The secondary genepool of Barley as gene donor for crop improvement

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Introduction

The wild species *H. bulbosum* L. is a potential resource of novel genes for barley improvement by introgression breeding. It represents the secondary genepool of barley. Among 110 accessions originated from Middle East, Europe, North- and South-America and from Australia there has been identified one tetraploid (2n=4x=28) *H. bulbosum* accession (*BAZ-3*) with resistance to barley yellow dwarf virus (BYDV-PAV 1 ASL), the soil-borne virus complex (BaMMV, BaYMV-1, -2), the cereal cyst nematodes (CCN) *Heterodera aveneae* Wollenweber and *H. filipjevi (*Madzhidov) Stelter*,* powdery mildew (26 races*)*, leaf rust and typhula blight. A crossing programme was initiated in 2001 to develop new recombinant *Hordeum vulgare x H. bulbosum* hybrids and to transfer desired characters into cultivated barley by introgression.

Material and Methods

- Interspecific hybridisation was carried out using the three diploid (2n=2x=14) *H. vulgare* cvs. 'Igri' (VV-1), 'Nikel' (VV-2) and 'Borwina' (VV-3), one tetraploid (2n=4x=28) cultivar 'Borwina' (VVVV) and the selected *H. bulbosum* accession (Michel, 1996) *BAZ-3* (BBBB). VV-1, VV-2 and VV-3 were used as female parents. Reciprocal crosses were also made between VV-1 and BBBB as well as VVVV and BBBB. VV-1 was used to produce BC1 offspring, with the triploid (2n=3x=21), partially fertile hybrid H2 (*BAZ-50.131*) used as pollinator.
- About 14-20 days after pollination, embryo rescue was performed.
  Using selfing, backcrossing with VV-1 and anther culture technique progenies were obtained from a BC1 genotype (*BAZ-60.001*) with resistance to BYDV- PAV 1 ASL.
- Chromosome counts were done on root tips using the Feulgen technique. The nuclear DNA content was analysed via flow cytometry.
- Resistance tests were performed as followed: BYDV-PAV 1 ASL: Niks et al. 2004; BaMMV/BaYMV-1, -2: Proeseler 1993; Leaf-rust: RUGE et al. (2004); powdery mildew: Flath 2000; cereal cyst nematodes: biotest, cit. Große.
- STS marker analysis was carried out as described by RUGE et al. (2003)
- Genomic in situ hybridisation (GISH) was performed according to Schrader et al. (2000).

Results and Discussion

- The seed set per flower and the yield of embryos and plantles from the caryopses and embryos, respectively, in *Hordeum vulgare* × *H. bulbosum* crosses highly depended on the *H. vulgare* cultivar that was used as a female parent (Table 1). Cv. 'Borwina' (2x, 4x) led to the highest frequency of embryos (VV-3: 67.9 %/VVVV: 44.3 %) compared to cv. 'Igri' (VV-1: 5.9 %) and cv. 'Nikel' (VV-2: 17.2 %). When cv. 'Borwina' (4x) was used as pollinator, however, only 7 % of the embryos could be obtained compared to 44.3 % achieved with the opposite direction. The regeneration rate of embryos into plants was also influenced by the genotype and the cross direction. When cv. 'Nikel' (VV-2) was used as female parent only 6 plants out of 69 embryos could be regenerated compared to 17 plants out of 20 embryos from cv. 'Igri' × *H. bulbosum* crosses. The regeneration rates of embryos into plants were much lower when *H. bulbosum* was used as female parent (36.4 %: BBBB × VV-1; 22.1 %: BBBB × VVVV; not shown in Table 1). MICHEL (1996) also reported a slower seed set in this cross dircetion. Apparently, there is an effect of the cytoplasm of *H. bulbosum* on the vigour of the embryos.
- All offspring plants from VV/VVVV × BBBB crosses that were analysed proved to be triploid (2n=3x=21). Offspring from BBBB × VV crosses were triploid or tetraploid and all offspring from BBBB × VVVV crosses were tetraploid (2n=4x=28). RUGE et al. (2003) demonstrated that fertile tetraploid VVBB hybrids are useful for gene transfer into *H. vulgare.*

Table 1: Yields of hybrid plants obtained from reciprocal crosses between *H. vulgare* (2n=14: VV-1, -2, -3; 2n=28: VVVV) and *H. bulbosum* (2n=28, BBBB)

Combinations	Florets pollinated	Embryos cultured Number	(%)	Hybrid Plants Number	(%)
VV-1 × BBBB	339	20	5.9	17	5.0
VV-2 × BBBB	401	69	17.2	6	1.5
VV-3 × BBBB	78	53	67.9	50	64.0
VVVV × BBBB	463	205	44.3	200	43.2
BBBB × VV-1	108	74	6.7	36	3.2
BBBB × VVVV	752	53	7.0	40	5.3

- Meiotic metaphase I analysis revealed high frequencies of trivalents (0-4 per PMC) in triploid interspecific VBB hybrids and of tetravalents (0-6 per PMC) in tetraploid interspecific BBVV hybrids (Fig. 1). Because of the high level of pairing between the parental chromosomes, these VBB (H2) and BBVV (H1, H3) hybrids may be useful for the transfer of desirable trais to *H. vulgare.*

