet 1971) to the 33 species or subspecies of the genus *Hordeum* listed by Blattner (2009), the primary and secondary gene pools of barley turn out to be composed in a simple manner. Since there are no incompatibility barriers between *Hordeum vulgare* subsp. *vulgare* and its wild progenitor *H. vulgare* subsp. *spontaneum*, these two taxa make up the primary gene pool. The secondary gene pool comprises a single species, *H. bulbosum*, which may be crossed to barley, however with some difficulty. Once hybrids have been obtained, though, the chromosomes of the two different parental genomes may pair in some instances and recombine in a homoeologous fashion. The remaining 31 *Hordeum* species fall into the tertiary gene pool. Hybrids between these species and *H. vulgare* have been achieved in some cases. However, there is no reported success in transferring genes from the tertiary gene pool into barley (Zeller 1998), nor is there any reported evidence for recombination between the parental genomes in those hybrids.

Based on the meiotic pairing of chromosomes in interspecific combinations, the species of the genus *Hordeum* have been assigned to four types of genomes (H, I, Xa, Xu), with *H. vulgare* and *H. bulbosum* belonging to the H-genome species (Blattner 2009). Meanwhile, it has become widely accepted that *H. vulgare* and *H. bulbosum* are the closest related species in the genus *Hordeum* (for an overview, cf. Blattner 2009). Congruently, *H. bulbosum* has remained the only *Hordeum* species not belonging to the primary gene pool, which was shown to allow for homoeologous chromosome pairing and recombination when crossed to cultivated barley.

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Hordeum bulbosum (*Hb*) comprises diploid and tetraploid cytotypes. The diploid cytotype is native to the northern and southern Mediterranean countries as far as Western Greece. Tetraploid cytotypes are found in the east of Greece, Turkey, the Middle East, the Caucasian countries, Turkmenistan, Tajikistan and Afghanistan. *H. bulbosum* has widely been used in cereal breeding for inducing the formation of doubled haploids, because its chromosomes normally are eliminated in the young interspecific hybrid embryos during the first days of development, leaving a single set of the seven chromosomes from the cultivated barley parent which subsequently can be doubled through application of colchicine (Kasha and Kao 1970). In addition to this role in enabling an important breeding method, *H. bulbosum* has long been investigated for its potential as a genetic resource for barley breeding. Since the primary gene pool has been extensively used as a genetic resource to improve disease resistance and other traits in barley, researchers started to turn to the secondary gene pool to dig for novel trait genes not available in *H. vulgare* (*Hv*). A growing number of reports now indicate that *H. bulbosum* carries resistance genes which are of potential interest to barley breeders and which may, indeed, more or less readily be introduced to barley germ plasm via introgression breeding.

17.2 Interspecific Hybrids

According to the widely accepted concept, the primary gene pool is made up of two closely related subspecies rather than different species. Crosses between the two can readily be accomplished and hybrids generally are viable. Consequently, *H. vulgare* subsp. *spontaneum* has long been (and continues to be) a rich resource for introgressing useful and hitherto unexploited trait alleles to cultivated barley. A large number of gene variants for resistance to leaf rust, powdery mildew, scald and the soil-borne virus complex (BaMMV, BaYMV-1, BaYMV-2) as well as for β-amylase activity, respectively, were introduced to barley breeding programmes and mapped to specific chromosomes by the use of molecular markers (Feuerstein et al. 1990; Ivandic et al. 1998; Schüller et al. 1992; Abbott et al. 1992; Ordon et al. 1997; Erkkila 1999; Eglinton et al. 1999). Little information is provided in the literature on the genetic and molecular aspects of recombination between the subspecies' genomes, probably because generally no severe problems are encountered in crossing experiments concerning sterility, introgression rate or linkage drag. Using the *H. vulgare* subsp. *spontaneum* accession Caesarea 26-24 as a donor, barley cv. Harrington as recipient and a total of 47 SSR loci as molecular markers, Matus et al. (2003) observed an average of 12.6 % of introgressed donor genome among 140 BC_2F_6 recombinant chromosome substitution lines (RCSL), which was not significantly different from the expected 12.5 %. Although there also were RCSL with a very low (3 %) or high (30 %) share of introgressed donor genome, this observation is concordant with the assumption of generally undisturbed recombination between the two genomes. The same authors report significant segregation distortions in some chromosome regions, namely, on

