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Katrin Humbroich

Identification and mapping of resistance genes against soil-borne viruses in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.)

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Head: Prof. Dr. Dr. h.c. W. Friedt

Identification and mapping of resistance genes against soil-borne viruses in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.)

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Katrin Humbroich
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Board of Examiners

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List of Abbreviations

A	adenine
ACGM	amplified consensus genetic markers
AFLP	amplified fragment length polymorphism
BaMMV	*Barley mild mosaic virus*
BaMMV-Sil	Barley mild mosaic virus strain found in Sillery, France
BaYMV	*Barley yellow mosaic virus*
BaYMV-2	*Barley yellow mosaic virus*-2
BMELV	Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz
BSA	bulked segregant analysis
bp	base pairs
C	cytosine
CAPS	cleaved amplified polymorphic sequences
cDNA	complementary DNA
CIA	Chloroform-Isoamylalcohol
cM	centiMorgan
CTAB	cetyltrimethylammonium bromide
DArT	Diversity Arrays Technology
DAS-Elisa	double antibody sandwich enzyme-linked immunosorbent assay
DGGE	heteroduplex analysis by density gradient gel electrophoresis
DHPLC	denaturing high-performance liquid chromatography
DI	diversity index
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide
EDTA	ethylene diamine tetra-acetate
e.g.	for example
ERAP	Exon-Retrotransposon amplification polymorphism
EST	expressed sequence tags
et al.	et alii
G	guanine
GS	genetic similarity
GST	gene specific tags
H’	Shannon-Weaver-Index
ha	hectare
HCL	hydrochloric acid
i.e.	id est
ISSR	Inter-simple sequence repeats
LD	linkage disequilibrium

LOD	logarithm of the odds value
M	molar
MALDITOF	Matrix Assisted Laser Desorption/Ionisation Time-of-Flight
MAS	marker-assisted selection
min	minute
mio	million
mg	milligram
ml	millilitre
$MgCl_2$	magnesium chloride
NaOAc	sodium acetate
NH_4OAc	ammonium acetate
Nlb	nuclear inclusion protein b
nm	nanometre
ORF	open reading frame
PAA	polyacrylamide
PCR	polymerase chain reaction
PIC	polymorphic information content
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RIL	recombinant inbred lines
RFLP	restriction fragment length polymorphisms
RGA	Resistance gene analogues
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
SBCMV	*Soil-borne cereal mosaic virus*
SBRMV	*Soil-borne rye mosaic virus*
SBWMV	*Soil-borne wheat mosaic virus*
SCAR	sequence characterised amplified region
SFP	single feature polymorphisms
SMC	simple matching coefficient
SNP	single nucleotide polymorphism
SSR	simple sequence repeats
ssRNA	single stranded ribonucleic acid
STS	sequence tag sites
T	thymine
TBE	Tris/Borat/EDTA-Puffer
TE	Tris-(hydroxylmethyl-)aminomethan
TEMED	tetremethylethylenediamide
t-RNA	transfer ribonucleic acid
U	unit
UPGMA	Unweighted paired group method using arithmetic averages

USA	United States of America
USDA	United States Department of Agriculture
V	volt
VPg	viral genome linked protein
vs	versus
v/v	volume /volume
WSSMV	*Wheat spindle streak mosaic virus*
WYMV	*Wheat yellow mosaic virus*
µg	microgram
µl	microlitre

1 Introduction

Barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) are two of the most important crop species. The worldwide acreage of wheat is first before rice maize and barley: In 2006 the global production was estimated at about 622 million (mio) tonnes of wheat and 138 mio tonnes of barley (USDA 2007). In Germany 3.12 mio hectares (ha) of wheat were harvested and the area under barley cultivation accrued to 2.03 mio ha (BMELV 2007).

Due to the predicted growth of the world's human population and the corresponding increased global food demand, it is a continuing challenge to improve varieties of crop plants, i.e. for disease resistance, to guarantee a stable harvest and yield production parallel to the decreasing acreage under cultivation worldwide, i.e. for barley in the last decades (USDA 2007). In spite of a permanent improvement of resistance in barley and wheat they are still confronted with many viral, bacterial and fungal pathogens, which cause significant damages and reduction in yield and quality due to a co-evolutional adaptation of respective pathogens. In the last decades several soil-borne virus diseases transmitted by the fungus *Polymyxa graminis* became increasingly important in Europe as pathogens of cereals, particularly of barley and wheat (HUTH 2002). These viruses are *Barley yellow mosaic virus* (BaYMV), *Barley mild mosaic virus*, *Soil-borne cereal mosaic virus* (SBCMV) and *Wheat spindle streak mosaic virus* (WSSMV), which cause high yield losses up to 80%. Therefore, because chemical treatments against *Polymyxa graminis* to prevent high yield losses are neither efficient nor economic, it is of prime interest to produce resistant varieties against these viral pathogens. The main objectives of the present study were on one hand to screen exotic genetic resources of barley for resistance and on the other hand to identify molecular markers for new resistance genes against *Barley yellow mosaic virus* (BaYMV) by screening seven different DH populations. With regard to wheat, the project aimed at the identification of sources of tolerance or resistance to *Soil-borne cereal mosaic virus* (SBCMV) by field tests carried out in France followed by genotyping of respective cultivars using *Eco*RI+*3/Mse*I+*3* AFLP primer combinations and microsatellite markers in order to achieve information on the genetic relatedness of resistant and susceptible cultivars and to identify SSR markers suitable for mapping respective genes or quantitative trait loci (QTL).

2 Literature survey

2.1 Soil-borne viruses of cereals

Several soil-borne viruses of cereals are known belonging to the plant virus family *Potyviridae*. This family consists of six genera designated as *Potyvirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus*, *Tritimovirus* and *Bymovirus* (REVERS & CANDRESSE 2004, ADAMS et al. 2005). Besides this, there are the *Furoviruses*, a genus which is not assigned to any specific family. Some of the most important viruses causing serious diseases of cereals like the *Barley yellow mosaic virus*, *Barley mild mosaic virus*, *Wheat spindle streak mosaic virus*, *Oat mosaic virus*, *Wheat yellow mosaic virus* and *Rice necrotic mosaic virus* belong to the *bymovirus* group, that are all transmitted by the fungus *Polymyxa graminis* (KANYUKA et al. 2003). Alike the *furoviruses, i.e. Soil-borne cereal mosaic virus*, *Soil-borne wheat mosaic virus* and *Oat golden stripe virus*, infect cereals via *Polymyxa graminis* (KANYUKA et al. 2003). Besides *Polymyxa graminis*, a related fungal vector *Polymyxa betae* transmits the furovirus *beet necrotic yellow vein virus* in sugar beets (RUSH 2003). All these *Polymyxa*-transmitted viruses have in common that high yield losses and important diseases are caused mainly in cereals (KANYUKA et al. 2003, ADAMS et al. 2004).

2.1.1 The Barley yellow mosaic virus complex

In Japan the Barley yellow mosaic virus disease is already known since the 1940's and it is epidemic since the 1970's (IKATA & KAWAI 1940, cited in INOUYE & SAITO 1975). After the first report in Germany in 1978 (HUTH & LESEMANN 1978) the disease also occurred in several other European countries and in Eastern China (HILL & EVANS 1980, LAPIERRE 1980, MAROQUIN et al. 1982, YILI & DENGDI 1983, LANGENBERG & VAN DER WAL 1986, FANTAKHUN et al. 1987, SIGNORET & HUTH 1993, KATIS et al. 1997, ACHON et al. 2005). The typical yellow patches appear in winter or early spring in the field as a result of the infection of roots in autumn by the different strains of the Barley yellow mosaic virus disease. The symptoms are mosaic pale green or yellow discolorations mostly on the youngest leaves. Sometimes infected plants show complete yellowing with necrotic patches and a stunted growth. Affected plants show fewer tillers, less reduction in grain yield

and grain size may be inhomogeneous. The severity of symptoms depends on the barley cultivar and the environmental conditions in autumn during the infection and in winter during the reproduction and spread of the virus within the plants. In general symptoms become less obvious with increasing temperatures and plant growth. Upper leaves are often free of symptoms. Typically, the symptoms appear in the newly emerging leaves when plants begin to grow again after a cold period in winter. This seems to be related to a temporary reversal of the major direction of phloem transport (SCHENK et al. 1995). Until now, the manner of virus movement has not been determined but virus RNA and the coat protein can be detected in root cells before symptoms appear in the leaves (PEERENBOOM et al. 1996). *Barley yellow mosaic virus* survives within resting spores that remain within root debris after crop harvest and can persist in soil for many years (HUTH 1991) even in the absence of a suitable host (USUGI 1988). The inoculum mostly becomes distributed as resting spores within soil or crop debris through soil cultivation and on machinery. Therefore, existing infected patches in the field enlarge and new ones may easily emerge. Resting spores may also spread by wind-blown soil particles and zoospores may travel short distances in soil water (HILL & WALPOLE 1989). Spring-sown barley normally does not develop symptoms of the disease in the field due to adverse environmental conditions for virus reproduction and spread and the viruses do not cause yield losses in spring barley. However, many spring barley cultivars turned out to be susceptible in laboratory resistance tests.

In Europe, the Barley yellow mosaic virus disease is caused by a complex of at least four viruses or virus strains, i.e. *Barley mild mosaic virus* (BaMMV), BaMMV-SIL (named according to the village Sillery in France, where the strain was first detected), *Barley yellow mosaic virus* (BaYMV-1), and BaYMV-2 (HUTH 1989, HARIRI et al. 2003), infecting barley individually or in combinations. BaYMV-2 was detected in Germany (HUTH 1989), in the United Kingdom (BEATON 1989), Belgium and France (HARIRI et al. 1990). A new strain similar to BaMMV-SIL and BaMMV has just recently been detected in Germany (HUTH et al. 2005, HABEKUSS et al. 2006). An even more complex situation is present in Japan where seven strains of BaYMV and two strains of BaMMV have been described (NOMURA et al. 1996). In Korea a strain biologically and serologically different from BaMMV strains known in Germany and Japan has been detected and several different strains have also been discovered in China (CHEN et al. 1996, LEE et al. 1996, LEE et al. 2006). Due to transmission by

the widespread soil-borne fungus *P. graminis* growing of resistant barley cultivars has to be considered as the only effective means to avoid high yield losses caused by BaMMV, BaMMV-SIL, BaYMV and BaYMV-2.

The viruses of this complex have a quite narrow natural host range limited to the *Poaceae*. The natural host is barley (*Hordeum* ssp.) but successful transmission by mechanical inoculation to *Aegilops* (PROESELER 1988)*, Eremopyrum, Lagurus* (ADAMS 2004)*, Triticosecale* (KEGLER et al. 1985), *Secale* (ORDON et al. 1992) and *Triticum durum* L. (PROESELER 1993) has been carried out.

The whole genus *Bymovirus*, family *Potyviridae*, is a well-defined group of viruses that resemble the aphid-transmitted potyviruses and other members of the family in having flexuous filamentous particles (12-13 diameters) with modal lengths of 270 and 568 nm causing pin wheel inclusions in infected cells (KANYUKA et al. 2003). The members have bipartite single stranded (ss) RNA genomes with a genome linked protein (VPg) at the 5'terminus. Each segment carries a single open reading frame (ORF) which encodes a polyprotein that is cleaved into functional proteins by virus-encoded proteases. The coding sequence of the coat protein is located in the C-terminus of the larger RNA1 polyprotein (KANYUKA et al. 2004a). Both RNA species are needed for infection (KASHIWASAKI 1996). BaMMV causes similar symptoms like BaYMV but the two viruses are serologically unrelated and their polyproteins share only about 36% identical amino acids (SCHLICHTER et al. 1993). Regarding these differences, serological methods or sequence tests are used to discern both viruses. BaYMV-2, a strain which is able to infect cultivars carrying the resistance gene *rym4* (see below chapter 2.2.1), is very closely related to BaYMV. The strains do not differ in the coding sequence of the coat protein and no diagnostic serological methods have been reported to distinguish them (HUTH & ADAMS 1990). The French BaMMV-SIL isolate is the only European BaMMV isolate able to infect barley cultivars with the *rym5* gene (see chapter 2.2.1, HARIRI et al. 2003). It is very similar to the BaMMV strain with only five amino acid exchanges consistently different between BaMMV and BaMMV-SIL. Two of these exchanges are in the viral genome linked protein (VPg) cistron and in the nuclear inclusion protein b (NIb) cistron region, respectively and seem to be functionally important (KANYUKA et al. 2004a).

2.1.2 Soil-borne cereal mosaic virus disease

Soil-borne wheat mosaic virus (SBWMV) is a member of the genus *Furovirus* which is also transmitted by the fungus *Polymyxa graminis*. Due to its high persistence the virus causes yield losses in winter wheat in many areas of the world, especially in the central and eastern part of the United States of America. SBWMV was first detected in 1919 in the USA (MCKINNNEY 1925) and furoviruses causing similar diseases in wheat and rye were later also found in Japan, China (DIAO et al. 1999), Italy (RUBIES-AUTONELL & VALLEGA 1990), France (LAPIERRE et al. 1985), UK (CLOVER et al. 1999a, CLOVER et al. 2001, BUDGE & HENRY 2002), several African countries (KAPOORIA et al. 2000), Belgium (VAIANOPOULOS et al. 2005) and in Germany (KOENIG et al. 1999). These isolates were thought to belong to the same SBWMV species, but it turned out that the global population of furoviruses on wheat consists of genetically divergent strains with a relatively high degree of polymorphisms at the nucleotide and amino acid level. The American, Chinese, European and Japanese isolates are now separately reclassified (KOENIG & HUTH 2000, SHIRAKO et al. 2000). The European virus isolate shares only 70% genome identity with SBWMV from the USA and Japan (DIAO et al. 1999) and due to the mainly infection of rye the name soil-borne rye mosaic virus was proposed in Germany (KOENIG et al. 1999). The natural hosts of this virus are bread wheat, durum wheat, rye, and triticale. In Germany, Poland and Denmark, the virus mainly infects rye, whereas in the United Kingdom, Italy and France wheat is the predominant host (HUTH 2002). Therefore, it was renamed as *Soil-borne cereal mosaic virus* (SBCMV, KOENIG & HUTH 2000, YANG et al. 2001) in Germany and Europe, respectively, which has recently been approved by the International Committee on Taxonomy of Viruses. In 2002, severe damage in wheat due to a furovirus infection was observed in a field near Heddesheim, Baden-Wuerttemberg, Germany. As a result of sequencing the disease causing virus it turned out to be closely related to the American strain of SBWMV. This was the first report of a type strain of *Soil-borne wheat mosaic virus* in Europe (KOENIG & HUTH 2003).

Symptoms caused by SBCMV on susceptible cultivars in the field are a pale green-yellow mosaic or streaks on the leaves and moderate to severe stunting. Young leaves appear mottled and develop pale discolorations that cover both the leaf lamina and the sheath (CLOVER et al. 2001, KASTIRR et al. 2004). The appearance and severity of soil-borne mosaic virus symptoms on wheat may vary considerably

depending on the plant genotype, the concentration and aggressiveness of the virus strain as well as the environmental conditions (BUDGE & HENRY 2002). Generally, late planting in autumn is recommended to reduce the number of infected plants and to minimise yield losses (HUTH 2002). All tolerant varieties are known to contain high virus levels in the root system and no or low to moderate levels in the leaf tissue (DRISKEL et al. 2002). Infected plants often occur in the field in circular patches of varying size. In field samples SBCMV frequently occurs in mixed infections with the bymovirus *Wheat spindle streak mosaic virus* (WSSMV) due to transmission of both viruses via *Polymyxa graminis* (see chapter 2.1.3, HUTH 2002). The primary zoospores of the vector penetrate root hairs or epidermal cells in autumn when there is sufficient moisture and soil temperature and the SBCMV is subsequently introduced into the host cytoplasm (KANYUKA et al. 2003). SBCMV consists of virus particles with a bipartite genome. All particles are rod-shaped with modal length of 120 to 130 and 200 to 230 nm. The genome consists of two positive-sense ssRNAs, with three open reading frames (ORFs, KOENIG et al. 1999) each. RNA1 and RNA2 have a cap structure at the 5'terminus and a tRNA-like structure at the 3'terminus. Three different strains (-G, -O, -C) of SBCMV which only differ in their aggressiveness (HUTH 2002) have been distinguished and showed after sequencing more than 90% sequence identity (KOENIG et al. 1999). SBCMV can be mechanically transmitted to several Poaceae like *Bromus secalinus* L., *Chenopodium quinoa* Willd., *Hordeum vulgare* L., *Secale cereale*, *Triticum aestivum*, *T. durum*, *T. turgidum* and *Triticale* (KASTIRR et al. 2004). Since virus-containing resting spores of *Polymyxa graminis* persists in soil and crop debris for several decades, cultural practises for virus control such as crop rotations or delayed planting are not effective, whilst chemical control measures are unacceptable for ecological reasons.

2.1.3 Wheat spindle streak mosaic virus disease

The appearance of *Wheat spindle streak mosaic virus* was reported for Africa, Canada, the USA and several European countries (RUBIES-AUTONELL & VALLEGA 1990, HAUFLER 1996, KAPOORIA & NDUNGURU 1998, CLOVER et al. 1999b, HUTH 2002, VAIANOPOULOS et al. 2006). The virus belongs to the Bymoviruses such as *Barley yellow mosaic virus, Barley mild mosaic virus, Oat mosaic virus* or *Wheat yellow mosaic virus* and is therefore also transmitted by the soil-borne fungus *Polymyxa graminis*. The symptoms are similar to SBCMV. Infected

plants show yellow-to-light green streaks which occur in parallel to the leaf veins. Besides the streaky symptoms fewer tillers are generated and the plants are dwarfed resulting in yield reductions (HUTH 2002). Infection of the roots and symptom expression are generally at temperatures between 5-17°C. Mixed infection with SBCMV and WSSMV in fields is widespread (see chapter 2.1.2.1). Reportedly, *Wheat spindle streak mosaic virus* reduces the level of field resistance to *Soil-borne cereal mosaic virus* (CLOVER et al. 1999a). The natural host is winter wheat, durum wheat, rye and triticale whereas some gramineous plants like *Hordeum vulgare* and *Avena sativa* can not be infected by WSSMV. Like BaMMV/BaYMV, WSSMV has a bipartite, positive ssRNA genome with two RNAs both encoding single polyproteins. The function of the polyprotein of RNA1 is unknown whereas RNA2 encodes one polyprotein, which is divided into two single proteins, i.e. P1 and P2. P2 is known to be involved in fungal transmission (SOHN et al. 2004). Until now, no different WSSMV strains have been detected.

2.2 Genetics of resistance

2.2.1 Barley Yellow Mosaic Virus Complex

On the basis of intensive screening programmes, mainly with barley germplasms derived from East Asia, resistance sources against the barley yellow mosaic virus disease have been identified (ORDON et al. 1993) and different reactions to the different strains of the BaYMV-complex have been observed (GÖTZ & FRIEDT 1993, ORDON & FRIEDT 1993). Up to now 16 resistance genes are known of which 14 derived from the primary barley gene pool are recessive, while *Rym14*^{Hb} and *Rym16*^{Hb} derived from *Hordeum bulbosum* are dominant (RUGE et al. 2003, RUGE-WEHLING et al. 2006). The resistance genes are distributed over the whole barley genome (GRANER et al. 2000, Ordon et al. 2005). An overview on all mapped resistance genes against barley yellow mosaic virus disease is given in table 1. In Europe barley yellow mosaic virus disease resistance is mainly based on two resistance genes, *rym4* and *rym5*, which are located on the long arm of chromosome 3H. *Rym4* and *rym5* represent two alleles of the same gene, the eukaryotic translation initiation factor 4E (*Hv-eIF4E*, STEIN et al. 2005, KANYUKA et al. 2005). The recessive resistance-encoding allele *rym4*, derived from the Dalmatian landrace ‘Ragusa’ (HUTH 1985), confers resistance against BaMMV and BaYMV-1 but it is not

effective against BaYMV-2. In contrast *rym5*, which is derived from the Chinese six-rowed land race 'Mokusekko 3' (KONISHI et al. 1997, GRANER et. al. 1999a), confers resistance against BaMMV, BaYMV-1 and BaYMV-2. However, *rym5* has been recently overcome by the new German BaMMV strain and BaMMV-SIL (HARIRI et al. 2003, HABEKUSS et al. 2006). On chromosome 4H KONISHI et al. (1997) identified another recessive resistance gene, *rym1*, which also derived from 'Mokusekko 3' and confers resistance against all BaMMV and BaYMV strains. The resistance of 'Mokusekko 3' to all strains of the barley yellow mosaic virus complex in Japan and Europe, including BaMMV-SIL and the new German BaMMV strain, is the result of the combination of at least two genes, i.e. *rym1* and *rym5* (OKADA et al. 2003, OKADA et al. 2004, HABEKUSS et al. 2006). Another gene that confers resistance against the European and Japanese BaYMV but not against BaMMV is *rym3*, which was detected in 'Haganemugi' and 'Ea 52', which is a mutant of the Japanese cultivar 'Chikurin Ibaraki 1' (UKAI 1984, KAWADA 1991, ORDON et al.1993). *Rym3* was mapped by RFLP analysis on the short arm of chromosome 5H (SAEKI et al. 1999). By using a Japanese strain of BaYMV, the resistance gene *rym2*, derived from the variety 'Mihori Hadaka 3', was mapped on chromosome 7HL and *rym6* of 'Amagi Nijo' on chromosome 3HL (TAKAHASHI et al. 1973, IIDA et al. 1999). Whereas *rym2* confers resistance against BaMMV, BaYMV-1 and BaYMV-2, *rym6* donors are completely susceptible against European strains of the barley yellow mosaic virus complex (KONISHI et al. 2002). The resistance gene *rym7*, which confers partial resistance to BaMMV, has been mapped to the centromeric region of chromosome 1HS (GRANER et al. 1999b). At the telomeric region of chromosome 4HL four resistance genes*, rym8, rym9*, *rym12* and *rym13* are mapped, whereas *rym8, rym9* and *rym13* forming a gene cluster. Thereof, resistance gene *rym8* derived from the cultivar '10247' shows partial resistance against BaMMV and BaYMV (BAUER et al. 1997, GRANER et al. 1999b). *Rym9* confers resistance exclusively against BaMMV, whereas *rym12*, derived from the Korean cultivar 'Muju covered 2', shows a complete resistance against all strains of the Barley yellow mosaic virus complex in Europe (ORDON et al. 1993, GRANER et al. 1996, BAUER et al. 1997, SCHIEMANN et al. 1998). Furthermore, *rym13*, derived from the Taiwanese cultivar 'Taihoku A', shows a complete resistance to the Barley yellow mosaic virus complex (WERNER et al. 2003b, HABEKUSS et al. 2006). Further on,

Table 1: Mapped resistance genes against barley yellow mosaic virus disease, their source, resistance of donor and virus used for mapping (from Ordon et al. 2005, mod.).