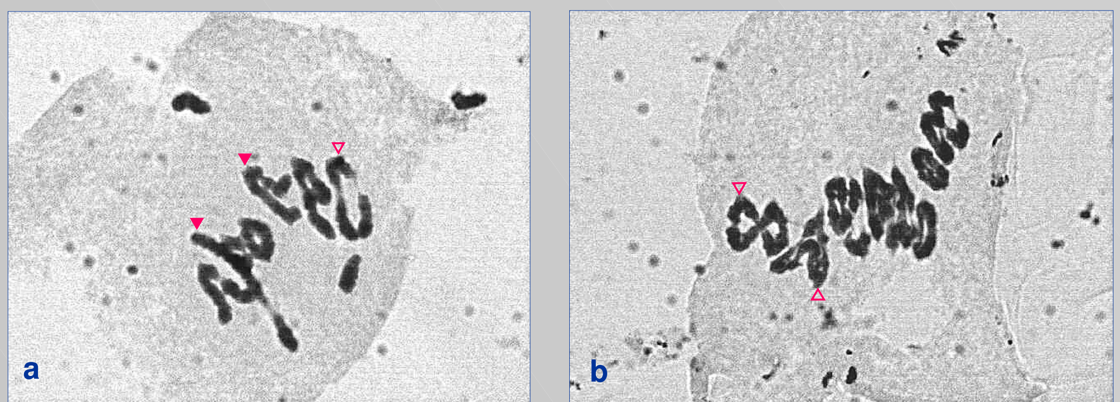

Fig. 1: Meiotic chromosome behavior in triploid (a) and tetraploid (b) interspecific *Hordeum vulgare* × *H. bulbosum* hybrids. (a) Metaphase I showing four univalents, one rod and three ring bivalents, two rod (closed triangles) and one ring trivalent (open triangle). (b) Metaphase I showing ten ring bivalents and two tetravalents (open triangles)

- A differentiation of molecular-marker alleles from *H. bulbosum* (*b* alleles) and *H. vulgare* (*v* alleles) in the hybrids and their parents was attempted by using STS anchor markers located on the seven chromosomes of barley (1HL: *ABG373*, 2HS: *MWG2133*, *MWG2146*, *MWG520*, 3HL: *MWG549*, 4HS: *WG622*, 5HL: *MWG877*, *MWG583*, 6HS: *MWG2218*, 6HL: *MWG934*, 7HS: *MWG530*, 7HL*: MWG2031*). The hybrid character of three interspecific F1 offspring individuals from BBBB × VV (H1, H3) and VV × BBBB crosses (H2), respectively, could be demonstrated (Fig. 2). However, in three instances (H2: *MWG530*, H3: *ABG373*; not shown in Fig. 2; H3: *MWG2031,* Fig. 2) anchor markers failed to verify the presence of the respective orthologous *H. bulbosum* chromosome or chromosome region.
- The absence of *b*-marker alleles may be indicative for partial elimination of *H. bulbosum* chromatin. Elimination of marker alleles of *H. bulbosum* in the two hybrids H2 and H3 appears to be independent on whether this parent was used as male or female.

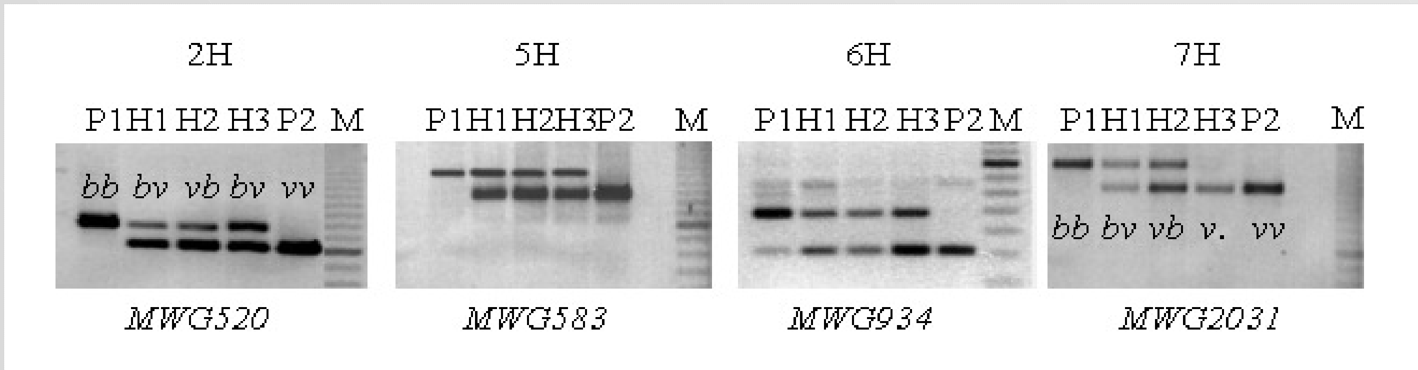

Fig. 2: Identification of interspecific hybrids by means of STS markers. P1, *H. bulbosum* parent, P2, *H. vulgare* parent; H1, H2 and H3, interspecific hybrids