chromosomes 1H, 5H and 7H. Referring to the taxonomic consensus of treating the two *Hordeum* forms as subspecies rather than separate species, they conclude that given this consensus, meiotic irregularities, which may manifest themselves in segregation distortion, would not be expected for crosses like the one used in their study. However, a conclusive statement on this item is not possible as long as no differentiation is feasible between the possible genetic causes—namely, meiotic vs. genotypic selection—of the observed segregation distortions.

A more complex situation concerning hybridisation and interspecific recombination with barley is met with the secondary gene pool, which is made up of *H. bulbosum*. In the following, focus is put onto utilising the secondary gene pool in barley breeding.

The first viable, albeit sterile, hybrid was reported by Kuckuck (1934) and resulted from a cross of a diploid *H. vulgare* as the female and a tetraploid *H. bulbosum* as the male parent. Hybrids obtained in early studies usually were not able to produce seeds, and hybrid offspring showing some pollen fertility and anther dehiscence was rare (Konzak et al. 1951). Later on, the yield in interspecific hybrids was substantially improved via embryo rescue (Szigat and Pohler 1982; Pickering 1991; Xu and Kasha 1992). Pickering (1988) obtained several triploid 'VBB' hybrids carrying one set of *H. vulgare* chromosomes (V) and two sets of *H. bulbosum* chromosomes (B). These hybrids possessed fully dehiscent anthers with 45–79 % germinable pollen grains. A tetraploid fertile 'BBVV' hybrid (*H. bulbosum* as female parent) was achieved by Szigat and Pohler (1982).

17.2.1 Homoeologous Recombination

From a breeder's viewpoint, introgressions at homoeologous positions are preferable, such that tailoring of the *H. bulbosum* segments via subsequent rounds of genetic recombination becomes feasible. Meanwhile, there is ample evidence that *H. bulbosum* segments introgressed into barley chromosomes result from homoeologous recombination between the parental genomes. Some of this evidence is outlined in the following.

17.2.1.1 Cytological Evidence

By backcrossing fertile triploid or tetraploid hybrids to barley or through anther culture of hybrids, recombination between the two genomes was achieved, as evidenced by chromosome pairing at meiotic metaphase (Pohler and Szigat 1991; Pickering 1991; Xu and Kasha 1992). For instance, when using the *H. bulbosum*-specific DNA probe pSc119.1 in FISH, Xu and Kasha (1992) detected five *Hb* sub-genomic fragments in a BC1 plant. Using the same probe, Gilpin et al. (1997) detected a *H. bulbosum* segment introgressed into barley chromosome 6HS. Recombination between *H. vulgare* and *H. bulbosum* chromatin was also demonstrated by genomic in situ hybridisation (GISH; Pickering et al. 1997).

17.2.1.2 Evidence from Molecular-Marker Analysis

At the genetic level, evidence for homoeologous recombination comes from genetic mapping studies using molecular markers. For instance, using RFLP markers genetically mapped in barley, Pickering et al. (1995) identified an introgression of a *Hb* segment on chromosome 2HS. Later on, PCR-based anchor markers such as SSR markers simplified the detection of *H. bulbosum* chromatin (Ruge-Wehling et al. 2006). In the majority of cases studied so far, molecular-marker alleles from either parental genome at molecular-marker loci inside or outside the introgressed segment recombine with each other more or less readily (Timmerman et al. 1993; Pickering et al. 1994, 2004, 2006; Ruge et al. 2003; Ruge-Wehling et al. 2006; Scholz et al. 2009), demonstrating that recombination between the two parental genomes is homoeologous.