Resistance gene	Chromosome	Source	Resistance of donor in Germany	Virus used for mapping	Reference
rym1	4HL	Mokusekko 3	BaMMV, BaYMV, BaYMV-2	BaYMV^{J}	Takahashi et al. 1973, Ordon et al. 1993, Konishi et al. 1997
rym2	7HL	Mihori Hadaka 3	BaMMV, BaYMV, BaYMV-2	BaYMV^{J}	Takahashi et al. 1973, Götz & Friedt 1993, Ordon et al. 1993
rym3	5HS	Ea 52	BaYMV, BaYMV-2	BaYMV^{J}	Götz & Friedt 1993, Ordon et al. 1993, Saeki et al. 1999
rym4	3HL	Ragusa,Franka	BaMMV, BaYMV	BaMMV, BaYMV	Götz & Friedt 1993, Graner & Bauer 1993, Ordon et al. 1993, 1995, Pellio et al. 2005
rym5	3HL	Mokusekko 3	BaMMV, BaYMV, BaYMV-2	BaYMV^{J}	Götz & Friedt 1993, Ordon et al. 1993, Graner et al. 1995, 1999a, Konishi et al. 1997, Pellio et al. 2005
rym6	3HL	Prior, Amagi Nijo	susceptible	BaYMV^{J}	Iida & Konishi 1994, Iida et al. 1999, Konishi et al. 2002
rym7	1HS	HHor 3365	BaMMV	BaMMV	Graner et al. 1999b
rym8	4HL	10247	BaMMV, BaYMV	BaMMV	Götz & Friedt 1993, Ordon et al. 1993, Bauer et al. 1997, Graner et al. 1999b
rym9	4HL	Bulgarian 347	BaMMV	BaMMV	Götz & Friedt 1993, Ordon et al. 1993, Bauer et al. 1997
rym10	3HL	Hiberna	BaYMV, BaYMV-2	BaYMV, BaYMV-2	Graner et al. 1995, 1999a
rym11	4HL	Russia 57	BaMMV, BaYMV, BaYMV-2	BaMMV	Götz & Friedt 1993, Ordon et al. 1993, Bauer et al. 1997, Nissan-Azzouz et al. 2005
rym12	4HL	Muju covered 2	BaMMV, BaYMV, BaYMV-2	BaMMV	Götz & Friedt 1993, Ordon et al. 1993, Graner et al. 1996
rym13	4HL	Taihoku A	BaMMV, BaYMV, BaYMV-2	BaMMV	Götz & Friedt 1993, Ordon et al. 1993, Werner et al. 2003b
Rym14^{HB}	6HS	*Hordeum bulbosum*	BaMMV, BaYMV, BaYMV-2	BaMMV, BaYMV, BaYMV-2	Ruge et al. 2003
rym15	6HS 5HS	Chikurin Ibaraki 1 Chikurin Ibaraki 1	BaMMV, BaYMV, BaYMV-2 BaMMV, BaYMV, BaYMV-2	BaMMV BaYMV, BaYMV-2	Le Gouis et al. 2004 Werner et al. 2003a
Rym16^{HB}	2HL	*Hordeum bulbosum*	BaMMV, BaYMV, BaYMV-2	BaMMV, BaYMV, BaYMV-2	Ruge-Wehling et al. 2006

^{J}Japanese strain of BaYMV

rym10, found in ‘Hiberna’, was assigned to chromosome 3HL (GRANER et al. 1995, GRANER et al. 1999a) and confers resistance against BaYMV-1 and BaYMV-2. Resistance gene *rym11* from the resistance donor ‘Russia 57’ has been mapped to the telomeric region of chromosome 4HL and confers resistance to all strains of the BaYMV complex (BAUER et al. 1997, NISSAN-AZZOUZ et al. 2005). The BaYMV/BaYMV-2 resistance of ´Chikurin Ibaraki 1´ has been located on chromosome 5HS (WERNER et al. 2003a) and the BaMMV resistance gene of this variety, called *rym15*, on chromosome 6H (LE GOUIS et al. 2004). In addition to these genes, two dominant resistance genes from *Hordeum bulbosum,* member of the secondary barley gene pool, are mapped on chromosome 6HS ($Rym14^{HB}$) and $Rym16^{HB}$ on chromosome 2HL (RUGE et al. 2004, RUGE-WEHLING et al. 2006). Regarding the new German BaMMV strain and BaMMV-Sil it turned out that *rym4, rym7, rym9, rym11, rym12, rym13, rym15,* $Rym14^{HB}$ and $Rym16^{HB}$, are effective against these strains (HABEKUSS et al. 2006).

2.2.2 Soil-borne cereal mosaic virus

Regarding the genetic base of resistance of bread wheat and durum wheat against *Soil-borne cereal mosaic virus* (SBCMV) several resistance tests were carried out and SBCMV resistant cultivars were identified (BUDGE & HENRY 2002, KANYUKA et al. 2003). These resistant varieties are reported to carry a translocation resistance, because all varieties show high virus levels in the roots (DRISKEL et al. 2002) but normally virus transmission to stems and leaves is restricted but may appear under certain environmental conditions (DRISKEL et al. 2002, HUTH & GOETZ 2007). The inheritance of *Soil-borne wheat mosaic viru*s (SBWMV) resistance, which is closely related to SBCMV, was investigated in several studies. The determinism of genetic resistance against this virus was described to be controlled by one dominant gene (MODAWI et al. 1982), two (BARBOSA et al. 2001) or even three genes (NAKAGAWA et al. 1959). In the United Kingdom SBCMV resistant cultivars were developed including genetic material of the resistant cultivars ‘Cadenza’, ‘Charger’ and ‘Claire’. Due to a recently established glasshouse-based resistance test, the monogenic inheritance of ‘Cadenza’ was identified (KANYUKA et al. 2004b). A study based on a doubled haploid (DH)-population of the cross ‘Avalon’ x ‘Cadenza’ reveals a 1:1 segregation ratio, giving hint to a monogenic mode of inheritance of the ‘Cadenza’ derived resistance. This resistance locus, referred to as *Sbm1*, was

mapped to the distal end of chromosome 5DL and closely linked microsatellite markers to the *Sbm1* locus were identified (BASS et al. 2006). Until now, it is still unknown whether the resistance of 'Cadenza' is related to a dominant, semi-dominant or a recessive inheritance due to the totally homozygous character of the used DH population (BASS et al. 2006). Regarding the pedigrees of 'Charger' and 'Claire' a genetic relation of these varieties to 'Cadenza' can be excluded. In this case, the Argentinean wheat cultivar 'Klein Rendidor', which shows also resistance against SBWMV, was identified as the resistance donor (MODAWI et al. 1982, BASS et al. 2006). Within the European wheat germplasm, two resistance sources against SBCMV are known, but further studies are necessary to confirm these presumptions (BASS et al. 2006).

With respect to WSSMV (see chapter 2.1.3) resistance sources have been found in some wheat species (COX et al. 1994, CADLE-DAVIDSON et al. 2006). In WSSMV resistance screenings a qualitative resistance was observed and therefore a high heritability controlled by a few dominant genes was assumed (KOEVERING et al. 1987). Due to difficulties in screening and mechanical inoculation of WSSMV, the identification of molecular markers is of high interest for the development of resistant cultivars. Hence, KHAN et al. (2000) identified one major gene resistance gene against WSSMV in a RIL population from a cross between the resistant variety 'Geneva' and the susceptible cultivar 'Augusta'. This resistance locus was mapped by RFLP markers on chromosome 2DL but due to the population type, the mode of inheritance could not be identified. Furthermore, a *Triticum aestivum-Haynaldia villosa* translocation line T4VS·4DL was developed, which shows resistance against WSSMV. The resistance locus was designated as *Wss1* and is located on 4VS (ZHANG et al. 2005). In several studies it has been demonstrated that the virus is detectable by DAS-ELISA in resistant varieties after mechanical inoculation in the greenhouse and even under natural conditions in the field (CARROLL et al. 2002, KANYUKA et al. 2003). Therefore, the WSSMV resistance has to be assigned as a tolerance, because distribution of the virus in the root system and virus transport from the roots into the leaves is limited (KANYUKA et al. 2003). These findings are in contrast to HUTH et al. (2002), who reported on immune wheat plants against WSSMV.

2.3 Molecular markers

Molecular markers or more generally speaking genetic markers detect genetic differences, i.e. polymorphisms, at the DNA level between individuals and species, respectively, whereas the variations are not visible in the phenotype except for morphological markers (JONES et al. 1997). Regarding a target gene or trait of interest, molecular markers act as flags because of their close localization to the gene of interest. Molecular markers, which are tightly linked to an agronomical important gene, can be used by breeders for marker-assisted selection (MAS), a tool for an early selection of difficult traits in plants (VARSHNEY et al. 2006). Random markers of unknown localisation and function can be used in pedigree studies and germplasm investigations to discover genetic relations based on the comparison of fingerprints. There are three different marker classes, mainly the morphological, the biochemical and the DNA-based markers (COLLARD et al. 2005). Morphological markers are visual traits, biochemical markers come up to differences in detected enzymes and are influenced by environmental factors. Therefore, DNA, respectively Polymerase chain reaction (PCR)-based molecular markers have been preferred in the last decades, because of their numerous occurrences in the genome and their neutral behaviour to environmental conditions (JOSHI et al. 1999).

2.3.1 DNA-based markers

2.3.1.1 Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs have primarily been used in human genome mapping (BOTSTEIN et al. 1980), the first organism for which polymorphisms were detected in coding sequences. The procedure of this molecular marker method is divided into two steps. The first step is based on the restriction endonuclease digestion of DNA, where the restriction enzyme recognizes and cleaves specific nucleotide sequences and therefore variations in the restriction site arise as a result of restriction fragments of different sizes (JONES et al. 1997). The whole range of different DNA fragments are separated by gel electrophoresis and transferred to a membrane by Southern blotting (SOUTHERN 1975). In a second step hybridisation to a labelled probe visualises DNA fragments of different size (polymorphisms). RFLPs were mainly used in the 1990s for creating linkage maps (GRANER et al. 1995, SAGHAI-MAROOF et al.

1996) or the assessment of genetic diversity in different crop plants like oilseed rape (for review cf. SNOWDON & FRIEDT 2004) or barley (RUSSELL et al. 1997). The major advantage of this method is its reliability and transferability to other populations although RFLPs are very time-consuming.

2.3.1.2 Random Amplified Polymorphic DNAs (RAPDs)

In 1983 the Polymerase chain reaction (PCR) was developed (MULLIS & FALOONA 1987), which facilitated the efficient development of molecular markers. The PCR is based on the amplification of a specific single nucleic acid sequence. To achieve this, three steps are needed. First of all double-stranded DNA is denaturated followed by an annealing step, where the primers attach to the single-stranded DNA template. The third step is the elongation of the DNA template. During the last step the Taq DNA polymerase isolated from a bacterium called *Thermus aquaticus* (CHIEN et al. 1976), synthesises a complementary DNA strand defined by the primers, and thus copies the DNA sequence between the primer annealing sites. RAPDs are based on using only a single primer of about 8-10 nucleotides for DNA amplification (WILLIAMS et al. 1990). This decamer-primer acts as forward and reverse primer. RAPDs are able to generate a large number of fragments of different size. Polymorphisms are detected by gel electrophoresis and thus RARD markers are identified due to the sequence differences in the primer binding sites. Therefore, RAPDs are dominant markers. Furthermore, the method is relatively cheap and easy to handle. The main disadvantages of these PCR-based markers are their lack of reproducibility and their non-transferability to other plants (SCHLÖTTERER 2004). Further on, RAPDs are used as specific markers in diversity studies (RUSSELL et al. 1997, SIMIONIUC et al. 2002) as well as in genetic mapping for identification and localisation of e.g. resistance genes (ORDON et al. 1995, SCHIEMANN et al. 1997, PELLIO et al. 2004).

2.3.1.3 Amplified Fragment Length Polymorphisms (AFLPs)

AFLPs are based on the selective PCR amplification of restricted fragments (ZABEAU & VOS 1993). This technique is divided into three different steps. In the first one, genomic DNA is digested by two different restriction enzymes, a frequently cutting enzyme (e.g. *Mse*I, 4bp recognition sequence) and another one cutting less

frequently (e.g. *Eco*RI, 6bp recognition sequence). The resulting fragments are ligated to restriction enzyme site specific adapters. The selective amplification of sets of restriction fragments follows in a second step. In the PCR reaction primers are used, which are complementary to the adapter sequences except for the presence of one to three additional bases at the 3' end arbitrarily chosen by the user. These selective amplifications lead to a reduction in the number of amplified fragments to 1/16 and 1/256, respectively. The third step complies with a gel analysis where the PCR products are visualised on a polyacrylamide (PAA) gel (VOS et al. 1995). The polymorphisms, which are observed, are the results of insertions, deletions and point mutations at the restriction sites, respectively. With AFLPs it is possible to detect more than 100 DNA fragments in just one PCR. The disadvantage of the AFLPs is their dominant inheritance and therefore the difficulty to identify homologous alleles. In this case their reduced informativeness leads to problems in mapping e.g. F_2 generations with heterozygous individuals (MUELLER & WOLFENBARGER 1999, SAAL et al. 2002). Nevertheless, the AFLP method has a lot of advantages like its high reproducibility, the quality of information, the ease of handling and the high grade of polymorphisms detected. Therefore, AFLP markers are often used for DNA fingerprinting, fine mapping of genes, genetic diversity analyses and for the construction of genetic linkage maps (SCHIEMANN et al. 1999, UPTMOOR et al. 2003, ABU-ASSAR et al. 2005, NISSAN-AZZOUZ et al. 2005, STODART et al. 2005, BRATTELER et al. 2006).

2.3.1.4 Simple Sequence Repeats (SSRs)

SSRs or microsatellites are tandemly arranged repeats of several nucleotides, which are present in the vast majority of eukaryotic genomes (DÁVILLA et al. 1999, RAKOCZY-TROJANOWSKA et al. 2004). The frequencies of SSRs vary significantly among different organisms. The most common SSRs in plants are dinucleotide repeats including $(AT)_n$, $(GT)_n$ and $(GA)_n$ (GUPTA & VARSHNEY 2000), whereas $(AC)_n$ is one of the most frequent SSRs in mammals (TOTH et al. 2000). SSRs are, due to their variation in the number of repeat units, highly polymorphic and flanked by highly conserved genomic regions. SSR markers are in general inherited codominantly, have a moderate abundance and good genome coverage. The main advantages of SSRs are their multi-allelic nature, the reproducibility, their unambiguous designation of alleles and their locus specificity (LI et al. 2000,

MACAULY et al. 2001, PARIDA et al. 2006). These properties have made SSRs a powerful tool for genetic mapping, genome analysis and population genetics (SCHLÖTTERER 2004). SSRs based linkage maps have been developed in all major cereals such as barley (RAMSAY et al. 2000, LI et al. 2003), wheat (ROEDER et al. 1998, SOMERS et al. 2004), maize (SHAROPOVA et al. 2002), and rice (MCCOUCH et al. 1997, 2002). In wheat and barley significant progress has been made by sequencing expressed sequence tags (ESTs) derived from SSRs for high density mapping (THIEL et al. 2003, STEIN 2007, VARSHNEY et al. 2007). Furthermore, SSRs have been used for genetic diversity studies in many plant species e.g. sorghum (UPTMOOR et al. 2003, ABU-ASSAR et al. 2005), oat (LI et al. 2000), wheat (HAMMER et al. 2000), and barley (ROUSSEL et al. 2004, PANDEY et al. 2006).

2.3.1.5 Single Nucleotide Polymorphisms (SNPs)

SNPs represent the marker of choice during the last years and are based on a single-base change in the DNA sequence (point-mutation), usually with an alternative of two possible nucleotides at a specific position (VIGNAL et al. 2002). In the human genome a total of ten million SNPs were detected, whereas over five million SNPs possess a minor allele frequency of more than 10% (BOTSTEIN & RISCH 2003). Furthermore, SNPs are distributed over the whole human genome at an estimated frequency of one SNP every 506 bp (CARLSON et al. 2003). SNPs are bi-allelic, codominant markers and regarding the modification or expression of a gene in non-coding regions they are mostly silent. Moreover, SNPs have great potential for automation and therefore for high-throughput screening (GUPTA et al. 2001). In general, SNPs are used for association studies due to their high frequency in the genome and their stability. Regarding the fully sequenced human genome the location of the allelic variations is known. In linkage analysis studies of different plants it could be confirmed that SNPs are very common in plant genomes. CHING et al. (2002) found one SNP per 60 bp in outbreeding maize, in wheat one SNP every 212 bp (RAVEL et al. 2006) was reported, one SNP per 300 bp was detected in rice and *Arabidopsis thaliana* (SCHMID et al. 2003, YU et al. 2005), and in barley SNPs were found every 200 bp (ROSTOKS et al. 2005), whereas there was one SNP every 50 bp (RUSSELL et al. 2004) and 58 bp (NEUHAUS et al. 2004), respectively, in samples including varieties of *Hordeum spontaneum* and *Hordeum vulgar*e.

To identify SNPs various strategies have been developed (LANDEGREN et al. 1998). One method is the heteroduplex analysis of DNA molecules by density gradient gel electrophoresis (DGGE). Electronic dot blot assays and denaturing high-performance liquid chromatography (DHPLC) are further well-suited methods (KOTA et al. 2001, SHIRASAWA et al. 2006). Furthermore, mass-spectroscopy using MALDI-TOF (Matrix Assisted Laser Desorption/Ionisation Time-of-Flight), microarray technology, EcoTilling and single strand conformation polymorphism (SSCP) are used to score SNPs (STOERKER et al. 2000, ANDERSEN et al. 2003, COMAI et al. 2004, WANG et al. 2005).

There are still a lot of other molecular markers, mostly variations of the mentioned procedures above, which are based on point mutations in the DNA sequence and are used for genetic diversity studies or linkage mapping. A few recently developed methods with high potential are listed. One of these techniques are the single feature polymorphisms (SFPs), which are identified in transcript profiling data by visualizing differences in hybridisation to individual oligonucleotide probes (VARSHNEY et al. 2005, WEST et al. 2006). The polymorphisms present in the DNA are transcribed into the messenger RNA and may affect hybridization to the microarray probes if located in a region complementary to the probe. SFPs detected using high density oligonucleotides microarrays such as the Barley1 Affymetrix GeneChip (CLOSE et al. 2004) can serve as function-associated markers for genetic analyses including quantitative trait loci (QTL) and linkage disequilibrium (LD) mapping. Further on, Diversity Arrays Technology (DArT) enables the profiling of the whole genome without any DNA sequence information. This method is based on the microarray hybridisation which detects the presence or absence of a specific DNA fragment from the whole genomic DNA of an individual or a whole population (JACCOUD et al. 2001, WENZL et al. 2004). Therefore, this technology generates a large number of high-quality markers in several crop species like barley (WENZL et al. 2004), *Arabidopsis thaliana* (WITTENBERG et al. 2005), cassava (XIA et al. 2005), wheat (AKBARI et al. 2006), and pigeonpea (YANG et al. 2006).
Besides the already described marker techniques, several methods are described to convert already existing unspecific PCR-markers to more robust ones, which are easier and less laborious to use. To specify point mutations-based AFLPs or RAPDs it is necessary to convert them into more stable single locus PCR markers like

Cleaved Amplified Polymorphic Sequences (CAPS), Sequence Characterised Amplified Region (SCAR) or Sequence Tag Sites (STS). These techniques are based on sequencing and design of specific primer pairs. Expressed Sequence Tags (ESTs), which are useful tools in gene discovery, comply with STS markers. Their sequence and location in the genome are known but ESTs derive from cDNA clones (JOSHI et al. 1999). In addition, there are several functional and gene targeted markers described like ACGMs (Amplified consensus genetic markers), GSTs (Gene specific tags), RGAs (Resistance gene analogues) or ERAP (Exon-Retrotransposon amplification polymorphism), which are ideal tools for marker-assisted selection (GUPTA & VARSHNEY 2000, ANDERSEN & LÜBBERSTEDT 2003, GUPTA & RUSTGI 2004, BAGGE et al. 2007).

2.4 Application of molecular markers in plant breeding

The development of molecular markers was an important step for plant breeding and opened a new area of molecular plant breeding. Molecular markers and especially PCR-based marker systems facilitate genotyping and the assessment of genetic diversity, the construction of linkage maps and the application in marker-assisted breeding. Further on, molecular markers ease pyramiding of genes, e.g. resistance genes, the detection of Quantitative trait loci (QTL) as well as the acceleration of back crossing procedures (ORDON et al. 2004b, WERNER et al. 2005).

2.4.1 Genetic linkage maps

To construct a genetic linkage map the grouping of linked markers into linkage groups and the arrangement of the known markers to each other within this group is necessary. This involves coding data for each marker on each individual of a segregating population, e.g. a DH population, and later on linkage analysis using software programmes like MapMaker (LANDER et al. 1987) or JoinMap (STAM & VAN OOIJEN 1995) to detect linkage groups and construct genetic maps. The linkage between two markers is usually measured by likelihood of odds ratio, which calculates the ratio of linkage versus no linkage (COLLARD et al. 2005). This ratio is worded as the logarithm of the ratio and is called a logarithm of the odds value (LOD) or LOD score (RISCH 1992). Usually, LOD values over 3.0 are taken for the construction of linkage maps, viz this value between two markers indicates that the

linkage is 1,000 times more likely than no linkage. The arrangement of markers is based on the frequencies of recombination between them. By means of mapping functions, recombination fractions are converted into genetic distances assessed in centiMorgan (cM), because of the non-linearity of recombination frequency, i.e. the frequency of crossing-over (COLLARD et al. 2005). The Kosambi mapping function (KOSAMBI 1944) and the Haldane mapping function (HALDANE 1919) are the most commonly used ones. Whereas Haldane expects no interference between crossing over, Kosambi assumes that a recombination event gains influence on the occurrence of a neighbouring recombination event (HARTL & JONES 2001). Genetic linkage maps are necessary for the identification of chromosomal regions, which possess 'genes of interest' or traits controlled by one or more genes, the identification of genetic markers closely linked to these important traits, for synteny studies (comparing genomes of different species) or for genome sequencing (MOHAN et al. 1997). The first barley linkage map was constructed by KLEINHOFS et al. (1988) with RFLP markers for chromosome 6H. A few years later more detailed maps of the whole genome were created based on different types of populations (GRANER et al. 1991, HEUN et al. 1991, KLEINHOFS et al. 1993). Other markers like AFLPs (WAUGH et al. 1997) or SSRs (BECKER & HEUN 1995, LIU et al. 1996, LI et al. 2003) were integrated in already existing maps to enhance the marker density. RAMSAY et al. (2000) established the first linkage map using only microsatellites. Further on, EST-derived SSRs were integrated into molecular maps (PILLEN et al. 2000, THIEL et al. 2003). Herefrom, a strong clustering of microsatellites markers around the centromeres of all chromosomes was observed (RAMSAY et al. 2000, LI et al. 2003), which results from suppressed recombination events in the centromeric regions (KÜNZEL et al. 2000) and leads further on to an incomplete genome coverage. Among others, WENZL et al. (2006) constructed a barley consensus map, which combines different maps with DArT markers to improve the genome coverage. Corresponding dense molecular linkage maps of other crops of worldwide importance like rice (MCCOUCH et al. 2002), maize (SHAROPOVA et al. 2002), sorghum (MENZ et al. 2002), wheat (SOMERS et al. 2004, SONG et al. 2005), rape seed (KIM et al. 2006) and grapevine (DOLIGEZ et al. 2006) are available.