- The hybrids H1 and H2 verified with molecular markers above proved to be non-infectable by BYDV-PAV 1 ASL. In addition, hybrids H1 and H2 also displayed resistance to leaf rust.
- After backcrossing the partially fertile hybrid H2 to VV-1, one BC1 plant (*BAZ-60.001*) could be obtained that did not develop any symptoms and stayed free of BYDV virus according to ELISA results. This BC1 plant is perennial, resembled *H. vulgare* morhpologically except the tillers were shorter and thinner than those of *H. vulgare* (Fig. 3). We identified four *H. bulbosum* signals at the terminal end of the *H. vulgare* chromosomes using GISH technique (Fig. 4 a). To regenerate plants with stable gene introgression selfing, backcrossing and anther culture techniques have been used. Haploid, diploid and triploid progenies having one (Fig. 4 b) up to seven introgressions have been produced from the BC1 plant. Some of the BC2 and BC1F2 genotypes displayed resistance to BYDV-PAV 1 ASL, the soil born virus complex (BaMMV, BaYMV-1, -2), the cereal cyst nematode *H. filipjevi* and nine isolates of powdery mildew.

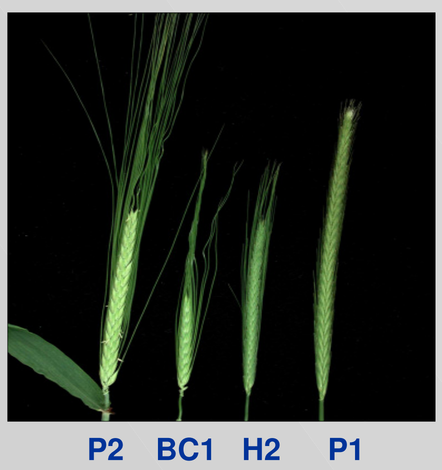

Fig. 3: Spikes of cross parents, P1, *H. bulbosum*; P2, *H. vulgare*; H2, triploid *H. vulgare* × *H. bulbosum* hybrid; BC1, progeny from backcrossing H2 to P2

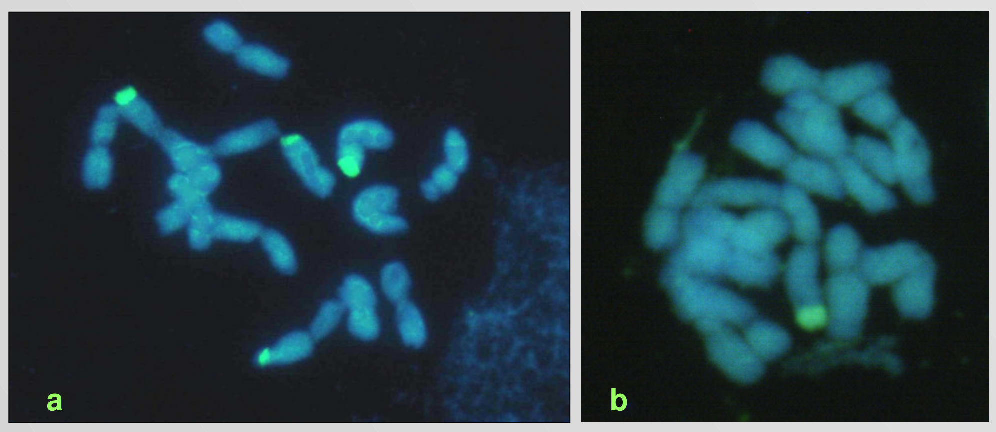

Fig. 4: Genomic in situ hybridisation of mitotic chromosomes from the diploid BC1 plant (*BAZ-60.001*) (a) showing four yellow signals of *H. bulbosum* chromatin at the terminal end of the *H. vulgare* chromosomes and b from the diploid BC2 plant showing only one signal of *H. bulbosum*

Conclusions

- The secondary genepool of barley can be used to introgress genes for immunity to BYDV, for resistances to the soil-borne virus complex (BaMMV, BaYMV-1, -2), for resistance to the cereal cyst nematode *H. filipjevi* and to powdery mildew effective against nine isolates and gene(s) for perenniality into cultivated barley.
- To utilize these novel genes in agriculture mapping populations are going to develop for genetic analysis together with molecular-marker analysis.

References

Flath, K. 2000. In: Bartels, G. and Backhaus, G. F. (eds.). Mitt. Biol. Bundesanst. Land- Forstwirtsch. Berlin-Dahlem 373: 6-8.
Michel, M. 1996. PhD Thesis, Technische Universität München, 116 p.
Niks, R. E. et al. 2004. Theor. Appl. Genet. 109: 1536-1543.
Proeseler, G. 1993. J. Phytopathol. 36: 262-264.
Ruge, B. et al. 2003. Theor. Appl. Genet. 107: 965-971.
Ruge, B. et al. 2004. Proc. 9th Int. Barley Genet. Symp., Brno, Czech Republic, 20-26 June 2004.
Schrader, O. et al. 2000. Theor. Appl. Genet. 100: 665-669.