17.2.1.3 Factors Influencing Homoeologous Recombination

Pickering and Johnston (2005) pointed out that *H. vulgare* × *H. bulbosum* hybrids with consistent and stable chromosome numbers and high intergenomic pairing at meiosis are needed for introgression breeding. In general, though, the number of recombinants obtained from *Hv* × *Hb* crosses has been low, and *H. bulbosum* chromosomes are subject to more or less rapid elimination in interspecific hybrids. There is evidence that both genetic and environmental factors have an influence on the chromosomal behaviour. Ho and Kasha (1975) detected factors on barley chromosomes 2H and 3H that influence the tendency for the elimination of *H. bulbosum* chromosomes in interspecific hybrids. Temperatures of <17.5 °C during the first few days after pollination were found favourable for the formation of diploid interspecific hybrids (Pickering 1985). Stability of *Hb* chromosomes in amphidiploid VVB hybrids was reported to depend on the genotype of both the *Hv* and *Hb* parents, and with regard to introgression breeding, use of parent lines selected for high and stable chromosome pairing was proposed (Thomas and Pickering 1983, 1985). As to the influence of the *Hv* parent, one or several dominant genes were suggested to be present in the barley cv. 'Vada' which prevented the elimination of *Hb* chromosomes from the amphidiploid VVB hybrid (Thomas and Pickering 1983). Using genomic in situ hybridisation (GISH), Zhang et al. (1999) demonstrated that recombination between the different parental chromosomes involved only distal chromosome regions. Furthermore, they observed significant differences between two diploid *H. vulgare* × *H. bulbosum* hybrids with regard to chromosome stability (i.e. retention of *H. bulbosum* chromosomes) and recombination frequency. Interestingly and for unknown reasons, while one hybrid led to significantly higher recombination frequency as compared with the second hybrid, the latter one had a ratio of recombination to meiotic pairing frequency, which was almost twice as high as in the former one. In both hybrids, meiotic pairing frequency greatly exceeded recombination. In summary, the results of Zhang et al. (1999) and others suggest that recombination frequency is genetically influenced and hybrid parents may be selected for optimising the yield of recombinants.

17.2.2 Introgression Sites and Sizes

Hordeum bulbosum introgressions are generally found at distal positions of barley chromosomes. Barley chromosomal arms greatly differ in the frequencies with which introgressions occur. Pickering et al. (2004) reported that mostly the chromosome arms 2HL and 4HL were found to carry *H. bulbosum* segments. In contrast, few introgressions were observed on chromosomes 1HS, 3HS, 3HL, 4HS and 5HS. Combining the results of cytological and molecular-marker-based analyses, it can be stated that meanwhile, *H. bulbosum* introgressions have been achieved on all the 14 chromosomal arms of barley (Pickering et al. 2004; Johnston et al. 2009; Scholz et al. 2009), thus making a large proportion of the *H. bulbosum* genome accessible to introgression breeding in barley.

Hordeum bulbosum sub-chromosomal segments introgressed into barley differ in their size. The two largest introgressions observed so far had approximately half the physical size of the long arms of chromosomes 4H and 6H, respectively (Pickering et al. 2004). Introgressions of comparably small sizes of 1.8 cM and 3.6 cM were reported by Ruge et al. (2003) and Pickering et al. (2006) on chromosome 6HS and 4HS, respectively.

With regard to plant breeding, though, accessibility of an introgressed *Hb* segment to size reduction via subsequent rounds of recombination appears more relevant than its initial size observed in the interspecific hybrid. Compared with the situation in a pure barley-genetic background, the relative ease with which recombination occurs in heterozygous introgression genotypes may vary between introgressions as well as along a given introgressed segment. In many cases, recombination frequencies were found to be similar or moderately suppressed relative to the pure-barley situation. But there are exceptions. This aspect is further explained below.