The knowledge of the position of molecular markers on these linkage maps is very useful for the identification of closely linked markers to genes encoding important

traits, and allows e.g. the precise localization of resistance genes. Several recessive resistance genes are mapped in barley using Bulked Segregant Analysis (BSA, see chapter 3.5, MICHELMORE et al. 1991). One of the first successful reports on the application of BSA in barley was the mapping of resistance genes against powdery mildew by GIESE et al. (1993), where the RFLP marker *ris16* was closely mapped to the resistance gene *MlLa* on chromosome 2H within a distance of 1 cM. Furthermore, GARVIN et al. (2000) mapped the scald resistance gene *Rrs14* by using BSA on chromosome 1H closely linked to the STS marker *Hor2* with a distance of 1.8 cM to the resistance locus (for an overview of all resistance genes already mapped by close association with DNA markers see CHELKOWSKI et al. (2003), WILLIAMS (2003) and ORDON et al. (2004b)). A high number of studies have demonstrated the identification of Quantitative Trait Loci (QTL) in many crop species based on existing genetic linkage maps. The principle of QTL analysis is to separate the mapping population into different groups with respect to the presence or absence of a genotype at a marker locus and to determine the differences, which exist between these groups on the phenotypic level with respect to a quantitative trait. If the phenotypes between groups differ significantly, the marker locus, which partitions the groups, is linked to a QTL. There are three different methods to detect a QTL: (1) single-marker analysis, (2) simple interval mapping (SIM) and (3) composite interval mapping (CIM, COLLARD et al. 2005), whereas CIM is the most common one (JANSEN & STAM 1994). In cereals, many QTL for major agronomic traits have been described. In barley, several markers for QTL of agronomic traits have been identified so far. These works include QTL for yield (VON KORFF et al. 2006), disease resistances like barley yellow dwarf virus (SCHEURER et al. 2000) or scald (ZHAN et al. 2007), and leaf rust (MARCEL et al. 2007). Further on, e.g. SOMERS et al. (1998) identified RAPD markers linked with linoleic acid desaturation in *Brassica rapa*, and AFLP and SSR markers could be detected for Fusarium head blight resistance in wheat (BUERSTMAYR et al. 2002, LIU & ANDERSON 2003). In other crop species NARASIMHAMOORTHY et al. (2007) recently found markers for QTL associated with the aluminium tolerance in alfalfa.

These molecular markers, which are closely linked to a gene of interest or to a QTL, can be used for marker-assisted selection (MAS). The specific DNA marker alleles can be applied for an indirect selection of DH populations, which are used for fixation of the traits, to identify genes of interest in the seedling stage and furthermore to

screen for the genetic background (VARSHNEY et al. 2006, TUVESSON et al. 2007). This method simplifies the work of plant breeders due to the great efficiency of marker assisted selection (MAS). One example of the sufficient use of MAS in barley breeding is the incorporation of resistances into the existent barley breeding materials against the barley yellow mosaic virus complex. Until now, several markers for the selection of resistance gene loci have been developed (ORDON et al. 2003, 2004b). The most common one is the SSR marker Bmac0029, which is used by many barley breeders for the selection of the *rym4* and *rym5* resistance genes (RAE et al. 2007). Furthermore, MAS offers the opportunity for the accomplishment of gene pyramiding. This has been shown in many crops like wheat (LIU et al. 2000), cotton (GUO et al. 2005), rice (ZHANG et al. 2006) and barley (WERNER et al. 2005, 2007). The use of tightly linked markers to a gene of interest is also the basis for map-based cloning, in which the marker is used as a probe for the screening of a genomic library (COLLARD et al. 2005), e.g. in barley based on a high resolution mapping (PELLIO et al. 2005). The resistance locus *rym4/rym5* was isolated (STEIN et al. 2005) facilitating the production of ideal diagnostic marker, i.e. allele specific markers. The map based cloning strategy has been applied in several crop species (for overview STEIN & GRANER 2004).

2.4.2 Genetic diversity

Genetic diversity represents the multifariousness within and between groups of individuals or populations. The knowledge of this pool of genetic variation for these individuals or within a population is necessary for breeding purposes (RAO & HODGKIN 2002). Genetic diversity is estimated based on differences in DNA sequences and these DNA-based marker data facilitate the reliable differentiation of genotypes. Molecular marker-based genetic diversity can be expressed and presented by different estimators and approaches like genetic diversity, genetic similarity respectively distance, population structure and cluster analysis (LABATE 2000).

Frequently used methods for the estimation of genetic similarity and distance, respectively, are the NEI and LI coefficient (1979), JACCARDs coefficient (1908), modified ROGERs' distance (WRIGHT 1978) and the *simple matching coefficient* (SMC, SNEATH & SOKAL 1973). All are based on binary data, which count the presence or absence of fragments or the allele frequency. The major differences

between these four methods are due to their emphasis of monomorphic or polymorphic alleles. Whereas JACCARD just considers fragments, which are present in all individuals, and ignores fragments, which are absent in both individuals. NEI & LI measures the proportion of alleles, which are present and shared in each individual. Modified ROGERs distance includes every locus scored as an orthogonal dimension and SMC considers the fragments, which are present and absent (MOHAMMADI et al. 2003). Due to this different emphasis on present and absent alleles, JACCARD is commonly used for dominant markers and NEI & LI for codominant markers (SCHÖN et al. 1997). Based on the matrix of genetic distances/similarities cluster analyses can be carried out. Cluster analysis is a statistical procedure, which groups individuals or populations into subsets or clusters based on their common traits. The clustering methods can be differentiated into two groups, herein after referred to as (1) the distance-based method and (2) the Bayesian model-based method. The main principle of the first one is the calculation with a pair-wise distance matrix as an input, whereas the model-based method assumes that the observations from each cluster are random draws from some parametric model (PRITCHARD et al. 2000). Distance-based methods are divided into two groups: (1) hierarchical procedure, where single individuals are treated separately before grouping into bigger clusters, and (2) non-hierarchical procedures, which is rarely used for the estimation of genetic diversity (MOHAMMADI et al. 2003). Among different hierarchical procedures known, the *Unweighted Paired Group Method using Arithmetic averages* (UPGMA) is due to the high level of accuracy the most frequently used one (MOHAMMADI et al. 2003).

The genetic diversity (H) is based on the number of alleles per locus and the frequency of alleles per locus. The most frequently used index is the gene diversity index by NEI (1973), which is a measure of the probability that two genotypes chosen randomly out of the population possess different alleles (KREMER et al. 1998). Another diversity measure is the Shannon-Weaver Index (H', SHANNON & WEAVER 1949). In contrast to the gene diversity index by NEI (1973) the Shannon-Weaver Index doesn't prerequisite the Hardy-Weinberg equilibrium (FRITSCH & RIESEBERG 1996). Genetic diversity is of prime interest for plant breeding. Due to the variation in allele frequency within species a selection is possible to change populations and to introduce new varieties into breeding populations. Furthermore, the breeding system of the species is significant for the evaluation of differences between populations

from different geographical regions (RAO & HODGKIN 2002). For the estimation of genetic diversity DNA-based markers are an efficient tool. Attention should be paid to the differences in genetic diversity resulting from different markers and their amount of genome coverage (STAUB et al. 1997). In cereals and other crop species, many studies about genetic diversity have been described, e.g. in barley (AHLEMEYER et al. 2006, PANDEY et al. 2006), wheat (REIF et al. 2005, HAI et al. 2007) or rapeseed (HASAN et al. 2006).

3 Material and Methods

3.1 Plant Material

3.1.1 Identification of new resistance resources of barley against the barley yellow mosaic virus complex

120 exotic barley germplasms, resistant against BaYMV in Japan, have been screened with the microsatellite marker Bmac0029 closely linked to the *rym4/rym5* locus (GRANER et al. 1999a) in order to identify new resistance donors carrying resistance genes different from *rym4* and *rym5* which are at present widely used in European barley breeding programmes. Most of the 120 barley accessions mainly originated from China, Nepal, Japan, Russia, Ethiopia and Turkey (Table 2) were provided by the Barley Germplasm Centre, Research Institute for Bioresources, Okayama University, Japan.

Table 2: New resistance resources of barley against the BaYMV-complex.

Name	Origin	Name	Origin
J. 20	Afghanistan	Debra Birhan 1	Ethiopia
9055	Austria	Debra Birhan 7	Ethiopia
Baku 3	Azerbaijan	Deder 2	Ethiopia
Shemakha 1	Azerbaijan	Dembi 3	Ethiopia
Shemaka 2	Azerbaijan	Ethiopia 14	Ethiopia
Shemakha 3	Azerbaijan	Ethiopia 53	Ethiopia
Chiuchiang	China	Ethiopia 65	Ethiopia
Chihchou Yinchiaai 3	China	Ethiopia 80	Ethiopia
Hsingwuke 2	China	Ethiopia 89	Ethiopia
Juichang 2	China	Ethiopia 506	Ethiopia
Liussuchiao 1	China	Ethiopia 510	Ethiopia
Liussuchiao 2	China	Ethiopia 534	Ethiopia
Paishapu 2	China	Gondar 6	Ethiopia
Paoanchen 1	China	Glyorgi 2	Ethiopia
Shanghai 1	China	Kulubi 1	Ethiopia
Tatung	China	Mota 1	Ethiopia
Addis Ababa 64	Ethiopia	Mota 7	Ethiopia
Adi Abun 2	Ethiopia	Nazareth 3	Ethiopia
Dabat 1	Ethiopia	Sululta 4	Ethiopia

Name	Origin	Name	Origin
Sululta 10	Ethiopia	Jungbori 20	Korea
France 7	France	Masan Covered 5	Korea
Tibilisi 1	Georgia	Sacheon Naked	Korea
Tibilisi 7	Georgia	Samcheog Dolbori	Korea
Mammuto	Germany	Suweon 31	Korea
Esfahan 1	Iran	Waegwan Covered 1	Korea
Esfahan 4	Iran	Yeoncheon Native	Korea
Gorgan 1	Iran	Zairaishu	Korea
Ramsar	Iran	Zairai Junkei 8	Korea
70 g	Iran	Chame 8	Nepal
Chikurin Ibaraki 3	Japan	Dhumpu 2	Nepal
Fushiguro	Japan	Keronja 2	Nepal
Hakusanmugi	Japan	Keronja 3	Nepal
Hanhadaka 2	Japan	Keronja 5	Nepal
Hayamugi	Japan	Sikha 10	Nepal
Hiroshima	Japan	Sipche 14	Nepal
Hosomugi 3	Japan	Thonje 16	Nepal
Iwate Hozoroi 1	Japan	Thonje 19	Nepal
Kinukawa Gozen 22	Japan	Tsumje 3	Nepal
Kobinkatagi 4	Japan	Katana 2	Syria
Koshimaki 40	Japan	Turkey 3	Turkey
Nagaoka	Japan	Turkey 29	Turkey
Oeyama Rokkaku 3	Japan	Turkey 33	Turkey
Sakaiwa Rokkaku 27	Japan	Turkey 39	Turkey
Sekitori 2	Japan	Turkey 41	Turkey
Shiro Omugi 79	Japan	Turkey 44	Turkey
Taishomugi	Japan	Turkey 45	Turkey
Tochigi Torano-o 1	Japan	Turkey 47	Turkey
Torano-o	Japan	Turkey 56	Turkey
Torano-o 7	Japan	Turkey 62	Turkey
Baegsan Santoku 1	Korea	Turkey 68	Turkey
Boseong Covered 3	Korea	Turkey 77	Turkey
Changweon Jecheon 5-1	Korea	Turkey 83	Turkey
Cheongyang Covered 2	Korea	Turkey 86	Turkey
Gangneung Covered 3	Korea	Turkey 101	Turkey
Gogseong Covered 4	Korea	Turkey 179	Turkey
Goheung Covered 2	Korea	Turkey 440	Turkey
Gwangju Baitori 1	Korea	Turkey 524	Turkey
Hamyang Covered 9	Korea	Turkey 581	Turkey
Hongcheon Anjeunbaengi 2	Korea	Turkey 723	Turkey
Hongseong Native	Korea	Russia 4	USSR

3.1.2 Mapping populations used for the development of new PCR-based DNA markers for resistance genes against BaMMV, BaYMV-1 and BaYMV-2

For the mapping approach of currently unknown resistance genes against the BaYMV complex seven different crosses with original exotic resistance donors have been generated and used. The mapping populations have been provided by the plant breeding companies Pajbjergfonden, Odder, Denmark, Florimond-Desprez, Cappelle en Pévèle, France and the Institute of Crop Science and Plant Breeding I, University of Giessen and herein referred to as MAP1-7.

3.1.2.1 Mapping population 1 (MAP 1)

The doubled haploid (DH) population MAP1 consists of 94 lines derived from a cross between the resistance donor ‘Cebada’ and the German susceptible two-rowed cultivar ‘Cleopatra’.

3.1.2.2 Mapping population 2 (MAP 2)

A number of 54 anther-derived DH lines of the Japanese cultivar ‘Shimane Omugi’ crossed with the susceptible cultivar ‘Sumo’ as well as 65 additional DH lines of the cross ‘Shimane Omugi’ with the German susceptible two-rowed cultivar ‘Gilberta’ were used for genetic mapping.

3.1.2.3 Mapping population 3 (MAP 3)

MAP 3 was developed by crossing the resistance donor ‘CI 3517’ with the susceptible two-rowed cultivar ‘Reni’ and comprises 80 DH lines.

3.1.2.4 Mapping population 4 (MAP 4)

A progeny of 131 DH lines of the cross between the resistance donor ‘Belts 1823’ and the German cultivar ‘Franziska’ were used for marker development. ‘Franziska’ is carrying *rym4* and is therefore known to be resistant against BaMMV and BaYMV-1 in Europe.

3.1.2.5 Mapping population 5 (MAP 5)

The Japanese resistant six-rowed cultivar ‘Chikurin Ibaraki 1’ was crossed with the German susceptible two-rowed winter barley cultivar ‘Igri’. The DH population, which

derived from the F1 generation by anther culture, comprised 163 DH lines. ‘Chikurin Ibaraki 1’ shows resistance against all three types of the BaYMV complex in Europe but is susceptible to BaYMV in Japan (GOETZ & FRIEDT 1993).

3.1.2.6 Mapping population 6 (MAP 6)

A subset of the original population from the cross between the Taiwanese six rowed cultivar ‘Taihoku A’ and the French susceptible cultivar ‘Plaisant’ (WERNER et al. 2003b) was used for the development of closer linked markers. The original subset comprised 90 DH lines which was later enlarged to 154 DH lines of the same cross. ‘Taihoku A’ is known to be resistant to BaMMV and BaYMV/BaYMV-2 (GOETZ & FRIEDT 1993).

3.1.2.7 Mapping population 7 (MAP 7)

MAP 7 is composed of 151 DH lines derived from a cross of the Korean resistance donor ‘Muju covered 2’ with the susceptible cultivar ‘Spirit’. Like ‘Taihoku A’, ‘Muju covered 2’ is resistant to BaMMV, BaYMV/BaYMV-2, and to the new German BaMMV-strain (GOETZ & FRIEDT 1993, HABEKUSS et al. 2006).

3.1.3 Wheat cultivars used for fingerprinting and studies on genetic diversity

Different wheat lines provided by different co-operation partners (W. von Borries-Eckendorf, Germany; Pajbjergfonden, Denmark; Florimond-Deprez, France) were screened for resistance against SBCMV and WSSMV in France in 2003 and 2004. Based on resistance screening in the field, 64 interesting wheat lines were selected and used for genotyping (Table 3).

Table 3: Selected wheat genotypes for fingerprinting.

Name	Provided by	Reaction to SBCMV/WSSMV	Name	Provided by	Reaction to SBCMV/WSSMV
Asperge	Florimond Desprez	resistant	701-477c	Pajbjergfonden	resistant
Autan	Florimond Desprez	resistant	701-481a	Pajbjergfonden	resistant
Bobino	Florimond Desprez	resistant	798-398b	Pajbjergfonden	susceptible
Brando	Florimond Desprez	resistant	BE01	W. v. Borries-Eckendorf	resistant
Cadenza	Florimond Desprez	resistant	BE02	W. v. Borries-Eckendorf	resistant
Charger	Florimond Desprez	resistant	BE03	W. v. Borries-Eckendorf	resistant
Claire	Florimond Desprez	resistant	BE04	W. v. Borries-Eckendorf	resistant
Enesco	Florimond Desprez	resistant	BE05	W. v. Borries-Eckendorf	resistant
Farandole	Florimond Desprez	resistant	BE06	W. v. Borries-Eckendorf	resistant
Gaspard	Florimond Desprez	resistant	BE07	W. v. Borries-Eckendorf	resistant
Gascogne	Florimond Desprez	resistant	BE08	W. v. Borries-Eckendorf	resistant
Igor	Florimond Desprez	resistant	BE09	W. v. Borries-Eckendorf	resistant
Intense	Florimond Desprez	resistant	BE10	W. v. Borries-Eckendorf	resistant
Levis	Florimond Desprez	resistant	BE12	W. v. Borries-Eckendorf	resistant
Rubens	Florimond Desprez	resistant	BE13	W. v. Borries-Eckendorf	resistant
Sponsor	Florimond Desprez	resistant	BE14	W. v. Borries-Eckendorf	resistant
Taldor	Florimond Desprez	resistant	BE15	W. v. Borries-Eckendorf	resistant
Tremie	Florimond Desprez	resistant	BE16	W. v. Borries-Eckendorf	resistant
701-37c	Pajbjergfonden	resistant	BE17	W. v. Borries-Eckendorf	resistant
701-42c	Pajbjergfonden	susceptible	BE18	W. v. Borries-Eckendorf	resistant
701-176a	Pajbjergfonden	resistant	BE19	W. v. Borries-Eckendorf	resistant
701-176c	Pajbjergfonden	resistant	BE20	W. v. Borries-Eckendorf	resistant
701-177a	Pajbjergfonden	resistant	BE21	W. v. Borries-Eckendorf	resistant
701-177c	Pajbjergfonden	resistant	BE22	W. v. Borries-Eckendorf	resistant
701-191a	Pajbjergfonden	susceptible	BE23	W. v. Borries-Eckendorf	resistant
701-210a	Pajbjergfonden	resistant	BE24	W. v. Borries-Eckendorf	resistant
701-210b	Pajbjergfonden	susceptible	BE25	W. v. Borries-Eckendorf	resistant
701-244c	Pajbjergfonden	resistant	BE26	W. v. Borries-Eckendorf	resistant
701-256b	Pajbjergfonden	resistant	BE27	W. v. Borries-Eckendorf	resistant
701-372c	Pajbjergfonden	resistant	BE28	W. v. Borries-Eckendorf	resistant
701-422b	Pajbjergfonden	resistant	BE29	W. v. Borries-Eckendorf	resistant
701-477b	Pajbjergfonden	susceptible	BE30	W. v. Borries-Eckendorf	resistant

3.2 Evaluation of virus resistance

The reaction against BaMMV was estimated after mechanical inoculation in the greenhouse according to FRIEDT (1983) in two replications comprising five plants per DH-line. The inoculation was carried out with plant sap extract of BaMMV-infected leaf material of the cultivar ‘Gerbel’. The sap was diluted 1:10 in K_2HPO_4

buffer (0.1 M; 9.1 pH), mixed with carborundum powder (0.5 g/25 ml) and applied by using a spray gun with 8 bar pressure. The youngest and second youngest leaves were sprayed from both sides with an average of 2.5 ml diluted sap. The inoculated plants were briefly rinsed under tap water and kept for one day in the shade at 18°C. Afterwards the plants were transferred to a cooled green house chamber at 16°C. Four weeks after inoculation resistance was estimated by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA, KOENIG 1985).

Since neither BaYMV nor BaYMV-2 can be transmitted mechanically at a sufficient infection level, field experiments were performed in 2003/2004 and in 2004/2005 at three locations which were either infested with BaMMV, BaYMV (Giessen, Hesse) or additionally with BaYMV-2 (Eikeloh, Northrhine-Westphalia and Lenglern, Lower Saxony). Besides visual assessment, the resistance reaction against the two different virus strains was determined by DAS-ELISA using specific antisera against BaMMV and BaYMV (kindly provided by Dr. Frank Rabenstein, Federal Centre for Breeding Research, Quedlinburg, Germany). Optical density was estimated photometrically at 405 nm and 620 nm reference wavelengths (Easy Reader 400 ATX, SLT-Labinstruments, Crailsheim).

Regarding the new German BaMMV strain the resistance reaction of MAP 6 was estimated by Dr. Antje Habekuß, Federal Centre for Breeding Research, Institute of Epidemiology and Resistance Resources, Quedlinburg.

Resistance against SBCMV and WSSMV was scored visually by two different breeders of the breeding companies Borries-Eckendorf and Florimond-Deprez at an infested field at Vatan, France, in the years 2003 and 2004. The cultivars and wheat lines were sown in two replications, whereas every replication comprised a double row. The severity of virus infection was easy to differentiate, so it was possible to score the symptoms using the complete range from 1 (resistant) to 9 (susceptible).