17.3 Transfer of Disease Resistance and Marker-Assisted Selection

During the past 20 years, it has become evident that *H. bulbosum* provides a rich source of resistances to a variety of pathogens, including fungal as well as viral diseases.

17.3.1 Fungus Resistance

Xu and Snape (1989) screened two tetraploid and two diploid *H. bulbosum* accessions with five isolates each of powdery mildew, yellow rust and leaf rust. All accessions proved to be immune to powdery mildew (PM), and the tetraploid accessions were also resistant to yellow rust (*Puccinia striiformis*) and leaf rust (*P. hordei*) isolates. Diploid and triploid hybrids of these accessions and susceptible barley parents were resistant to PM, and the single tested triploid hybrid also expressed resistance to

yellow rust. Xu and Kasha (1992) produced backcross progeny (BC1) of resistant triploid *Hv* × *Hb* hybrids and demonstrated that PM resistance could be transferred from the wild species to barley as a single dominant resistance factor, thereby opening up the perspective of using *H. bulbosum* as resistance resource in plant breeding. Using GISH and RFLP analysis, Kasha et al. (1996) showed that this PM-resistance gene had been transferred to the 2HL chromosome arm in barley.

Another dominant PM-resistance gene was described by Pickering et al. (1987, 1995) and Michel (1996) in segregating F2 offspring obtained from a selfed tetraploid hybrid that was derived from a cross of tetraploid *H. vulgare* and tetraploid *H. bulbosum*. This PM-resistance gene was located to barley chromosome 2HS by the use of the barley RFLP anchor marker cMWG862 (Pickering et al. 1995) and was found to be inherited together with resistance to leaf rust, which could be explained by both genes being located on the same introgressed *H. bulbosum* sub-genomic fragment on barley chromosome 2HS (Pickering et al. 1998). Evidence was presented by the authors that the two genes may be separated via recombination and, hence, the original *Hb* introgression may be reduced in its size. Ruge et al. (2004) came to the same conclusion when analysing a 2HS introgression derived from a differing *Hb* cross parent. Again, two resistance genes to powdery mildew and leaf rust, namely Ml^{Hb} and $Rph20^{Hb}$, respectively, had jointly been transferred to barley; however, they could be separated from each other via recombination.

Taken together, there is ample evidence that the secondary gene pool holds a variety of resistances to fungal diseases in barley, among them powdery mildew, leaf rust, yellow rust, stem rust, *Rhynchosporium secalis*, *Septoria passerinii* and *Typhula incarnata*. A summary of introgressed resistance genes is given in Table 17.1. The majority of resistances described so far are dominantly expressed, with one exception concerning resistance to stem rust which was reported by Fetch et al. (2009) to be inherited as a recessive trait (Table 17.1).

In some cases, resistance genes introgressed from *H. bulbosum* into barley represent novel genes, which reside at loci different from those known from the primary gene pool. This is the case for the two PM-resistance factors reported by Pickering et al. (1995) and Michel (1996). Whereas these factors seem to be allelic or else closely linked to each other, they are nonallelic to PM-resistance genes drawn from the primary gene pool. Even when independently derived introgressions turn out to carry allelic resistances, each of these may contribute to broaden the genetic basis for disease resistance. Since *H. bulbosum* is strictly outcrossing, populations and individuals are expected to be genetically heterogeneous or heterozygous; there is a chance that introgressions carrying allelic resistance genes will introduce alleles, which react differently to races or pathotypes in barley. This was demonstrated for PM resistance. Michel (1996) showed that the two independent albeit allelic introgressions mentioned above represent different alleles, which react differently to a set of PM isolates. Moreover, when combined in F1, the two introgressions complemented each other to give a more robust, immune-like resistance to a variety of PM isolates as compared to the reaction of each single introgression (Michel 1996). Independent introgressions from differing *Hb* parents have been provided for resistance to powdery mildew (Xu and Kasha 1992; Pickering et al. 1995; Michel 1996), leaf rust (Pickering et al. 1998; Ruge et al. 2004), stem rust (Pickering

Table 17.1 Resistances to fungal or viral diseases in barley introgressed from *H. bulbosum*

Resistance to pathogen	Gene designator	Introgressed on barley chromosome	References
Erysiphe graminis	–	2HL	Kasha et al. (1996)
	–	2HL	Pickering et al. (1995)
	–	2HL	Michel (1996)
	–	2HS	Pickering et al. (1998)
	–	7HL	Pickering et al. (2004)
	Ml^{Hb}	2HS	Ruge et al. (2004)
Puccinia hordei	–	1HL	Pickering et al. (2004)
	–	2HS	Pickering et al. (1998)
	$Rph20^{Hb}$	2HS	Ruge et al. (2004)
	$Rph21^{Hb}$	2HL	Ruge et al. (2004)
	$Rph22^{Hb}$	5HL	Ruge et al. (2004)
Puccinia graminis	*Rpg6*	6HS	Fetch et al. (2009)
	–	7HL	Pickering et al. (2004)
Septoria passerinii	–	4HL	Toubia-Rahme et al. (2003)
Rhynchosporium secalis	$Rrs16^{Hb}$	4HS	Pickering et al. (2006)
BaMMV, BaYMV-1, -2	$Rym14^{Hb}$	6HS	Ruge et al. (2003)
	$Rym16^{Hb}$	2HL	Ruge-Wehling et al. (2006)
BYDV	$Ryd4^{Hb}$	3HL	Scholz et al. (2009)

et al. 2004; Fetch et al. 2009) and the soil-borne virus complex (BaMMV, BaYMV-1, BaYMV-2) (Ruge et al. 2003; Ruge-Wehling et al. 2006).

17.3.2 Virus Resistance

The secondary gene pool has proven a valuable source also in respect to virus resistance. Michel (1996) provided evidence for two dominant barley mild mosaic virus (BaMMV)-resistance genes that had been transferred from a tetraploid *H. bulbosum* accession to barley. Later on, these two genes were designated as $Rym14^{Hb}$ and $Rym16^{Hb}$, mapped relative to molecular markers and assigned to barley chromosomes 6HS and 2HL, respectively, using FISH and molecular anchor markers (Ruge et al. 2003; Ruge-Wehling et al. 2006). $Rym14^{Hb}$ was found to cosegregate among 168 individuals with two RFLP anchor markers and one codominant STS marker, *Xiac500*(*STS*). This marker had been derived from a differential cDNA analysis of two bulks made of resistant vs. susceptible individuals, respectively, of a segregating F5 mapping population. From this analysis, a cDNA-AFLP fragment was obtained which was detectable only in the resistant bulk and only following BaMMV inoculation. Regarding this specific origin as well as the cosegregation with

BaMMV resistance, *Xiac500*(*STS*) potentially provides a diagnostic marker for marker-assisted selection of *Rym14*^{Hb} carriers in plant breeding programmes.

Among the BaMMV-resistance genes described in barley so far, *Rym14*^{Hb} and *Rym16*^{Hb} are unique in that they are dominantly expressed. All other BaMMV resistances derived from the primary gene pool are known to be recessive. This poses the question as to the biological resistance mechanism underlying the two genes. The question still remains to be answered. As pointed out by Ruge-Wehling et al. (2006), the resistance is effective following mechanical inoculation with BaMMV, which suggests that post-transmission steps are influenced by each of the two genes. In any case, while other genes, i.e. *rym3*, *rym5* and *rym6* (Kanyuka et al. 2004), have been overcome by novel virus strains, *Rym14*^{Hb} and *Rym16*^{Hb} appear to be more durable in their effectiveness (Habekuß et al. 2005).