3.3 Molecular analysis

3.3.1 DNA extraction and measurement of DNA concentrations

DNA was isolated from two weeks old leaves as described by Doyle & Doyle (1990). For this purpose, the frozen plant material was grounded with liquid nitrogen to a fine powder. 200 mg of plant material together with 700 µl of the CTAB-extraction buffer

were homogenised and incubated at 65°C for 20 to 30 minutes. To separate polysaccharides, 700 µl of chloroform/isoamylalcohol (CIA, 24:1 [v/v]) were added to the solution and shaken for 5 minutes. After a centrifugation step at 4°C during 10 min at 10,000 rpm the upper phase was removed and mixed with 600 µl of CIA. After shaking the samples for 5 minutes, centrifugation was again carried out and the liquid phase was transferred to a new 1.5 ml reaction tube and loaded with 50 µl 10 M ammonium acetate (NH_4OAc), 60 µl 3 M sodium acetate (NaOAc) and 500 µl cold isopropanol. Upon slight swivelling, the DNA precipitated and formed a DNA pellet after centrifugation at 4°C and 4,000 rpm for 4 minutes. The supernatant was discarded and the DNA pellet was washed with 200 µl washing buffer (70 % ethyl alcohol/10 mM ammonium acetate) for at least 10 minutes. After drying, the DNA was dissolved in 100 µl TE-Buffer (10 mM Tris HCl, 1 mM EDTA, pH 8). RNA impurities were removed by supplying 1 µl of RNAse (1mg ml^{-1}) per 100 µl DNA solution. The composition of the different buffers used for DNA extraction is shown in Table 4.

Table 4: Composition of buffers used for DNA extraction.

CTAB-Extraction buffer		**Washing buffer**	
CTAB	2 %	ethyl alcohol	70 %
Na_2EDTA [pH 8.0]	20 mM	NH_4OAc	10 mM
ß-mercaptoethanol	0.2 %		
NaCl	1.4 M	**TE-buffer**	
$Na_2S_2O_5$	1 %	Tris-HCl [pH 8.0]	10 mM
Tris-HCl [pH 8.0]	0.1 M	Na_2EDTA [pH 8.0]	1 mM

DNA concentration was determined using a Fluorometer (Model TK 100, Hoefer Scientific Instruments, San Francisco, USA) and diluted to a final concentration of 25 ng/µl. The measurement is based on the attachment of the fluorescent dye H33258 (Hoechst) to the double stranded DNA. At 365 nm wavelength, this complex emits light at 458 nm wavelength, which is measured by the fluorometer. For calibration of the instrument, a calf thymus DNA solution (100 ng/µl) was used. Buffers and solutions used for determining DNA concentration are listed in Table 5.

Table 5: Solution for the determination of DNA concentrations.

10 x TNE		**Dye-Solution**	
Na_2EDTA	10 mM	H33258	10 mg
NaCl	1 M	H_20_{dd}	10 ml
Tris-HCl	100 mM		
pH 7.4			

3.3.2 RAPD-analysis

According to WERNER et al. (2003b) two identified decamer-primers (Operon technologies) OP-C13 and OP-E14 linked to the resistance gene *rym13* were included in the mapping approach. AmpliTaq Stoffel-Fragment DNA-polymerase (Perkin Elmer Applied Biosystems, Weiterstadt, Germany) was used to perform the RAPD amplification, which, due to the higher thermostability, is different from unmodified Taq-polymerase. PCR reaction and PCR cycler program used are described in table 6-7. The amplification was carried out in a thermocycler type GenAmp® PCR System 9700 (Applied Biosystems, Darmstadt, Germany). The RAPD PCR products were separated on a 2 % agarose gel (Ultra Pure, Gibco BRL Life Technologies™, Karlsruhe, Germany) via horizontal gel electrophoresis (BioRad Sub-Cell GT, Munich, Germany) in 0.5 x TBE–buffer solution with 4 V/cm (Table 8). Each reaction mix was completed with 5 µl of loading buffer (bromophenol blue: orange G = 3:1) and an aliquot of 10 µl was loaded. The size of the resulting RAPD fragments were determined by means of a standard DNA ladder ranging from 100 bp to 2072 bp (Gibco BRL Life Technologies™, Karlsruhe, Germany). The visualization of the amplificats was achieved by staining the agarose gel for 15 min in an ethidium bromide solution (2 µg/ml) followed by exposure to UV light (254 nm) on an UV-transilluminator.

Table 6: Reaction components of a 25 µl-PCR reaction mix for RAPD amplification.

Components	Per reaction
DNA (5 ng/µl)	25 ng
decamer-primer (5 pmol/µl)	7.5 pmol
dNTPs (10 mM)	0.4 mM
MgCl2 (100 mM)	6.0 mM
PCR buffer 10x (Stoffel)	1x
AmpliTaq Stoffel-Fragment polymerase (10 U/µl)	1.5 U
H_2O_{dd} add	25 µl

Table 7: Amplification cycles of the RAPD reaction.

Cycles	Phase	Temperature	Duration
1 x	Denaturation	94°C	4 min
	Denaturation	94°C	1 min
45x	Annealing	36°C	1 min
	Extension	72°C	2 min
1 x	Fill in	72°C	7 min

Table 8: Composition of ingredients used for RAPD analysis.

10x TBE-buffer		Loading Buffer	
Tris HCl (Roth)	0.89 M	Bromophenolblue	0.15 %
Boric acid (Roth)	0.89 M	Ficoll	15 %
EDTA 0.5 M pH 8.0	0.5 M	EDTA	100 mM
		Orange G	0.15 %
		Ficoll	15 %
		EDTA	100 mM

3.3.3 Microsatellite-analysis

A total of 45 simple sequence repeats (SSRs, microsatellites) were used for genotyping the different barley populations (MAP 1-7). Out of these, 26 SSRs (Table 1-2, Appendix) evenly distributed on the seven barley chromosomes, were used for BSA (see chapter 3.5). All microsatellites were amplified according to LIU et al. (1996), RAMSAY et al. (2000) and THIEL et al. (2003). The diagnostic marker Bmac0029 (*rym4*, *rym5*) was amplified according to GRANER et al. (1999a). PCR reaction for each SSR which turned out polymorphic in BSA is shown in Table 10. The different PCR cycling programs are shown in Table 3 in the appendix. PCR amplifications of 65 wheat SSRs (Table 4-5, Appendix) were carried out according to ROEDER et al. (1998), GUPTA et al. (2002) and SOMERS et al. (2004). In some cases, the forward primer was 5'-end labelled with the fluorescence dye IRD 700 or IRD 800 whereas in other cases a 'tailed primer method' (OETTING et al. 1995) was used (Table 4, Appendix). This method employs a two-part primer. A standard sequencing primer M13 or 'tail' is added to the 5'-end of the forward primer. The forward primer binds specifically to the DNA sequence and can be amplified together with the SSR-motif by a universal fluorescence labelled primer (M13) complementary to the 'tail', thereby saving costs for labelling each SSR forward primer. All microsatellites used for mapping are listed in Table 1 of the appendix including sequence information, repeat motif, labelling, fragment size, PCR recipe, PCR program and chromosomal localisation. SSR-amplification products were separated on a denaturing polyacrylamide gel based on an 8 % Long Ranger Gel Solution (FMC Biozym, Hessisch Oldendorf, Germany). The fluorescence-labelling allowed the detection on a LI-COR DNA Sequencer GenReadir 4200 (MWG Biotech AG, Ebersberg, Germany). An equal amount of formamide loading buffer was added to the PCR-samples, which afterwards were denatured in a thermocycler at 95°C for 90 s. The electrophoresis was conducted in 1 x TBE Long Run Buffer under specific conditions: 1500 V, 50 W, 35 mA and 48°C. Determination of the microsatellites allele sizes was achieved by utilising a labelled standard ladder ranging from 50 to 350 bp. The chemical composition of gels and buffers used for SSR detection are listed in Table 9. The EST derived microsatellite GBM 1015 was separated on a 2 % agarose gel via horizontal gel electrophoresis (BioRad Sub-Cell GT, München, Germany) in 0.5 x TBE–buffer with 4 V/cm like described before for the RAPD

amplification (see chapter 3.3.2).

Table 9: Compounds of solutions and buffers used for gel electrophoresis.

PAA-gel solution 8%		10 x TBE Long Run Buffer	
Long Ranger PAA Solution 50 % (FMC, Biozym, Hessisch Oldendorf)	16 ml	tris-HCl (Sigma)	1340 mM
		boric acid (Sigma)	450 mM
		EDTA (Sigma)	25 mM
urea (USB, Cleveland, USA)	42 g	H_20_{dd}	add 1 l
10 x TBE	10 ml		
H_20_{dd}	add 100ml		

Gel Solution for a PAA-Gel (0.25 mm, 25 cm)		Formamide-Loading-buffer	
PAA-Gel Solution 8 %	25 ml	formamide (Sigma)	95 ml
TEMED (Sigma)	25 µl	EDTA (Sigma)	2 ml
DMSO (Sigma)	250 µl	basic fuchsine (Sigma)	0.1 g
APS, 10 % (Roth)	175 µl	bromophenol blue	0.01 g
		H_20_{dd}	add 100 ml

Table 10: Chromosomal localisation, PCR programs and PCR recipes of all polymorphic SSRs used for mapping in barley.

SSR	Chromosome	DNA (µl)	H_20 (µl)	10 x PCR-buffer (µl)	dNTPs (µl) (10 mM)	$MgCl_2$ (µl) (100 mM)	Reverse-primer (2 pmol/µl)	Forward-primer (2 pmol/µl)	Taq-polymerase	PCR program
Bmac0029	3H	2.0	13.2	2.0	0.2	0.4	1.0	1.0	0.2	Bmac0029
Bmac0181	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	E
Bmac0310	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	E
Bmag0353	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	F
Bmag0384	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	F
Ebmac0788	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	D
Ebmac0906	4H	4.0	8.7	2.0	0.4	0.8	2.0	2.0	0.1	Ebmac0906
GBM 1015	4H	4.0	9.4	2.0	0.4	-	2.0	2.0	0.2	GBM
HVM03	4H	2.0	11.0	2.0	0.4	0.4	1.0	1.0	0.2	HVM03
HVM14	4H	2.0	13.1	2.0	0.4	0.4	2.0	2.0	0.1	A
HVM67	4H	2.0	13.0	2.0	0.4	0.4	1.0	1.0	0.1	A
HVM68	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	HVM15
HVM74	4H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	C
Bmac0018	6H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	D
Bmac0127	6H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	F
Bmag0001	6H	2.0	6.7	2.0	0.4	0.8	1.0	1.0	0.1	B
Ebmac0639	6H	2.0	10.7	2.0	0.4	0.8	2.0	2.0	0.1	F
Ebmac0806	6H	2.0	6.7	2.0	0.4	0.8	1.0	1.0	0.1	F
Ebmac0874	6H	2.0	6.7	2.0	0.4	0.8	1.0	1.0	0.1	F

3.3.4 AFLP-analysis

AFLP analysis was essentially carried out according to VOS et al. (1995). DNA restriction and ligation was performed using the AFLP Core Reagent Kit (Gibco Life Technologies, Eggenstein, Germany). 150 ng of genomic DNA was digested with the restriction enzymes *Eco*RI (5'-G/AATTC-3') and *Mse*I (5'-T/TAA-3') according to the manufacturers' instructions in a thermocycler at 37°C for two hours and a final enzyme inactivation at 70°C for 15 min. Adapters with complimentary sequences to the restriction enzymes' recognition sites were ligated to the specific restriction sites of the DNA fragments by T4-ligase. Incubation of the samples was carried out in a thermocycler at 20°C for two hours. A 1:10 dilution in TE-AFLP-buffer was used as DNA template for the following pre-amplification steps. Ligation was followed by two pre-amplification steps using primers complementary to each of the two adapter sequences. First, non-selective AFLP primers E-00 and M-00 were used in order to reduce unspecific background on polyacrylamide gels (+0 pre-amplification), followed by an amplification using primers (E01 and E02 as well as M01 and M02) complementary to each of the two adapter sequences with one additional selective nucleotide (+1 pre-amplification). Thus, amplification of only 1/16th of *Eco*RI-*Mse*I fragments occurred. The PCR-reaction of the +0 pre-amplification was diluted 1:10 and used as DNA template for the +1 pre-amplification. The components of the PCR-reaction and the PCR cycle profiles of the +0/+1 pre-amplification are listed in Table 12+13. The sequences of the primers are listed in Table 11.

Table 11: AFLP sequences for the +0/+1 pre-amplification.

Primer	Primer name	Sequence
+0-*Eco*RI-primer	E00	5' - GAC TGC GTA CCA ATT C - 3'
+0-*Mse*I-primer	M00	5' - GAT GAG TCC TGA GTA A - 3'
+1-*Eco*RI-primer	E01	5' - GAC TGC GTA CCA ATT CA - 3'
+1-*Eco*RI-primer	E02	5' - GAC TGC GTA CCA ATT CC - 3'
+1-*Mse*I-primer	M01	5' - GAT GAG TCC TGA GTA AA - 3'
+1-*Mse*I-primer	M02	5' - GAT GAG TCC TGA GTA AC - 3'

Table 12: Composition of the +0/+1 pre-amplification reaction mix.

	+0 pre-amplification	+1 pre-amplification	per reaction
DNA template	5µl of a 1:10 dilution of the ligation	5µl of a 1:10 dilution of the +0 pre-amplification	
polymerase-buffer 10x	5µl	5µl	1x
dNTPs (10 mM)	1µl	1µl	0.2 mM
*Eco*RI-primer (50 ng/µl)	1.5 µl E00	1.5 µl E01 or E02	75 ng
*Mse*I-primer (50 ng/µl)	1.5 µl M00	1.5 µl M02 or M01	75 ng
Taq-polymerase	0.2 µl	0.2 µl	1 U
H_20_{dd}	add 50 µl	add 50 µl	

Table 13: Amplification cycles of the +0/+1 analyses.

Steps	Reaction	Temperature	Time	Cycles
1	Denaturation	94°C	3 min	1 x
2	Denaturation	94°C	30 s	
	Annealing	56°C	60 s	20 x
	Polymerisation	72°C	60 s	
3	Fill in	72°C	5 min	1 x

The PCR reaction of the +1 pre-amplification was diluted 1:20 with TE buffer and used as template for the selective amplification (+3-amplification). This amplification was carried out using primers with three additional selective nucleotides (Table 14). The compounds of the PCR reactions and the PCR-cycle profile are listed in Table 15+16. For AFLP-detection the PCR products were separated on a polyacrylamide (PAA)-gel using the same protocol as described before for SSR-detection (see chapter 3.3.2). In each case the *Eco*RI primer was labelled at the 5'-end with fluorescence dye IRD700 or IRD800 (MWG Biotech). Electrophoresis was conducted in 1 x Long Run TBE buffer at 1.500 V, 40 W, 40 mA and 48°C. Determination of the generated fragment sizes was achieved using the 50 to 700 bp standard ladder.

Table 14: AFLP-sequences for the +3 amplification.

Primer	Selective bases	Primer	Selective bases
E31	5' - ... AAA - 3'	M47	5' - ... CAA - 3'
E32	5' - ... AAC - 3'	M48	5' - ... CAC - 3'
E33	5' - ... AAG - 3'	M49	5' - ... CAG - 3'
E36	5' - ... ACC - 3'	M50	5' - ... CAT - 3'
E39	5' - ... AGA - 3'	M51	5' -… CCA - 3'
E40	5' - ... AGC - 3'	M52	5‘ -… CCC - 3'
E43	5' - ... ATA - 3'	M53	5' -… CCG - 3'
E51	5’ - ... CCA - 3’	M54	5' -… CCT - 3'
E53	5’ - ... CCG - 3’	M55	5' -… CGA - 3'
E56	5’ - ... CGC - 3’	M56	5' -… CGC - 3'
M36	5’ - ... ACC - 3’	M57	5' - ... CGG - 3'
M39	5’ - ... AGA - 3’	M58	5' - ... CGT - 3'
M40	5’ - ... AGC - 3’	M59	5' - ... CTA - 3'

Table 15: Composition of the +3 pre-amplification reaction mixes.

	+3 amplification	per reaction
DNA template	5µl of a 1:20 dilution of the +1 pre-amplification	
polymerase-buffer 10x	2 µl	1x
dNTPs (10 mM)	0.4 µl	0.2 mM
*Eco*RI-primer (50 ng/µl)	0.25 to 1.5 µl	7.5 to 12.5 ng
*Mse*I-primer (10 ng/µl)	3 µl	30 ng
Taq-polymerase	0.08 µl	0.4 U
H_20_{dd}	add 20 µl	

Table 16: Amplification cycles of the +3 amplification.

Steps	Reaction	Temperature	Time	Cycles
1	Denaturation	94°C	3 min	1 x
2	Denaturation	94°C	30 s	
	Annealing	65°C (-0.7°C/cycle)	30 s	12 x
	Polymerisation	72°C	60 s	
3	Denaturation	94°C	30 s	
	Annealing	56°C	30 s	22 x
	Polymerisation	72°C	60 s	
4	Fill-in	72°C	5 min	1 x

3.4 Bulked segregant analysis (BSA)

According to the phenotypic data, bulks comprising equal amounts of 10 barley DNAs of the respective DH lines (susceptible/resistant) were constructed for BSA (MICHELMORE et al 1991). For the identification of polymorphic SSRs, 26 microsatellites (Table 1, Appendix) uniformly distributed over the seven barley chromosomes were screened for polymorphisms between these two bulks. To detect linkage of the polymorphic microsatellites, the 10 DH lines included in each bulk were tested. In case linkage was detected, the whole population was analysed with this SSR and additional SSRs located in the same region were screened on the bulks and mapped accordingly (see chapter 3.3.3). The same procedure was applied to AFLPs.

3.5 Data analysis

3.5.1 Genetic mapping of BaMMV/BaYMV resistance loci

Linkage analysis of the barley mapping populations was performed with the JoinMap 3.0 software (STAM & VAN OOIJEN 1995). Crossover units were converted into map distances (cM) by applying the Kosambi function (KOSAMBI 1944). By using the chi-square test it was determined, whether the observed data were compatible to the expected values of a 1:1 segregation ratio of the DH populations or whether there was a distorted segregation ratio. A threshold log likelihood ratio (LOD) of 3.0 was used to arrange markers into linkage groups.

3.5.2 Estimation of genetic diversity and genetic relatedness

Based on the analyses of 40 SSRs and 30 AFLP primer combinations the genetic diversity and genetic similarity of wheat breeding lines and cultivars was estimated based on the presence (1) or absence (0) of bands using the software package RFLP-Scan 2.0. The resulting 0/1 matrix includes both monomorphic and polymorphic bands. The genetic similarity was estimated according to NEI and LI (1979), which is corresponding to the Dice coefficient (DICE 1945):

$$GS=2a/2a+b+c$$

whereby *a* refers to alleles shared between two varieties, and *b* and *c* refer to alleles present in either one of the two varieties. On the basis of the Dice similarity matrix, *Unweighted Pair Grouped Method Arithmetic Average* (UPGMA-) clustering of the different wheat genotypes was carried out using the *Sequential Agglomerative Hierarchical and Nested* (SAHN) method of the software package NTSys-pc 1.7. The genetic diversity of SSR data was estimated based on the number of alleles per locus and the mean diversity index (DI) over all loci was calculated according to NEI (1973):

$$DI= 1/n\sum_{j}\left(1-\sum_{i}x_{ij}^{2}\right)$$

where x_{ij} is the frequency of the i^{th} allele of locus j and n is the number of loci.

The Shannon-Weaver Index (also called Shannon-Wiener Index, SHANNON-WEAVER 1949) H' was used to analyse genetic diversity of the AFLP data due to the

dominant character of this marker type. This index takes into account the phenotypic frequency:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

whereas *S* is the number of species and p_i is the relative abundance of each species. The analysis was performed by the software POPGENE 1.32.

In order to get information of the usefulness of the SSRs the polymorphic information content (PIC) of the different microsatellites was calculated (see Chapter 4.3). The polymorphic information content (PIC) is a tool to measure the informativeness of a given SSR marker. According to WEBER (1990) and ANDERSSON et al. (1993), the PIC-value was calculated as follows:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$$

whereby *k* is the total number of alleles detected for a microsatellite and P_i is the frequency of the i^{th} allele in the set of wheat genotypes investigated.

4 Results

The main objective of this work was to identify and characterise new or already known resistances resources in barley and wheat against soil-borne viruses, i.e. *Barley yellow mosaic virus* (BaYMV), *Barley mild mosaic virus* (BaMMV) in barley and *Wheat spindle streak mosaic virus* (WSSMV) and *Soil-borne cereal mosaic virus* (SBCMV) in wheat. Therefore, molecular markers were used to map resistance genes of diverse origin in different DH populations of barley and fingerprint resistant or tolerant cultivars and landraces in wheat.

4.1 Screening of germplasms for the presence of *rym4/rym5*

In order to identify donors of new resistance genes against barley yellow mosaic virus disease carrying genes different from *rym4* and *rym5,* 120 gene bank accessions, resistant against BaYMV in Japan, were analysed by using the SSR marker Bmac0029 being closely linked to the *rym4/rym5* locus and being to some extent diagnostic for these different alleles.

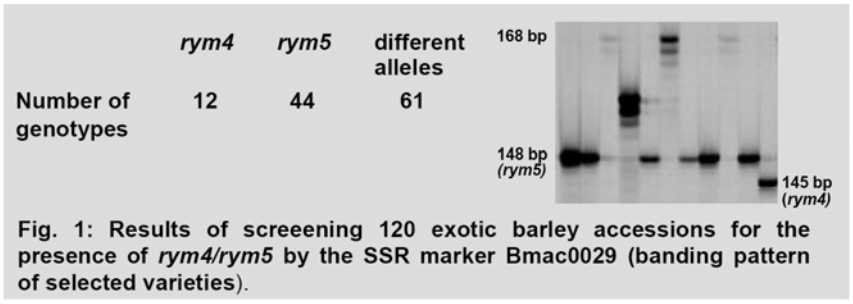

Fig. 1: Results of screeening 120 exotic barley accessions for the presence of *rym4/rym5* by the SSR marker Bmac0029 (banding pattern of selected varieties).

In these studies it turned out, that out of the screened exotic germplasm 12 genotypes revealed a fragment size of 145 bp indicative for *rym4*, 44 showed the size of 148 bp indicative for *rym5* and 61 genotypes carried different fragment sizes ranging from 140 to 170 bp (Fig. 1). Those remaining genotypes not carrying *rym4* or *rym5* are potential candidates for detecting new resistance genes. Detailed results of detected fragment size after screening the barley accessions are given in Table 17.

To identify new resistance resources, the remaining 61 genotypes were evaluated for resistance against the European strains of BaYMV and BaMMV in a one year trial at three locations. After the screening, ‘Chikurin Ibaraki 3’, ‘Hakusanmugi’, ‘Hongcheon Anjeunbaengi 2’, ‘Ramsar’, ‘Sekitori 2’, ‘Turkey 3’ and ‘Turkey 179’ turned out to be resistant to the common European strains BaMMV, BaYMV and BaYMV-2 (Heidi Jaiser, personal communication). Therefore, these accessions represent useful

Table 17: Screening of 120 gene bank accessions with the SSR marker Bmac0029.

Name	Fragment size	Name	Fragment size	Name	Fragment size
Adi Abun 2	168	Hiroshima	148	Sipche 14	145
Addis Ababa 64	168	Hongcheong Anjeunbaengi 2	168	Sululta 4	168
Baku 3	157	Hongseong Native	148	Sululta 10	168
Baegsan Santoku 1	148	Hosomugi 3	148	Suweon 31	148
Boseong Covered 2	148	Hsingwuke 2	-*	Taishomugi	172
Chame 8	168	Iwate Hozoroi 1	148	Tatung	168
Cheongyang Covered 2	139	Juichang 2	148	Thonje 16	145
Changweon Jecheon 51	148	Jungbori 20	148	Thonje 19	168
Chihchou yinchiaai 3	148	J. 20	145	Tibilisi 1	148
Chikurin Ibaraki 3	168	Katana 2	159	Tibilisi 7	145
Chiuchiang	148	Keronja 2	145	Tsumje 3	145
Dabat 1	168	Keronja 3	145	Tochigi Torano-o 1	148
Debra Birhan 1	168	Keronja 5	145	Torano-o	148
Debra Birhan 7	168	Kinukawa Gozen 22	148	Torano-o 7	148
Deder 2	168	Kobinkatagi 4	148	Turkey 3	168
Dembi 3	168	Koshimaki 40	148	Turkey 29	164
Dhumpu 2	168	Kulubi 1	168	Turkey 33	168
Esfahan 1	168	Liussuchiao 1	148	Turkey 39	145
Esfahan 4	168	Liussuchiao 2	148	Turkey 41	168
Ethiopia 14	168	Mammuto	168	Turkey 44	164
Ethiopia 53	168	Masan Covered 5	148	Turkey 45	141
Ethiopia 65	168	Mota 1	168	Turkey 47	168
Ethiopia 80	168	Mota 7	168	Turkey 56	168
Ethiopia 89	168	Nagaoka	168	Turkey 62	168
Ethiopia 506	148	Nazareth 3	168	Turkey 68	168
Ethiopa 510	168	Oeyama Rokkaku 3	148	Turkey 77	168
Ethiopia 534	168	Paishapu 2	148	Turkey 83	168
France 7	168	Paoanchen 1	148	Turkey 86	145
Fushiguro	148	Ramsar	140	Turkey 101	145
Gangneung Covered 3	148	Russia 4	148	Turkey 179	168
Glyorgi 2	168	Sacheon Naked	148	Turkey 440	168
Gogseong Covered 4	148	Sakaiwa Rokkaku 27	148	Turkey 524	170
Goheung Covered 2	148	Samcheog Dolbori	148	Turkey 581	-*
Gondar 6	168	Sekitori 2	168	Turkey 723	168
Gorgan 1	148	Shanghai 1	148	Waegwan Covered 1	148
Gwangju Baitori 1	148	Shemakha 1	158	Yeoncheon Native	148
Hakusanmugi	168	Shemaka 2	165	Zairai Junkei 8	148
Hamyang Covered 9	148	Shemakha 3	-*	Zairaishu	168
Hanhadaka 2	148	Shiro Omugi 79	148	70 g	168
Hayamugi	148	Sikha 10	145	9055	168

* - = unverifiably

sources for broadening the genetic base of barley yellow mosaic virus disease in Europe.

4.2 Identification and mapping of BaMMV resistance genes in different DH-populations

4.2.1 Mapping the resistance gene of 'Cebada' (MAP 1)

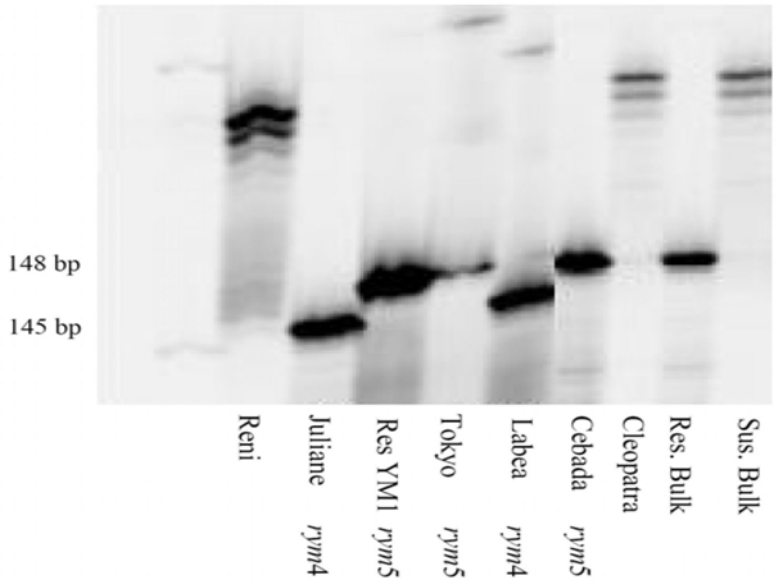

Fig. 2: Results of screening 'Cebada' for the presence of *rym4/rym5* by the SSR-marker Bmac0029.

Due to breeders information it was supposed that the resistance of 'Cebada' is not due to *rym4* or *rym5*. The phenotyping of resistance against BaMMV after mechanical inoculation suggested the presence of one resistance gene in this DH population due to the observed segregation ratio of resistant *vs.* susceptible plants of 46:48 (Chi^2 = 0.42, p=0.650). However, since checking respective bulks with markers of each chromosome did not result in any polymorphisms, resistant and susceptible bulks were screened with Bmac0029 being closely linked to the *rym4/rym5* locus. As can be seen in Figure 2 the analysis revealed that 'Cebada' carries *rym5* because a fragment of 148 bp was detected in 'Cebada' being indicative for *rym5* and a clear differentiation between the susceptible and resistant bulk was observed. Therefore, no further molecular work was carried out on this DH population.

4.2.2 Mapping the resistance gene of 'Shimane Omugi' (MAP 2)

In the DH population 'Shimane omugi' x 'Sumo' and 'Shimane Omugi' x 'Gilberta' (MAP 2) a segregation ratio of resistant *vs*. susceptible plants of 51: 46 (Chi^2 = 0.257;

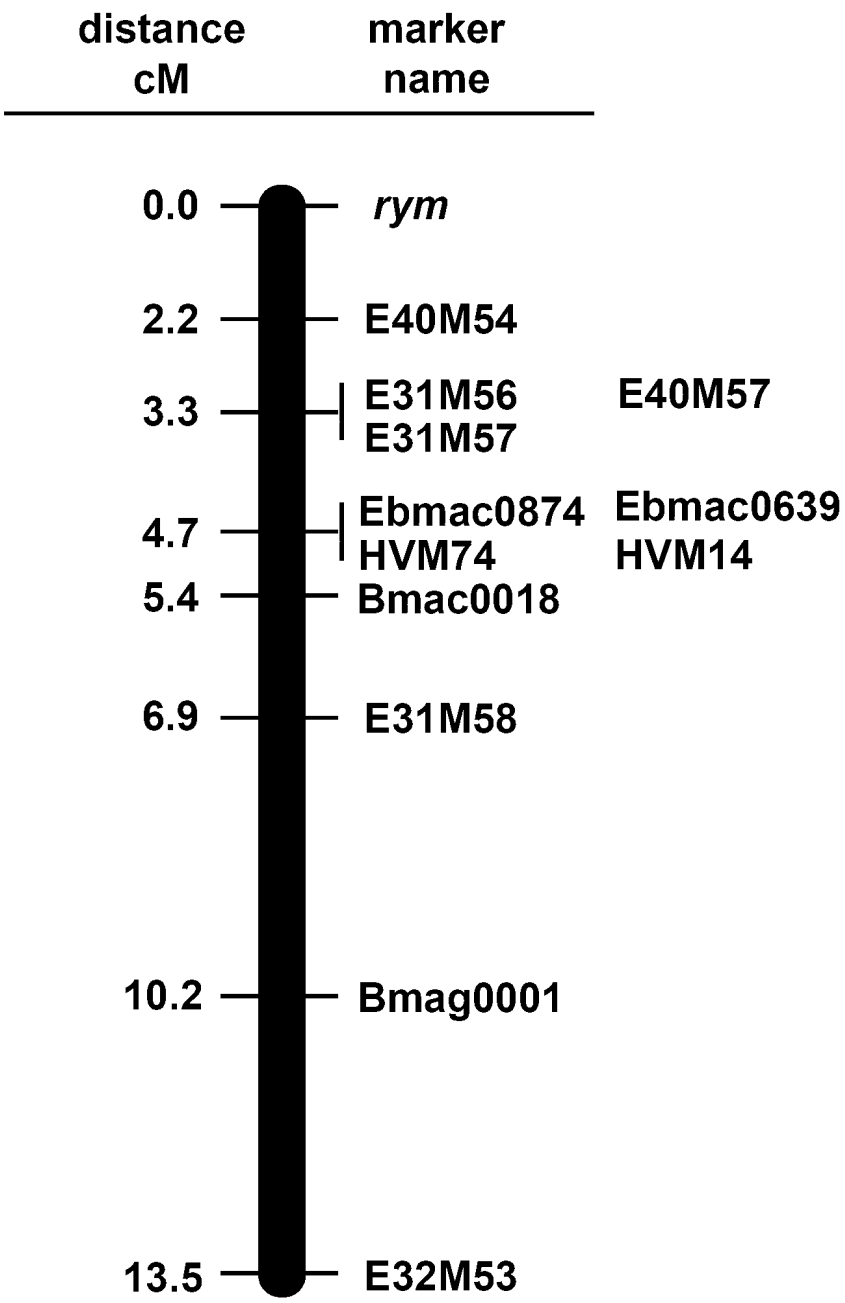

Figure 3: Partial map of chromosome 6H including the BaMMV-resistance of 'Shimane Omugi'.

p=0.612) was observed giving hint to a single recessive gene effective against BaMMV. In the initial screening using BSA the BaMMV resistance of 'Shimane Omugi' was mapped on chromosome 6H. Polymorphisms between the bulks containing susceptible and resistant lines, respectively, were observed with Bmac0018. Linkage of the BaMMV resistance to Bmac0018 has been confirmed by analysis of the single lines included in these bulks. Furthermore, additional SSRs located in the same chromosomal region of 6H were analysed on the bulks. In this

respect well defined polymorphisms between bulks differing in their resistance to BaMMV were detected for Bmac0127, Bmag0001, Ebmac0639, Ebmac0874, HVM14, and HVM74. In order to achieve further marker saturation in this chromosomal region AFLP based BSA was conducted with 96 *Eco*RI+*3*/*Mse*I+*3* AFLP primer combinations. Sixteen AFLP primer combinations revealed polymorphisms between the parents as well as the susceptible and resistant bulks. Out of these 16 promising primer combinations just six combinations E31M56, E31M57, E31M58, E32M53, E40M54, and E40M57 revealed linkage on the DH lines included in the bulks. The whole population was screened with these AFLP primer combinations and the microsatellites mentioned above. The resulting linkage group (Fig. 3) located on chromosome 6H comprises a length of 13.5 cM with six SSR markers plus six AFLP markers. The marker with the closest linkage to the BaMMV resistance locus is E40M54, which has been mapped in a distance of 2.2 cM. E40M54 generated an additional fragment on lines carrying the resistance encoding allele at 274 bp. Furthermore, three AFLP markers were detected to co-segregate at a genetic distance of 3.3 cM. All three AFLP marker show an additional fragment in resistant DH lines namely E31M56 at 234 bp, E31M57 at 508 bp and E40M57 at 500 bp. A second cluster comprises four microsatellite markers. These are HVM14, Ebmac0874, Ebmac0639 and HVM74, which have been mapped at a distance of 4.7 cM from the resistance locus. HVM14 generated a resistant fragment at 157 bp whereas susceptible lines reveal a fragment at 161 bp. HVM74 amplifies a fragment of 216 bp in resistant lines and 228 bp in susceptible lines. The SSR markers Ebmac0639 and Ebmac0806 amplified a fragment of 147 bp and 173 bp, respectively, in resistant lines and 167 bp and 198 bp, respectively, in susceptible lines.

4.2.3 Mapping the resistance gene of ‘CI 3517’ (MAP 3)

Alarmed by the results obtained in MAP1, ‘CI 3517’ and MAP 3 were screened with Bmac0029 in a first step in order to exclude that ‘CI 3517’ may also carry *rym5* or *rym4*. Although it was shown that ‘CI 3517’ did not carry *rym5* or *rym4*, 15 DH lines out of 80 were identified in this DH population to carry *rym4* and were thus excluded from further analyses. In the remaining DH lines a segregation ratio of resistant (r) *vs*. susceptible (s) of 1:1 (26r:38s; Chi^2= 2.25; p= 0.134) was observed based on the

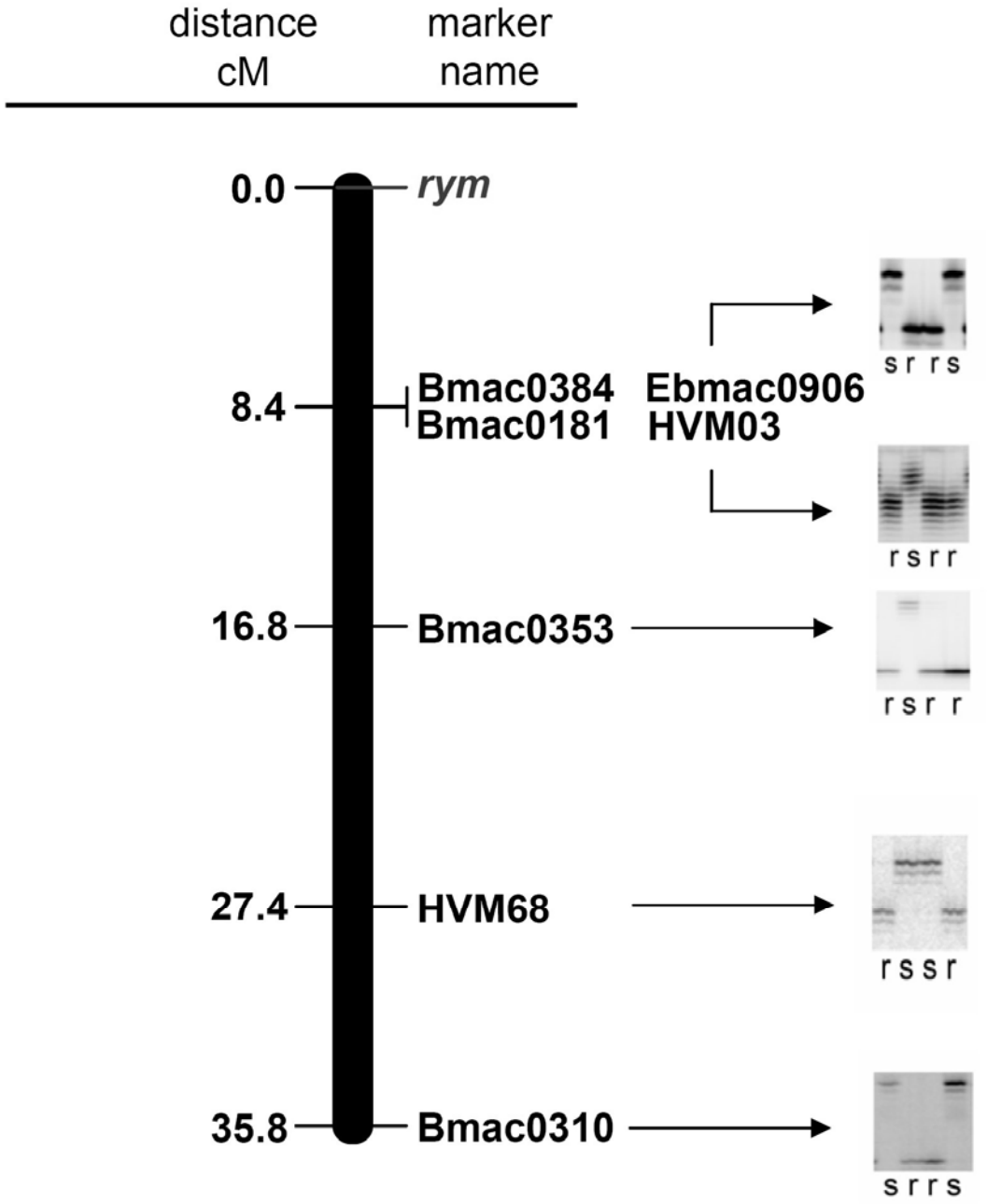

Figure 4: Partial map of barley chromosome 4H including the BaMMV resistance of ´CI 3517´.

DAS-ELISA-results giving hint to a single recessive resistance gene effective against BaMMV. Bulks were composed of six susceptible and six resistant lines of the DH-population. In order to assign the resistance gene to a chromosome SSRs (listed in Table 1, Appendix) were analysed in a first step. Polymorphisms between the bulks were revealed by microsatellite Bmag0353 on chromosome 4H. Additional microsatellites located in the same chromosomal region were analysed in order to identify more closely linked markers. In this respect, additional polymorphisms

between the bulks were detected for Bmac0384, Ebmac0906, Bmac0181, HVM03, HVM68 and Bmac0310. The remaining population comprising 65 DH lines was genotyped with these markers. Based on the genotypic data, the BaMMV resistance was mapped with the closest linkage at a distance of 8.4 cM to the co-segregating SSR markers Bmac0384, Bmac0181, Ebmac0906, and HVM03 (Fig. 4). The SSR markers HvOle and HVM40, which are located in the direction of the centromer, turned out to be monomorphic.

4.2.4 Mapping the resistance gene of 'Belts 1823' (MAP 4)

It was known that 'Franziska', one of the parents of the population MAP 4, carries *rym4.* Therefore, the population has been primarily screened with SSR marker Bmac0029 in order to identify lines carrying the resistance encoding allele at the *rym4/rym5* locus, which had to be excluded from mapping as they are not informative for mapping the resistance of 'Belts 1823'. In this respect it turned out that 'Belts 1823', which is the donor of the assumed new resistance of MAP 4, possesses *rym5.* Therefore, no further analysis was performed on this population.

4.2.5 Mapping the resistance gene of 'Chikurin Ibaraki 1' (MAP 5)

The phenotyping of resistance against BaMMV after mechanical inoculation suggested the presence of one resistance gene in the MAP 5 DH population due to a detected segregation of 78 resistant to 85 susceptible lines fitting a 1:1 segregation ratio (Chi^2 = 0.301; p=0.583). In order to localise the BaMMV resistance, DNA bulks were composed and analysed by microsatellite markers. Primary screenings revealed polymorphisms between the bulks consisting each of 15 completely resistant lines and susceptible lines with Bmac0018 and Ebmac0806 located on barley chromosome 6H. Further analysis, first on the members of the bulks then on the whole population confirmed linkage between the resistance locus and these two markers. Consequently, additional microsatellite markers located in the same region of chromosome 6H were screened. Additional polymorphisms between the single lines included in these bulks were detected for Bmac0127, Bmag0001, Ebmac0639 and Ebmac0874. Therefore, all 163 DH lines of the cross were analysed with these SSR markers resulting in a linkage group of six mapped SSRs (Fig. 5). The map

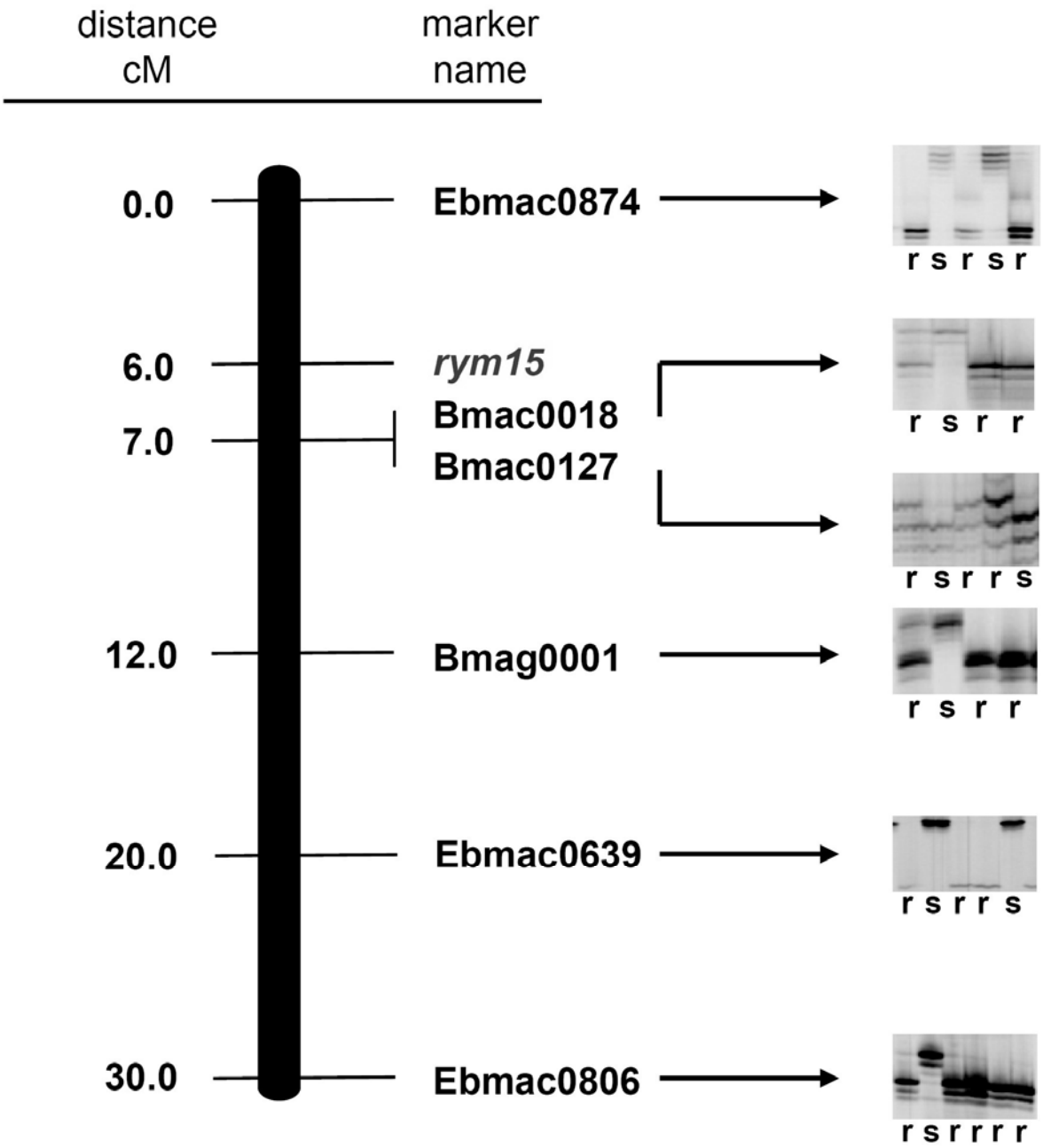

Figure 5: Partial map of chromosome 6H including the resistance locus *rym15* of 'Chikurin Ibaraki 1'.

shows the BaMMV resistance gene flanked by three markers whereby the closest co-segregating SSR markers are Bmac0018 and Bmac0127 located proximal at a distance of 1.0 cM. Furthermore, Ebmac0874 shows linkage to the resistance gene with a distance of 6.0 cM. In this cross Bmac0018 shows a fragment of 132 bp in resistant lines whereas susceptible lines reveal a fragment at 138 bp. Bmac0127 amplifies a fragment of 120 bp in resistant lines and 118 bp in susceptible lines. The

two co-segregating microsatellites Bmac0018 and Bmac0127 are ideal DNA markers for marker assisted selection due to their small genetic distance of 1.0 cM.