Another dominant resistance introgressed from the secondary gene pool is effective against barley yellow dwarf virus (BYDV), a disease of growing economic importance in many regions where winter barley is cultivated. In contrast to the BaMMV/BaYMV virus complex, which is transmitted by the soil-borne fungus *Polymyxa graminis*, BYDV is transmitted by aphids (*Rhopalosiphum padi*, *Sitobion avenae*). A BYDV resistance was introgressed from a tetraploid *H. bulbosum* accession to the susceptible barley cv. 'Igri'. Resistance was reported to be inherited as a monogenic dominant trait and was assigned to a novel resistance gene, *Ryd4*^{Hb}. In plants homozygous or heterozygous for *Ryd4*^{Hb}, this gene confers immunity against the strain BYDV-PAV around Aschersleben, as demonstrated by ELISA values of zero or close to zero. Using cytogenetic detection methods (FISH, GISH) as well as molecular anchor markers, *Ryd4*^{Hb} was assigned to barley chromosome 3HL (Scholz et al. 2009). Due to its immune-like mode of action, *Ryd4*^{Hb} is of potential interest to barley breeders and farmers, because plants carrying this gene are expected not only to be tolerant in terms of BYDV-mediated yield reduction. Rather, *Ryd4*^{Hb} carriers are expected to have nonhost properties to the virus and thus would not support virus loading and spreading by aphid vectors. However, before a practical use of *Ryd4*^{Hb} in plant breeding programmes can be launched, some additional work will have to be done to tailor the introgressed segment, as outlined in the following section, and to devise tools for marker-assisted selection (MAS) of suitable *Ryd4*^{Hb} carriers in the breeder's nursery. MAS is especially rewarding in the case of virus resistance because direct assessment of this trait is expensive to do.

17.3.3 *Genetic Tailoring of* Hb *Introgressions in Barley Germ Plasm*

An introgressed segment carrying a valuable trait gene from the secondary gene pool into barley may be quite large (if defined in size at all) in its original version and, thus, be of doubtful value for a plant breeder. Usually, the introgression has to be reduced in size by substituting *Hv* chromatin for most of the *Hb* chromatin, while retaining the valuable trait gene. This is done by a marker-assisted search for

recombinants among selfed or backcrossed offspring. To find suitable recombinants may be accomplished with more or less ease, depending on the degree of homoeologous chromosomal pairing, and hence the probability for genetic recombination of *Hb* and *Hv* chromatin along the introgressed sub-chromosomal segment. The final proof whether or not an introgressed segment has been sufficiently downsized must, of course, come from comparative yield trials which usually are performed under 'common practice', in the absence of severe infection pressure.

An estimate of how much the recombination activity of a homoeologous pair of chromosomes is altered due to the existence of an introgressed *Hb* sub-chromosomal segment may be obtained by mapping molecular markers along the introgressed segment and comparing their distances with a consensus map set up in a pure barley-genetic background.

For instance, the *Hb* introgression reported to harbour the scald-resistance gene $Rrs16^{Hb}$ (Pickering et al. 2006) was estimated 3.6 cM in size, as judged from the genetic distance of the most distant molecular anchor markers which still segregated with a *Hb* allele. In comparison, the same marker interval extends over 5 cM in a barley consensus map, suggesting that in this case, the introgression is relatively small and there is little, if any, suppression/reduction of recombination along the introgressed segment. Thus, further downsizing of the introgression should readily be accomplished via several rounds of recombination. Molecular markers flanking $Rrs16^{Hb}$ in 0.1 cM and 0.3 cM distance, respectively, are available for starting a marker-assisted pre-breeding project.