4.2.6 Mapping the resistance gene of 'Taihoku A' (MAP 6)

On the basis of earlier work (WERNER et al. 2003b) it was known that 'Taihoku A' contains a new BaMMV resistance gene also referred to as *rym13* located on chromosome 4H. Recently, HABEKUSS et al. (2006) described a new German BaMMV strain, against which 'Taihoku A' also confers resistance after mechanical infection. Using a subset of the original mapping population 'Taihoku A' x 'Plaisant', which was enlarged by 64 DH lines of up to 154 DH lines, mechanical inoculation and DAS-ELISA was carried out. A segregation ratio of 87r: 67s (p=0,107; Chi^2= 2.59) confirmed that *rym13* also confers resistance against the new German strain of BaMMV. BSA was carried out with so far untested SSRs and with AFLPs to identify more closely linked markers to the resistance gene. At that time the closest SSR marker (WMS06) had been mapped proximal of *rym13* at a distance of 15.2 cM (WERNER et al. 2003b). Furthermore, a marker cluster comprising the AFLP markers E53M36, E53M40 and the RAPD marker OP-C13 located 6.7 cM distally was identified (WERNER et al. 2003b).

Unfortunately, in this region of chromosome 4H only few microsatellites are known. Therefore, EST derived SSRs' (THIEL et al. 2003), kindly provided by Prof. Andreas Graner, IPK Gatersleben, were used for BSA. Polymorphisms between the two bulks containing 10 resistant and 10 susceptible DH lines were only observed with GBM1015. To find polymorphism on the different bulks a subset of 256 *Eco*RI+*3/Mse*I+*3* AFLP primer combination was applied for BSA. Eleven *Eco*RI+*3/Mse*I+*3* AFLP primer combinations showed polymorphism on these bulks. Three combinations differentiated between the several DH lines included in the bulks and were used for mapping. E33M56 (250 bp) and E43M59 (285 bp) generated an additional fragment on the resistant lines (Figure 6). The map comprises a length of 39.1 cM with seven AFLP markers, three microsatellite markers and two RAPD markers, with the closest markers being linked at a distance of 1.0 cM to *rym13*. This marker is E53M36, which shows an additional fragment on resistant DH lines at 105 bp. GBM1015, E51M40 and the RAPD-marker OP-C13, which are co-segregating,

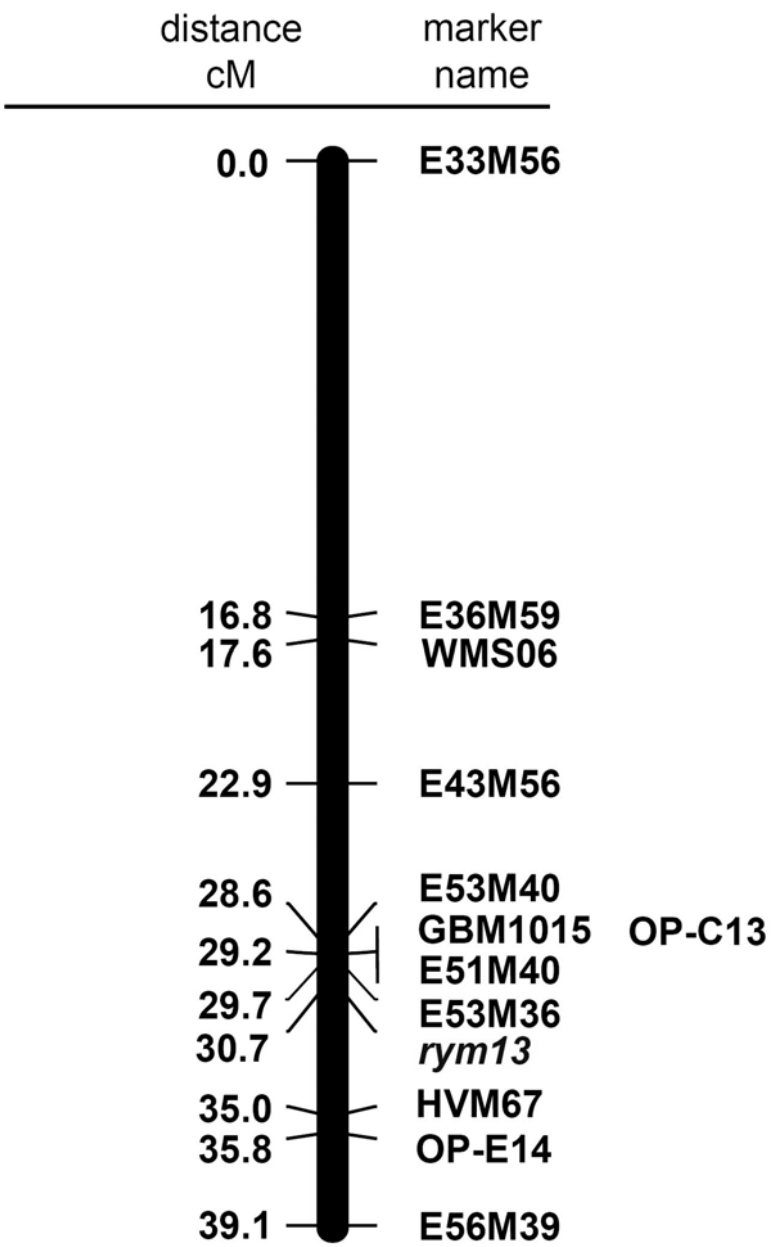

Figure 6: Partial map of chromosome 4H including *rym13* of 'Taihoku A'.

mapped at a genetic distance of 1.5 cM proximal to *rym13*. OP-C13 generated bands of 900 bp in the resistance donor 'Taihoku A'. The AFLP primer combination E51M40 (120 bp) showed an additional fragment on lines carrying the resistance encoding allele. GBM1015 amplified a fragment of 100 bp in resistant lines and bands of 200 bp were detected in susceptible lines. They are all located proximal to the resistance locus. Furthermore, linkage was detected for the microsatellite marker HVM67 with a recombination rate of 4.3 cM. DH lines with the susceptibility encoding allele revealed

a fragment of 115 bp and the resistant lines showed a smaller fragment of 112 bp after using HVM67.

4.2.7 Mapping the resistance gene of 'Muju covered 2' (MAP 7)

In the DH population 'Muju covered 2' x 'Spirit' a segregation ratio of 51 resistant to 100 susceptible lines (Chi^2= 15.90, p= 6.675) was found after a resistance test against BaMMV. This segregation ratio does not fit to the expected 1r:1s segregation as an excess of susceptible plants was observed which may be due to different suitability of the parental lines for tissue culture procedures. Due to former analyses by GRANER et al. (1996) it was known, that the resistance of 'Muju covered 2' is localised on chromosome 4H. In order to map this BaMMV resistance bulks were composed and analysed by SSR markers located on this chromosome. Polymorphisms were found only with HVM67 and Ebmac0788 because of the limited availability of microsatellites in this region of chromosome 4H. WMS06 located on the long arm of chromosome 4H was monomorphic between the bulks. Therefore, EST derived SSRs, kindly provided by Prof. Andreas Graner, IPK Gatersleben, were additionally analysed. Out of these only GBM1015 was polymorphic and used besides the two above mentioned SSRs for mapping. Based hereon, a genetic map was constructed based on 154 DH lines with a length of 38.7 cM (see Figure 7). The SSR marker with the closest linkage to the BaMMV-resistance is Ebmac0788 mapped within a distance of 7.8 cM. Furthermore, linkage to the resistance gene was detected for GBM1015, located distally with a distance of 23.9 cM. The linkage of HVM67 is rather loose with a recombination rate of 30.9 cM to the resistance locus.

4.3 Estimation of genetic relatedness of wheat cultivars and breeding lines

The aim of this work was to reveal the genetic relatedness within a subset of wheat genotypes and breeding lines resistant against *Soil-borne cereal mosaic virus* (SBCMV) compared to a few tolerant varieties. Therefore, 1146 wheat cultivars had been evaluated by different breeders (see Material and Methods chapter 3.2) in field trials in Vatan, France, for resistance. Out of all screened wheat genotypes 64 interesting, predominantly resistant wheat genotypes were selected for analysis of genetic relatedness by fingerprinting with 40 SSRs and 30 +3-AFLP primer

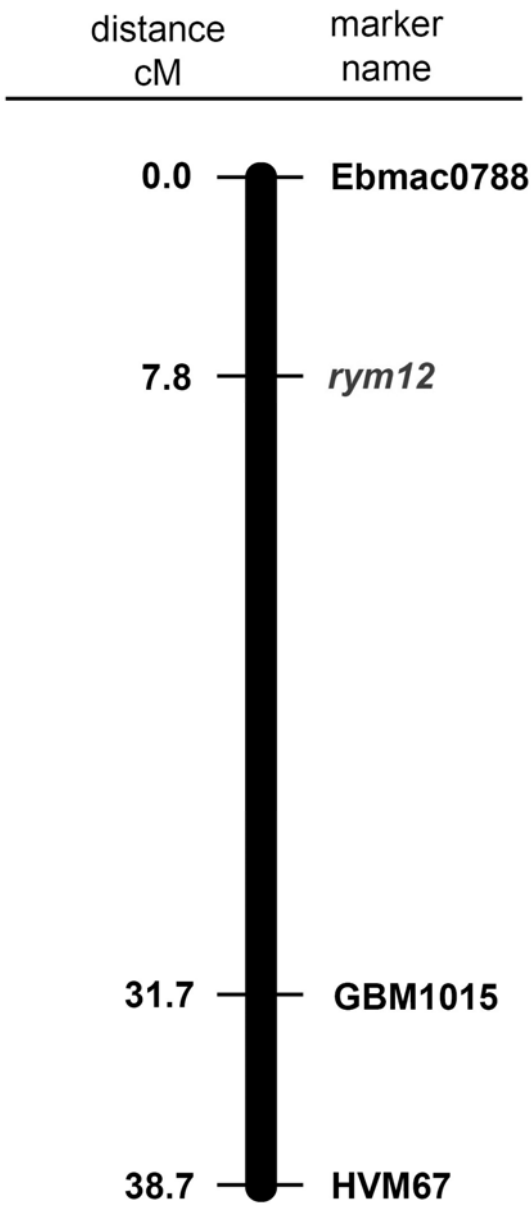

Figure 7: Partial map of barley chromosome 4H including the BaMMV resistance of ‘Muju covered 2’.

combinations. Genetic analyses with the 40 SSRs resulted in the detection of 305 alleles, whereas the number of alleles per locus was on average 7.5 within the range of 1 to 17. In addition, the Polymorphic Information Content (PIC) value was estimated, which gives information about the usefulness of a SSR regarding marker development in breeding programmes and estimation of genetic diversity. The SSR marker with the highest PIC-value is wmc276 (0.89) whereas the monomorphic wmc41 shows the lowest value (0.00). All results and further information of the 40 SSRs are given in Table 18. Based on the presence or absence of the amplification of alleles, the pair-wise genetic similarity (GS) according to NEI and LI (1979), which

corresponds to the likelihood that an allele is generated in a second genotype, was analysed ranging from 0.19 to 0.86 with an average of GS=0.49. The minimum genetic similarity of 0.19 was observed between the genotypes ‘Enesco’ *vs.* ‘Sponsor’ and the maximum genetic diversity of 0.86 was found between the Danish breeding lines ‘701-176c’ *vs*. ‘701-177c’. The mean genetic diversity (DI) across the loci within the analysed set of wheat genotypes was DI=0.57. An UPGMA-cluster analysis, based on the 0/1-matrix derived GS, was carried out (Fig. 8). The dendrogram reveals a strong differentiation of the French cultivars (from ‘Tremie’ to ‘Gaspard’) from the rest of the analysed wheat lines due to their origin. However, no clear grouping could be observed within the remaining genotypes, but the high level of genetic diversity in the analysed set indicated a sufficient level of genetic diversity within these SBCMV resistant lines.

Regarding AFLP data, 1847 fragments were detected in total. The genetic similarity (GS) was estimated between 0.50 and 0.97 with an average of GS=0.74. The maximum similarity was observed between the French cultivars ‘Tremie’ *vs.* ‘Taldor’, whereby the minimum genetic similarity of 0.50 was found between the cultivars ‘Sponsor’ *vs.* ‘Enesco’. Genetic diversity according to the Shannon-Weaver Index was H’=0.521, whereas the percentage of polymorphic loci added up to 88.2%. Within the wheat accessions of the three different breeding companies the genetic diversity was calculated on a similar level between the lines of the German (H’=0.439) and the Danish (H’=0.443) breeding company. The genetic diversity of the genotypes within the French group was clearly higher with H’=0.524. The UPGMA cluster analysis based on UPGMA is shown in Figure 9. Similar results as mentioned for the SSR analysis were obtained with AFLPs. The French cultivars of the group ‘Tremie’ to ‘Gaspard’ are separated from the rest of wheat genotypes. A stronger grouping according to their origin, respectively to the breeding companies, was observed for the rest of the lines. Detailed information about the different genotypes has to be concealed with respect to further breeding programs at each breeding company.

Table 18: Chromosomal location, number of alleles and the PIC-values per locus for 40 wheat SSRs.

SSR	Chromosome	Alleles	PIC-value
wmc24	1A	13	0.67
wmc254	1A	07	0.48
wmc177	2A	07	0.69
wmc264	3A	06	0.75
gwm513	4A	05	0.57
psr6465	4A	02	0.17
wmc219	4A	05	0.26
barc117	5A	04	0.65
gwm129	5A	06	0.56
gwm304	5A	10	0.88
gwm415	5A	06	0.70
wmc215	5A	10	0.74
wmc398	6A	05	0.55
wmc168	7A	07	0.59
wmc44	1B	13	0.76
wmc149	2B	13	0.76
wmc245	2B	02	0.49
barc147	3B	05	0.43
wmc78	3B	07	0.77
wmc307	3B	07	0.57
wmc322	3B	06	0.68
wmc418	3B	05	0.67
wmc625	3B	08	0.41
wmc754	3B	13	0.85
wmc777	3B	04	0.22
barc20	4B	07	0.75
wmc47	4B	09	0.40
wmc238	4B	10	0.86
wmc710	4B	11	0.68
gwm539	5B	07	0.40
wmc104	5B	06	0.59
wmc276	7B	17	0.89
wmc147	1D	08	0.28
wmc41	2D	01	0.00
wmc167	2D	09	0.48
wmc601	2D	14	0.85
wmc52	4D	04	0.12
wmc331	4D	15	0.67
psr6394	5D	08	0.70
wmc161	5D	07	0.61

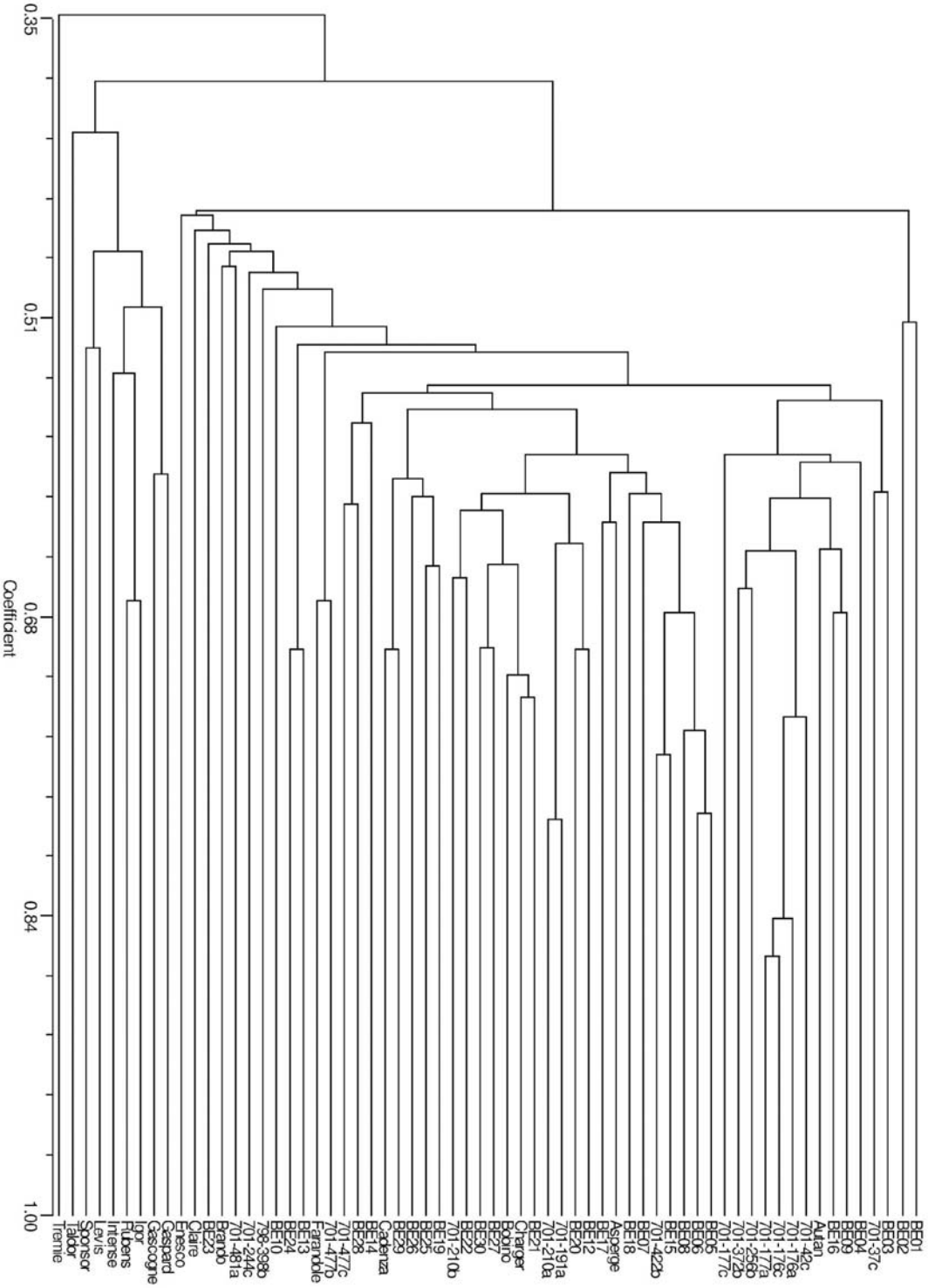

Figure 8: Dendrogram of 64 wheat cultivars and breeding lines based on UPGMA cluster analysis of genetic diversity estimated on SSRs.

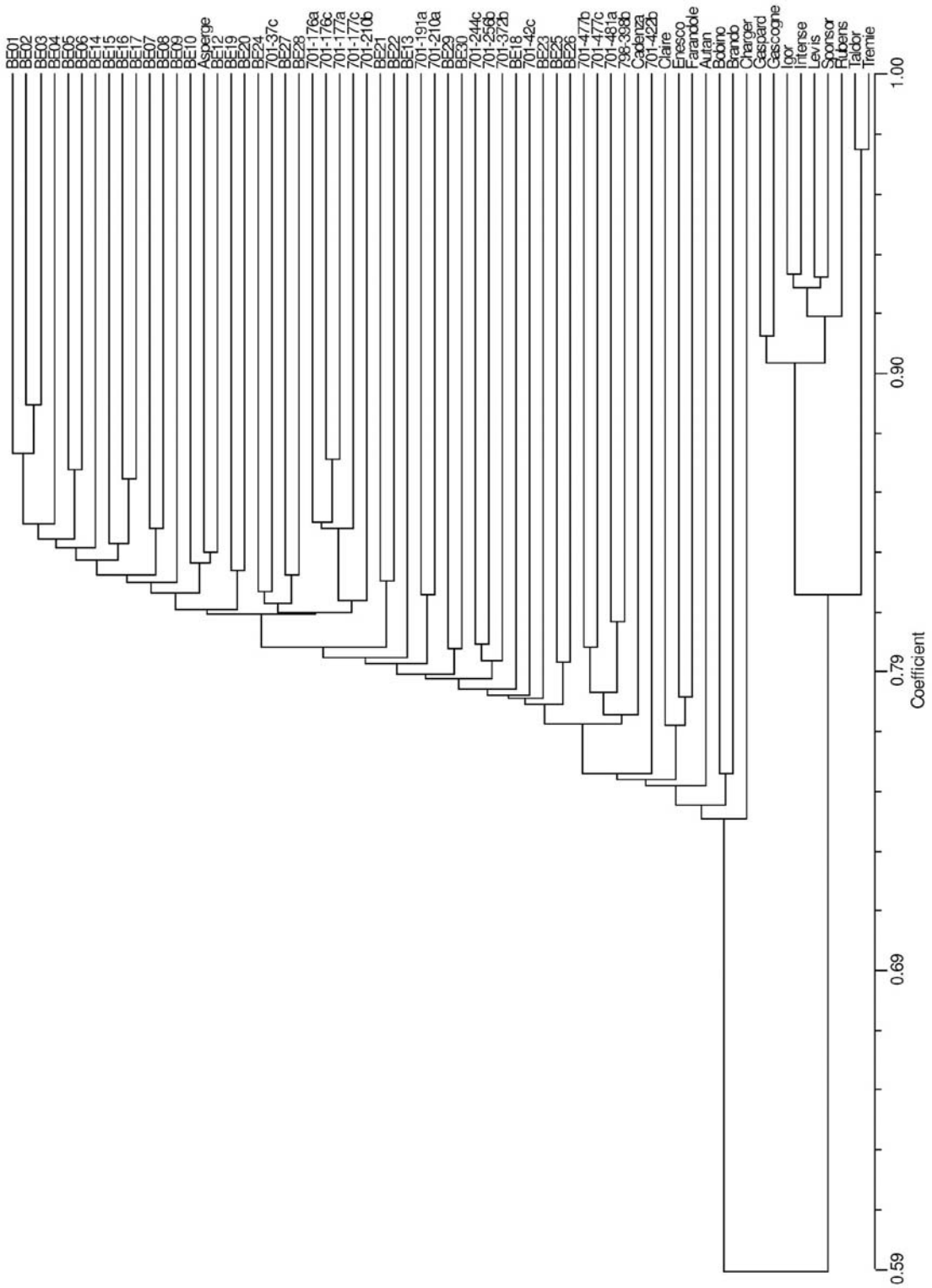

Figure 9: Dendrogram of 64 wheat cultivars and breeding lines based on UPGMA cluster analysis of genetic diversity estimated on AFLPs.

5 Discussion

5.1 Identification of new resistance donors against barley yellow mosaic virus disease

As a result of extensive screening programmes several exotic germplasms were identified within the primary barley gene pool showing resistance against all known strains of the barley yellow mosaic virus disease (GÖTZ & ORDON 1993, ORDON et al. 1993), but due to co-evolution of the virus (HARIRI et al. 2003, HABEKUSS et al. 2006), i.e. the detection of new resistance breaking virus strains, new sources of resistance have to be identified. Therefore, it is of prime interest to identify new varieties possessing a BaMMV/BaYMV resistance, which is not allelic to the BaMMV resistance genes *rym4* (ORDON & FRIEDT 1993) or *rym5*, because these have already been overcome by new strains of these viruses. In this context, exotic germplasms, although their agronomic traits are not outstanding, become more and more important for broadening the genetic base of resistance against BaYMV disease (ORDON & FRIEDT 1994). The main objective of the present study was to identify new resistance genes against BaMMV/BaYMV and respective molecular markers by screening resistant genetic resources for known PCR-based markers for *rym4/rym5* and analysing segregating DH populations. In order to identify new resistance donors against BaYMV/BaMMV 120 exotic gene bank accessions, which are resistant against BaYMV in Japan, were analysed in the present study with the diagnostic SSR marker Bmac0029 for *rym4* and *rym5* resistance. The genotypes, which are not carrying *rym4* or *rym5,* are potential candidates for the identification of new resistance genes. After evaluation of the BaYMV/BaMMV resistance of these exotic germplasms in greenhouse and field trials the varieties ‘Chikurin Ibaraki 3’, ‘Hakusanmugi’, ‘Hongcheon Anjeunbaengi 2’, ‘Ramsar’, ‘Sekitori 2’, ‘Turkey 3’ and ‘Turkey 179’ were identified to be resistant against BaMMV, BaYMV and BaYMV-2 (H. JAISER personal communication) and carrying genes different from *rym4/rym5*. Therefore, these are useful sources for further breeding programmes to broaden the genetic base of resistance against the barley yellow mosaic virus complex. These germplasms can now be crossed to high yielding barley varieties to develop new resistant cultivars. However, in tests for allelism it has to be verified, if these exotic genotypes possess already known resistance genes like *rym11*, *rym12* and *rym13*,

which impart resistance against all known strains of BaYMV, or if the varieties possess new, not yet identified, resistance genes. Despite only a few newly detected virus strains which are able to overcome already known resistance genes in Europe up to now, the search of new resistance donors is an ongoing task, because of the enduring risk of co-evolution. Regarding the present situation seven strains of BaYMV and two of BaMMV have been described in Japan (NOMURA et al. 1996), whereas in France and Germany new variants of BaMMV have been reported which have overcome the resistance genes being effective so far (HARIRI et al. 2003, KANYUKA et al. 2004, HABEKUSS et al. 2006). Beside the primary barley gene pool, the secondary gene pool, i.e. *Hordeum bulbosum*, is used to improve BaYMV resistance. *Hordeum bulbosum* possesses a lot of useful traits like several disease resistances (PICKERING et al. 2000, WALTHER et al. 2000). Due to problems with hybrid instability, interspecific incompatibility and endosperm degeneration the transfer of genetic material was previously limited, but these problems have been solved almost completely (PICKERING & JOHNSTON 2005). Through interspecific crosses loci from *Hordeum bulbosum*, which confer resistance against BaYMV, scald, stem rust, and powdery mildew, were transferred into the *Hordeum vulgare* genome (PICKERING et al. 1995, RUGE et al. 2003, RUGE-WEHLING et al. 2006, PICKERING et al. 2006, SHTAYA et al. 2007).

5.2 Mapping of new resistance genes against *Barley yellow mosaic virus*

The aim of the present work was to identify and localize new resistance genes and to develop closely linked molecular markers in addition to those genes already known. Therefore, seven different DH populations were used for mapping purposes. The total offspring of all seven crosses between a new resistance donor and a susceptible variety were screened with the diagnostic marker Bmac0029 to identify *rym4* and *rym5* resistance donors. By this approach, the offspring of the cross ‘Cebada’ x ‘Cleopatra’ and the cross ‘Belts 1823’ x ‘Franziska’ were identified to carry the recessive resistance genes *rym5* and *rym4*, respectively. Both genes have successfully been mapped already (SCHIEMANN et al. 1997, GRANER et al. 1999a, PELLIO et al. 2005). Map based cloning and sequencing revealed that *rym4* and *rym5* are two alleles of the same gene and encode a eukaryotic translation initiation factor 4E (Hv-eIF4E, STEIN et al. 2005). Since sequence information is already available and *rym4* and *rym5* are no longer effective against certain BaYMV strains

(HARIRI et al. 2003, HABEKUSS et al. 2006), no further analysis and mapping was carried out on these DH populations.

5.2.1 Mapping resistance genes on chromosome 4H

The resistance locus of the variety ‘CI 3517’ was mapped on chromosome 4H, with the closest linkage revealed by a cluster of SSR markers (Bmac0384, Bmac0181, Ebmac0906 and HVM03) in a distance of 8.4 cM. In comparison to the map position of the recessive resistance locus *rym11* of the DH mapping population ‘IPK1’ and ‘IPK2’ on chromosome 4H (NISSAN-AZZOUZ et al. 2005) allelism with the resistance locus of ‘CI 3517’ can be hypothesized because all three maps (see Fig. 10) show the same SSR markers linked, located all in the centromeric region of chromosome 4H. The map of ‘CI 3517’ compared with the partial map of ‘IPK1’ published by NISSAN-AZZOUZ et al. (2005) and the barley consensus map (VARSHNEY et al. 2007) revealed only some slight rearrangements of the marker order (see Fig. 10). The SSR markers Bmac0384 and Bmac0181 have been mapped proximally to *rym11* (NISSAN-AZZOUZ et al. 2005) contrary to the DH population of ‘CI 3517’, where a co-segregation with other SSR markers distally to the resistance locus was observed (NISSAN-AZZOUZ et al. 2005). Furthermore, the order of the flanking marker located distally is inversed compared to the barley consensus map published by VARSHNEY et al. (2007). The SSR marker HVM03 has been mapped distally to the resistance gene in the DH population of ‘CI 3517’, whereas the marker has been mapped proximally to *rym11* in the ‘IPK2’ map. These differences between the order of the markers and the map distances are assumed to be due to the size of the mapping populations, because in smaller mapping population estimations of recombination frequencies are not as accurate as in larger populations. Therefore rearrangements may be due to the higher resolution of the *rym11* region of ‘IPK1’ (191 DH lines) and ‘IPK2’ (161 DH lines) in comparison to the *rym* region of 65 DH lines of the population ‘CI 3517’ x ‘Reni (see Fig. 10). In addition, it has to be taken into account that SSR markers are clustering in the centromeric region (RAMSAY et al. 2000, LI et al. 2003) and a suppressed recombination occurs in proximal chromosome regions (KÜNZEL et al. 2000) leading to differences in the estimations of genetic distances. Furthermore, the order of SSR markers can vary due to the application of the AFLP markers in mapping of genes like applied to map the resistance genes of ‘IPK1’ and ‘IPK2’. Based on earlier works by WERNER et al.

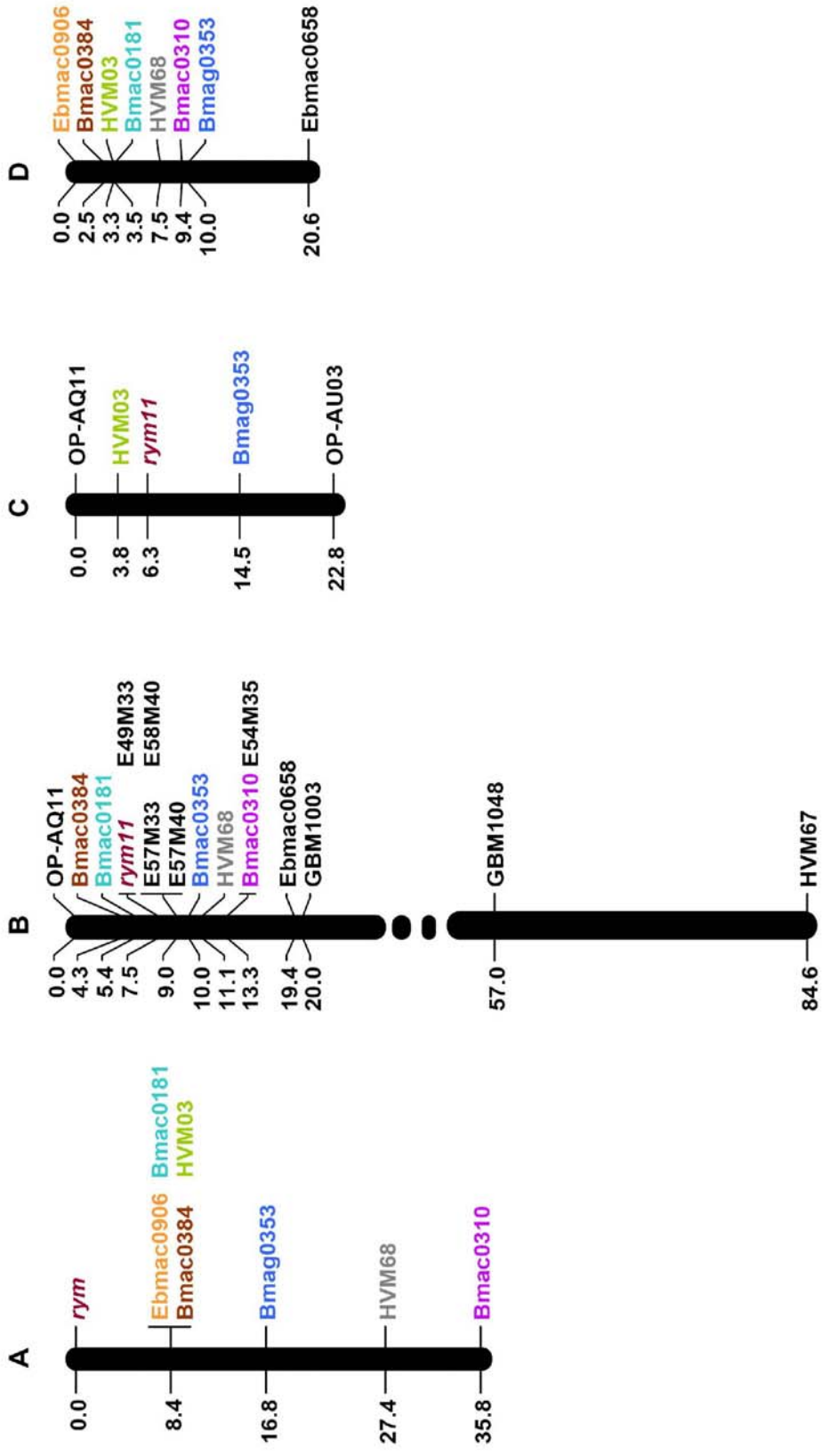

Figure 10: Genetic map of chromosome 4H including the BaMMV resistance of ‚CI 3517' based on the analysis of 65 DH lines of the cross ‚CI 3517' x ‚Reni' (A) in comparison to the partial chromosome map ‚IPK1' and ‚IPK2' with the resistance gene *rym11* (B, C, NISSAN-AZZOUZ et al. 2005) and the barley consensus map (D, VARSHNEY et al. 2007).

(2003b), the resistance gene *rym13* of 'Taihoku A' mapped on chromosome 4H, like the earlier described resistance genes *rym1* (OKADA et al. 2004), *rym8* (BAUER et al. 1997) *rym9* (WERNER et al. 2000a), the above mentioned *rym11* (BAUER et al. 1997, NISSAN-AZZOUZ et al. 2005) and *rym12* (GRANER et al. 1996). The closest linked markers in the mapping approach of WERNER et al. (2003b) revealed a cluster comprising the AFLP markers E53M36, E53M40 and the RAPD marker OP-C13 located 6.7 cM distally of the resistance gene *rym*13. Therefore, in the present work more closely linked single markers were identified by enlarging the mapping population to an entire DH population of 154 lines and mapping of additional AFLP markers. In doing so, a new linkage map was generated, comprising seven new AFLP markers, three microsatellite markers and two RAPD markers with the closest one present in a distance of 1.0 cM to the BaMMV resistance locus *rym13* (Fig. 11 B). Regarding the mapped SSR markers HVM67 and WMS06, the results suggest that the resistance gene *rym13* is located within the same genomic region like the resistance gene *rym9* from 'Bulgarian 347' (WERNER et al. 2000b, see Fig. 11). In both cases, HVM67 shows a closer linkage to the resistance genes compared to WMS06. On the basis of a preliminary allelism test (WERNER 2002) it can be deduced that *rym13* and *rym9* are not allelic like the *rym4/rym5* resistance locus (STEIN et al. 2005), but additional test are necessary to confirm these results.

As a result of previous studies by GRANER et al. (1996) it is known, that the resistance gene *rym12* is located on chromosome 4H, too. In the present study, *rym12* is mapped in the cross 'Muju covered 2' x 'Spirit' by the use of SSR markers of the long arm of chromosome 4H. Due to the limited availability of microsatellites in this region, polymorphisms between the bulks were only found by using the markers HVM67, Ebmac0877, and the EST derived SSR GBM1015. Ebmac0877 is distally the closest linked marker to the resistance locus with a distance of 7.8 cM (D, Figure 11). With respect to the other linkage maps mentioned above the SSR marker WMS06 does not reveal polymorphisms in the 'Muju covered 2' x 'Spirit' map. BAUER et al. (1997) mapped the resistance gene *rym8,* which shows only partial resistance against BaMMV and BaYMV (GRANER et al. 1999b), in the telomeric region of chromosome 4H in the map interval between the RFLP markers MWG051 and MWG616 (C, Fig. 11). Due to double-crossover events it was not possible to determine the exact map position. The mapping of the RFLP markers MWG051 and MWG616 suggest that the recessive resistance genes *rym8* and *rym9* are located in

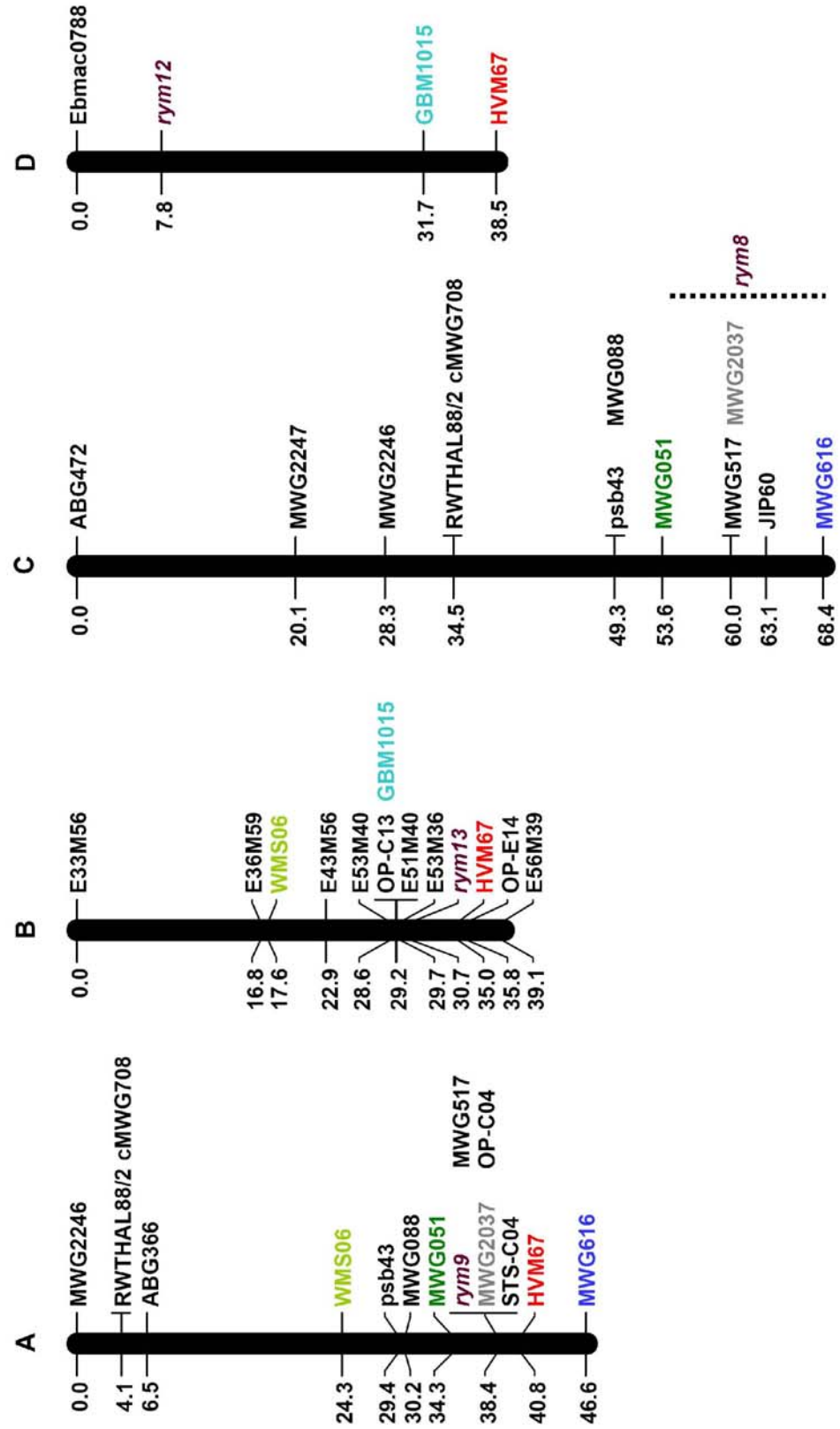

Figure 11: Genetic map of chromosome 4HL with the resistance gene *rym9* of 'Bulgarian 347' (A, WERNER et al. 2000b) in comparison with the genetic map of chromosome 4HL with the resistance gene *rym13* of 'Taihoku A' (B), the resistance gene *rym8* of '10247' (C, BAUER et al. 1997) and the resistance gene *rym12* of 'Muju covered 2' (D).

the same genomic region of chromosome 4H, because both RFLP markers are linked to the two resistance loci (BAUER et al. 1997). Therefore, the three resistance genes *rym8, rym9* and *rym13* form a gene cluster on the long arm of chromosome 4H in the telomeric region. To find out, if these resistance genes are allelic additional tests have to be carried out. With respect to *rym12*, which is located in the same telomeric region like *rym8*, *rym9*, and *rym13* (ORDON et al. 2004a), it may be concluded that it is pertinent to the gene cluster mentioned above. Further marker saturation with AFLP marker has to be done to find more closely linked markers to confirm this hypothesis. Due to the great distance of the SSR marker HVM67 of 77.7 cM to the resistance gene *rym11* from 'Russia 57' (see Fig. 10 C, NISSAN-AZZOUZ et al. 2005), it exemplifies the different position of *rym11* in contrast to *rym13*, where HVM67 showed a close linkage of 4.3 cM (Fig. 11 B).

5.2.2 Mapping resistance genes on chromosome 6H

The BaMMV resistance gene of the Japanese variety 'Chikurin Ibaraki 1' could be mapped on the short arm of chromosome 6H. The two closest PCR markers are the co-segregating SSRs Bmac0018 and Bmac0127, which have been mapped in a distance of 1.0 cM from the resistance gene *rym15*. These results are in accordance with the results of LE GOUIS et al. (2004) based on 217 DH lines of the cross 'Chikurin Ibaraki 1' x 'Plaisant'. Regarding the results of LE GOUIS et al. (2004), the SSR marker Bmac0173 shows the closest linkage to *rym15*. Unfortunately, Bmac0173 is monomorphic in the 'Chikurin Ibaraki 1' x 'Igri' population used in the present work (see Fig. 12). Besides the co-segregating SSRs Bmac0018 and Bmac0127 *rym15* was found to be flanked distally by the SSR Ebmac0874, which is in accordance with the results of LE GOUIS et al. (2004). The molecular marker order of the two already mentioned maps of chromosome 6HS are confirmed by maps published by RAMSAY et al. (2000) based on the DH population 'Lina' x '*H. spontaneum* Canada Park' composed of 86 DH lines and by the barley consensus map recently published by VARSHNEY et al. (2007). Both maps reveal a highly comparable clustering of the markers Ebmac0874 and Ebmac0806 with only slightly different genetic distances in between. Bmac0173, which is closely linked to *rym15* in the 'Chikurin Ibaraki 1' x 'Plaisant' population (LE GOUIS et al. 2004), is located distally in both maps. The two common SSR markers closest to *rym15* Bmac0127 and Bmag0018 also form a cluster in the 'Lina' x '*Hordeum spontaneum* Canada

Park' map of RAMSEY et al. (2000), but are mapped in a distance of 0.5 cM in the barley consensus map of VARSHNEY et al. 2007. Furthermore, the marker order of the 'Chikurin Ibaraki 1' x 'Igri' population used in the present study is highly similar when compared to other maps (LE GOUIS et al. 2003, RAMSAY et al. 2000, VARSHNEY et al. 2007), except for Ebmac0639, which is more closely linked to the centromeric region. A strong clustering of SSR markers close to the centromeric region of chromosome 6H was observed (RAMSAY et al. 2000, LI et al. 2003), which is probably due to suppressed recombination in the centromeric regions and which likely impede further marker saturation. This results in a gap of 14-22 cM without any mapped SSR markers at the short arm of chromosome 6H (RAMSAY et al. 2000, VARSHNEY et al. 2007). All markers shown in the four different linkage maps (see Fig. 12), are suitable for the marker assisted selection (MAS), whereas Bmac0127 and Bmac0018 can be used for a fine-mapping approach of the BaMMV resistance gene of 'Chikurin Ibaraki 1', because these markers are flanking the gene in a distance of 1.0 cM (see Fig. 12A). To check whether the majority of plants selected on the basis of these markers will carry the resistance-encoding allele, the two flanking markers Bmac0018 and Bmac0127, respectively, and Ebmac0874 can be chosen instead of only one. In addition, the markers Bmac0018, Bmac0127 and Ebmac0874 possess high diversity indices with 0.59, 0.83 and 0.62 (RAMSAY et al. 2000), which make them powerful tools for MAS (LE GOUIS et al. 2004) due to their high polymorphic character in European barley cultivars.