In the case of $Rym16^{Hb}$ (Ruge-Wehling et al. 2006), the introgressed *Hb* sub-chromosomal segment extended over 30 cM on the long arm of barley chromosome 2H, with $Rym16^{Hb}$ mapping at the distal end of the introgression. At its proximal end, the introgressed segment had a region of approx. 7 cM in size, which corresponded to approx. 28 cM in a barley consensus map, indicating that recombination of *Hb* with *Hv* chromatin was suppressed in this section by a factor of 4. This proximal part carried a lethality factor which prevented the formation of viable homozygous-resistant offspring. A larger, distal part (20.4 cM) of the introgressed segment appeared not to be subject to pronounced recombination suppression/reduction, since the extension of the respective molecular-marker interval compared to 18 cM in a pure-barley map. As a consequence, in a first round of marker-assisted selection, recombinant resistant offspring could readily be identified which was devoid of the proximal part of the original introgression.

In the case of $Rym14^{Hb}$, size reduction will probably be more time-consuming since the introgressed segment corresponds to a 13-cM marker interval in the consensus map [equivalent to 21 Mb in the physical map by Künzel et al. (2000)], while in segregating offspring from a $Hv \times Hb$ hybrid, its genetic size is only 1.8 cM, which means a suppression/reduction of recombination by a factor of 7 (Ruge et al. 2003).

Association of an introgressed trait gene with otherwise negative effects may pose a challenge to pre-breeding. This is exemplified by the $Ryd4^{Hb}$ introgression (Scholz et al. 2009), which was found to bring about a number of negative side effects. Firstly, a segregation-distorting locus (SDL) was linked to resistance, leading to selection against gametes carrying the *Hb* introgression and, hence,

preventing the formation of homozygous-resistant offspring. While this SDL could finally be separated from $Ryd4^{Hb}$ via recombination, a recessive sublethality factor remained linked to the resistance. This factor leads to severe growth depression of homozygous-resistant plants, thus preventing their use in a plant breeding programme at present. Additional molecular markers will have to be developed to perform a high-resolution mapping of the residual introgressed segment and to use this information in a marker-assisted pre-breeding programme.

Conclusion

The past 20 years has seen a major breakthrough in the utilisation of plant genetic resources for barley breeding. In 1991, von Bothmer and Jacobsen came to the conclusion that 'the plant breeding potential for improvement of barley using wide hybridization is, at the present state of knowledge, to utilize the gene pool available within *H. vulgare*, i.e. landraces, wild and weedy form of subsp. *spontaneum*, where no sterility barriers are operating in combinations with cultivated barley'. Since then, a number of reports have been published on the successful introgression of *H. bulbosum* genes into barley, their usefulness as novel trait genes in barley breeding and the tailoring of introgressed segments via marker-assisted selection. Today we can be confident in stating that the secondary gene pool of cultivated barley has been made accessible to the plant breeder as a novel resource for enriching the genetic diversity of the barley germ plasm. For obvious reasons, monogenic disease resistances were among the first traits improved in barley by using the secondary gene pool. At least some of these disease resistances (e.g. the BYDV resistance mediated by $Ryd4_{Hb}$) seem to be controlled by genes different from those described so far in the primary gene pool and are expected to confer relatively durable resistance. What remains to be done is to evaluate the secondary gene pool with respect to other possibly quantitative traits like tolerance to abiotic stresses such as drought, cold and salinity.

The future prospects of approaching the secondary gene pool in a more systematic way will depend on the progress that can be achieved in two major directions. Firstly, the protocols for generating fertile interspecific $Hv \times Hb$ hybrids in sufficiently high frequency and for deliberately achieving introgressions in cultivated barley will have to be further optimised and refined. Secondly, genomic tools will have to be used for a more efficient genetic tailoring of *H. bulbosum* introgressions. Such tools will make use of the tremendous gain of knowledge achieved with regard to genome orthology among the grasses including rice, to the sequencing of candidate genes in grasses and to constructing a 'genome zipper' which exactly predicts the position of genes and markers in barley and related genomes (Mayer et al. 2011). As a result, optimised species hybridisation, marker-assisted size tuning of introgressed segments and selection markers diagnostic for the gene of interest will make the utilisation of the secondary gene pool a less random and more predictable approach.

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