For the identification and mapping of a new resistance gene against the barley yellow mosaic virus disease the DH populations of the cross 'Shimane Omugi' x 'Gilberta' and 'Shimane Omugi' x 'Sumo' were characterised concerning their BaMMV reaction in greenhouse trials. Thereby, a BaMMV resistance gene could also be mapped on the short arm of chromosome 6H. In this case additional AFLP markers were used where E40M54 reveals the closest linkage within a distance of 2.2 cM, followed by a cluster of co-segregating AFLP markers. In comparison to the results of LE GOUIS et al. (2004), the barley consensus map of VARSHNEY et al. (2007), and the map of 'Chikurin Ibaraki 1' from the study discussed before, the two BaMMV resistance genes seem to be located within the same genomic region of chromosome 6HS. The map of the 'Shimane Omugi' resistance shows some rearrangements regarding the molecular marker order but there are still some of the same SSRs mapped. Only the SSR marker Ebmac0874 has been mapped distally of the resistance locus derived of

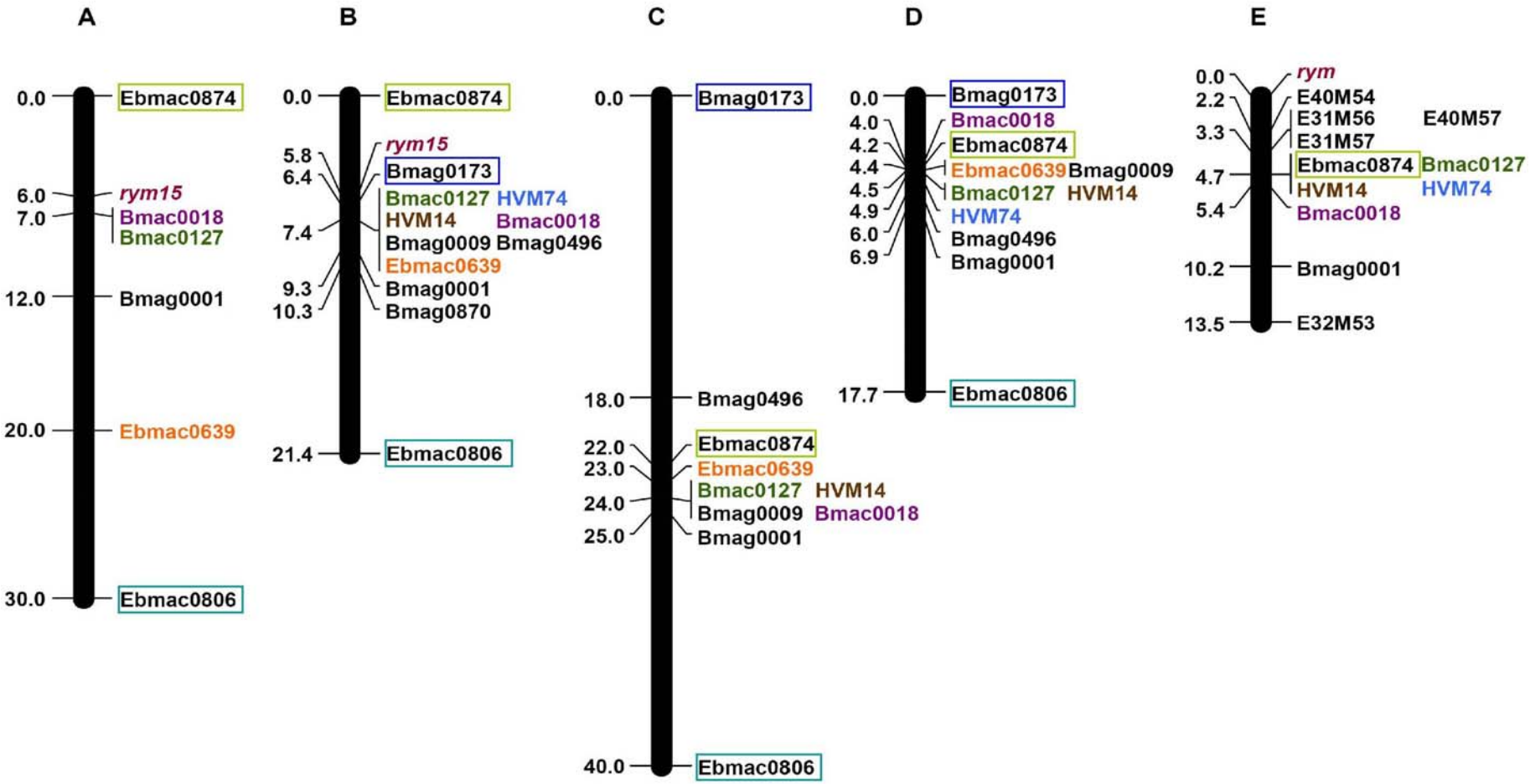

Figure 12: Genetic map of barley chromosome 6H including the BaMMV resistance gene *rym15* of 'Chikurin Ibaraki 1' based on the analysis of 163 DH lines derived from a 'Chikurin Ibaraki 1' x 'Igri' cross (A), 217 DH lines of a 'Chikurin Ibaraki 1' x 'Plaisant' cross (B) in comparison to the partial map of the 'Lina' x '*Hordeum spontaneum* Canada Park' map (C, RAMSAY et al. 2000), the barley consensus map of VARSHNEY et al. (2007, D) and to the partial map of 'Shimane Omugi' (E).

'Shimane Omugi' in opposite direction compared to the results of LE GOUIS et al. (2004). However, results are still in accordance to the population of 'Lina' x '*H. spontaneum* Canada Park' (RAMSAY et al. 2000). Furthermore, the SSR markers Bmac0127, HVM74 and HVM14 are also co-segregating in the DH population of 'Chikurin Ibaraki 1' x 'Plaisant', whereas the markers Bmac0127 and HVM14 have been mapped within a distance of 0.4 cM to HVM74 in the barley consensus map. Only two markers, HVM14 and Bmac0127, could be mapped by RAMSAY et al. (2000), but are also co-segregating. Molecular markers like Ebmac0806 and Bmac0173, which are located distantly from the centromeric region, turned out to be monomorphic in the 'Shimane Omugi' population. Due to a still concordant order of the SSRs of the maps of the 'Shimane Omugi' and 'Chikurin Ibaraki 1' resistance (Fig. 12A+B), it can be assumed that the different localization of the resistance gene *rym* is likely due to the included AFLP markers. In summary, it can be hypothesised, that the locus conferring resistance in 'Shimane Omugi' is the same like the resistance locus in 'Chikurin Ibaraki 1', which has to be proven by tests for allelism.

5.3 Application of doubled haploids and molecular markers in plant breeding

Molecular markers, in particular microsatellite markers (SSRs), are important tools to facilitate the effective selection on a single plant level in an early developmental stage independently of the symptom development in the field. The use of molecular markers for the breeding companies is time-saving, and therefore cost-effective which is a major aspect in developing new improved varieties (FRIEDT & ORDON 2004, ORDON et al. 2005) especially for a private profit-oriented breeding company. In the present study the usefulness of the SSR marker Bmac0029 as a diagnostic selection marker facilitated e.g. the identification of the already known resistance genes *rym4 and rym5.* Especially for mapping the 'CI 3517' resistance on chromosome 4H it was a prerequisite to eliminate DH lines carrying the *rym4* resistance gene to be able to map the new resistance gene. Furthermore, the usefulness of SSR markers in gene mapping and in MAS has been proven already in many different crop species like barley (WERNER et al. 2003a), wheat (PENG et al. 1999), and soybean (MUDGE et al. 1997). In comparison to RFLPs (GRANER et al. 1991), SSRs facilitate a much faster mapping and compared to AFLPs (VOS et al. 1995) specific SSR markers can be used by breeding companies directly and easily in plant breeding programs without the conversion into STS markers. Further on,

SSR markers are very useful tools for the location of a gene of interest on chromosomes (JOSHI et al. 1997) and therefore giving hint to the specific map position.

Regarding the present study, it was observed that the use of DH populations as mapping populations is well suited for the development and application of PCR-based markers to identify resistance genes against Barley yellow mosaic virus disease. DH populations are advantageous in comparison to F_2-populations, because DHs represent totally homozygous lines, with a defined segregation ratio of recessive to dominant genotypes in 1:1, which can be easily phenotyped (TUVESSON et al. 2007). The DH-technology, starting from F_1 donor plants, leads immediately to homozygous DH lines without further segregation and facilitate a more accurate selection compared to F_2-generations (WERNER et al. 2007). Further on, DH populations can be easily reproduced. Reliable phenotypic data are of high importance for marker development. These data can be obtained for BaMMV on segregating DH populations by mechanical inoculation in the greenhouse followed by DAS-ELISA (FRIEDT 1983), a prerequisite for the estimation of the segregation ratio (GÖTZ & FRIEDT 1993). Furthermore, DH populations are advantageous in comparison to recombinant inbred lines (RIL), because they can be produced in a shorter period of time. The DH-technology is already used for practical breeding in several crop species like rapeseed, wheat and barley (CUSTERS 2003, JACQUARD et al. 2003, TUVESSON et al. 2003, DEVAUX & PICKERING 2005). This procedure has also been developed for rye, triticale, oat, and cabbage, but is still rarely used (MANNINEN et al. 2004). Based on the DH-technology, the resistance genes *rym12*, *rym13*, *rym15*, the BaMMV resistance of ‘Shimane Omugi’ and of ‘CI 3517’ have been mapped. Furthermore, *rym4* (GRANER & BAUER 1993), *rym5* (GRANER et al. 1999), *rym13* (WERNER et al. 2003b), *rym15* (LE GOUIS et al. 2004) and the BaYMV/BaYMV-2 resistance of ‘Chikurin Ibaraki 1’ (WERNER et al. 2003a) have been identified by using DHs.

The availability and combination of molecular markers and doubled haploids facilitate an efficient combination of different resistance genes in one breeding line (pyramiding) against the barley yellow mosaic virus complex (ORDON et al. 2004, WERNER et al. 2005, 2007). Pyramiding may lead to durable and broad spectrum resistance (WERNER et al. 2000b, ORDON et al. 2005), which is of prime interest due to resistance breaking strains described in the last years in Europe and Japan

(NOMURA et al. 1996, HARIRI et al. 2003, HABEKUSS et al. 2006). There are several possibilities to create durable resistances due to the application of molecular markers, which were developed e.g. for the resistance genes *rym13* and *rym15* in this study. WERNER et al. (2005) reported on two strategies, which involve one and two DH steps, respectively, to combine the resistance genes *rym4* or *rym5* with *rym9* and *rym11.* Many of the resistance genes described before (see Chapter 2.2.1) – except *rym11* and *rym13* – are not effective against all strains of the barley yellow mosaic virus complex, *rym9* for example is only effective against BaMMV and BaMMV-SIL and *rym5* shows resistance against BaMMV, BaYMV and BaYMV-2 (KANYUKA et al. 2004) and are therefore appropriate genes for pyramiding strategies. The identified resistance genes in the present work like *rym13* or *rym15* can easily be incorporated into pyramiding strategies due to the availability of closely linked markers. The combination of genes is a useful approach for extending the usability of these resistance genes in barley breeding. For example the combination of *rym5* and *rym9* should result in a resistance against all strains of barley yellow mosaic virus known in Europe (KANYUKA et al. 2004). Pyramiding of genes has been applied in several crop breeding programmes leading to the development of varieties possessing multiple and durable resistances (ORDON et al. 2005, BOYD 2006, ZHANG et al. 2006). The successful marker-assisted pyramiding has already been reported for wheat with respect to three powdery mildew resistance genes *Pm3*, *Pm4a* and *Pm21* (LIU et al. 2000) and two cereal cyst nematode resistance genes of *Aegilops variabilis* (BARLOY et al. 2007). Furthermore ZHANG et al. (2006) published the combination of the two dominant resistance genes *Xa7* and *Xa21* against *bacterial blight* in hybrid rice. The combination of the two resistance genes *Bph1* and *Bph2* against the brown planthopper (*Nilaparvata lugens* Stål) into rice by means of pyramiding has also been reported (SHARMA et al. 2004).

5.4 Wheat genetic diversity

The wheat data presented in this study are the basis for ongoing breeding programmes for *soil-borne cereal mosaic virus*, because detailed knowledge on the genetic diversity between genotypes in the frame of a breeding programme is of prime interest and facilitates a more efficient selection of parental genotypes. Furthermore, parental lines can be selected based on the cluster analysis and molecular markers can be used for the identification of suitable wheat genotypes.

The objective of the studies on wheat was to analyse the genetic relatedness between 64 wheat genotypes, provided by different co-operation partners from Denmark, France and Germany, using 40 SSR markers and 30 *EcoRI+3/MseI+3* AFLP primer combinations. Both types of molecular markers were able to distinguish the 64 accessions examined and therefore found to be suitable for assessing the genetic diversity within this material. The set of 40 (39 polymorphic ones) SSRs produced a total number of 305 different alleles, which can be considered as sufficient to get stable and reliable estimations of the genetic relatedness (STACHEL et al. 2000). Although ZHANG et al. (2002) insist upon the need of 350 to 400 alleles to distinguish between wheat materials, STACHEL et al. (2000) required only 202 alleles to get a cluster analysis, which clearly differentiated between the wheat accessions according to their agroecological areas. Furthermore, STĘPIEŃ et al. (2007) came to the conclusion that 166 alleles are sufficient for the successful assessment of the genetic diversity in Polish wheat varieties. Thus the necessary number of polymorphic alleles can vary and depends highly on the investigated numbers of included varieties and their evolutionary relatedness (STACHEL et al. 2000, ROUSSEL et al. 2004). GAO et al. (2003) reported only on 163 alleles for the effective characterisation of 108 rice accessions and PANDEY (2006) suggested that 237 alleles are enough to cluster 161 barley varieties. Based on the polymorphic information content (PIC) value, which is a tool to measure the informativeness of a given SSR marker, 27 SSR markers used in the present study turned out to be highly polymorphic (PIC value > 0.5, STODART et al. 2005) and are therefore well suited for the use in genetic diversity studies and discrimination of varieties. In comparison to previous studies on genetic diversity of wheat cultivars, it could be shown that the average number of alleles per locus in the present work (7.5) was lower. The mean number of alleles per locus reported by RÖDER et al. (2002), studying 502 recent European wheat varieties, was 10.5, whereas ROUSSEL et al. (2005) detected the mean average of 16.4 alleles per locus in 480 European wheat cultivars released from 1840 to 2000. Furthermore ROUSSEL et al. (2004) assessed the genetic diversity of 559 French bread wheat varieties with 41 SSRs and found the average number of alleles with 14.5. These differences and the higher variation respectively are probably due to the analyses of old varieties and landraces in comparison to the present study where breeding lines and newer cultivars were used. This result could be explained by the intensive use of related species during the last decades

(ROUSSEL et al. 2004). In addition to this, the extended geographic distribution of the investigated genotypes is a further explanation (STACHEL et al. 2000, ROUSSEL et al. 2004). Further on, the number of genotypes, which were used in other studies for the estimation of genetic diversity, was usually higher when compared to the 64 genotypes described in this project. Therefore, a higher variation within the wheat material was expected. STACHEL et al. (2000) reported the mean number of alleles with 4.8 for studying genetic differentiation in only 60 wheat cultivars originating from Austria, Germany and Hungary. This value is comparable to the present study and to results of different authors, who detected 5.5 alleles per locus in 43 Chinese wheat varieties (ZHANG et al. 2002) and found the average number of 4.7 alleles per locus in 30 parents (LIU et al. 2007). The PIC values for each SSR marker (see Table 18, chapter 4.3) is comparable to the PIC values published in earlier works (PRASAD et al. 2000, MCCARTNEY et al. 2004). Only the wheat SSRs wmc167, wmc177, and wmc254 used in the present study were less informative compared i.e. to the work of PRASAD et al. (2000) and MCCARTNEY et al. (2004), who showed the use of SSR markers for detecting DNA polymorphism and haplotype diversity in wheat.

Regarding the use of AFLPs as genetic markers, one major advantage is the large number of scorable bands (ROY et al. 2004), which increases the power for the detection of polymorphisms. In the present study more than 1800 fragments were detected and 88.2 % of scorable AFLP loci turned out to be polymorphic, which is relatively high when compared to other studies. The mean level of polymorphism reported by HAZEN et al. (2002) or ROY et al. (2004) was 14 % and 46 %, respectively. However, both authors used only 8 *EcoRI+3/MseI+3* AFLP primer combinations in genetic diversity studies with 44 and 55 genotypes, respectively. The genetic similarity of the different AFLP markers was 0.74 in contrast to the SSR markers, where a wider range was found leading to a lower GS value of 0.54. Similar results were published by ROY et al. (2004) for bread wheat, RUSSELL et al. (1997) for barley, and UPTMOOR et al. (2003) for sorghum. In accordance with these results similar levels of the mean genetic diversity were observed with both marker systems. Regarding the SSR data the diversity index (DI) value, which is the mean number of alleles detected over all loci, is 0.57 and the genetic diversity within the set of the 64 wheat cultivars analysed with AFLP is 0.521. The comparison of AFLP markers with SSRs showed that microsatellite markers have a higher specificity while

AFLPs possess the highest marker index resulting from the large number of loci detected by one AFLP primer combination. This comparison was described in different studies of crop species like barley, wheat and soybean (POWELL et al. 1996, RUSSELL et al. 1997, BOHN et al. 1999). Furthermore, the knowledge about the genome location of SSRs is useful for future studies and for sampling the genome, but the efficiency in detection of polymorphism and therefore the generation of well saturated maps is much higher for the AFLP markers (MORAGUES et al. 2007). The results on genetic relatedness after UPGMA cluster analysis within the 64 wheat accessions revealed a clear grouping of the cultivars regarding their origin, respectively their breeding companies. With respect to the genetic diversity estimated by the Shannon-Weaver-Index for the 64 genotypes, which are separated into three different groups according to their breeding companies, it was observed, that the group with varieties of the French breeding company had the highest diversity with H'=0.524, whereas the genetic diversity was calculated on a similar level in the German and Danish accessions with H'=0.439 and H'=0.443, respectively. These differences can be considered to be due to their different pedigrees. For the cultivars of the French company it could be shown that wheat varieties like 'Tremie' or 'Cadenza' were used, which had already been released in France and the UK in contrast to the German and Danish breeding companies, where breeding lines with a similar genetic background were taken for the estimation of genetic relatedness.

6 Summary

Barley yellow mosaic virus (BaYMV) and *Barley mild mosaic virus* (BaMMV) have spread to the most winter barley growing areas in Europe and have become a serious threat to winter barley cultivation. Besides, an increasing spread of soil-borne viruses of wheat, i.e. *Soil-borne cereal mosaic virus* (SBCMV) and *Wheat spindle streak mosaic virus* (WSSMV), respectively, was observed in the last decade. Due to transmission of these viruses by the ubiquitous soil-borne fungus *Polymyxa graminis* chemical measures are neither efficient nor economically and environmentally acceptable to prevent high yield losses. The only way to ensure high crop yields in infested areas is breeding and cultivation of resistant cultivars. Therefore, the aim of the present study was to identify PCR-based markers for new resistance genes against BaYMV by analysing seven DH populations and to evaluate barley germplasm for new resistance donors by screening them with already known molecular markers. With respect to wheat the main objective was to identify sources of tolerance or resistance to SBCMV followed by marker-based genotyping of resistant and tolerant cultivars as the starting point of a breeding program.

After screening 120 exotic barley germplasm by using the SSR marker Bmac0029 for the identification of *rym4/rym5*, seven genotypes were detected, which carry neither *rym4* nor *rym5* and showed complete resistance against BaYMV/BaMMV in field trials. Those barley accessions are potential candidates for detecting new resistance genes. By analysing different DH populations the resistance locus of barley stock ‘CI 3517’ was mapped on the long arm of chromosome 4H, just like the resistance gene *rym13* of variety ‘Taihoku A’. The new closest linked marker E53M36 for *rym13* was mapped at a distance of 1.0 cM and can be used for MAS in the future. Furthermore, *rym12* of the resistant cultivar ‘Muju covered 2’ was localised by SSR markers on the long arm of chromosome 4H. However, closer molecular markers have to be developed for MAS. Using bulked segregant analysis (BSA) the resistance genes of Japanese varieties ‘Chikurin Ibaraki 1’ and ‘Shimane Omugi’ were mapped on chromosome 6H. Regarding *rym15* of ‘Chikurin Ibaraki 1’ the SSR markers Bmac0127 and Bmac0018 are closest linked with a distance of 1.0 cM. With respect to ‘Shimane Omugi’ E40M54 is the closest marker mapping in a distance of 2.2 cM. Based on the mapped SSR markers it can be hypothesised that the locus conferring

resistance in ‘Shimane Omugi’ is the same as the resistance locus in ‘Chikurin Ibaraki 1’. However, this has to be further proven by allelism tests.

In addition, 64 wheat accessions derived from a set of 1,146 cultivars tested for resistance to SBCMV of three different breeding companies were analysed for genetic relatedness using SSR markers and *EcoRI+3/MseI+3* AFLP primer combinations. The application of 40 genome covering microsatellites revealed a high level of genetic diversity (DI=0.57) and genetic similarity (GS) was estimated to range from GS=0.19 to GS=0.86, with an average of GS=0.49. The genetic diversity according to the Shannon-Weaver Index based on 30 AFLP primer combinations amounts to H’=0.521, whereas genetic similarity was estimated to vary between 0.50 and 0.97, with an average of GS=0.74. Furthermore, genetic diversity was measured among the wheat lines of the different breeding companies revealing a similar level between the German (H’=0.439) and the Danish materials (H’=0.443). Regarding the varieties of the French breeding company, a much higher genetic diversity (H’=0.524) was estimated, probably due to the incorporation of susceptible accessions and already released cultivars.

The results on genetic diversity in the breeding materials of barley and wheat developed by different European breeding companies presented here allow conclusions on the potentials for future progress. Above that, the identification of molecular genetic markers for different virus resistance genes enables the confirmation of the chromosomal location of resistance genes and an indirect selection for these major-gene resistances based on the respective molecular markers (“Smart Breeding”).

7 Zusammenfassung

Eine der bedeutendsten Viruskrankheiten im europäischen Wintergerstenanbau ist die bodenbürtige Gelbmosaikvirose. Die Krankheit wird in Europa durch einen Erregerkomplex verursacht, dem die Viren *Barley Mild Mosaic Virus* (BaMMV), BaMMV-SIL, *Barley Yellow Mosaic Virus* (BaYMV) und BaYMV-2 zugerechnet werden (HUTH 1989, HUTH & ADAMS 1990). Weiterhin gehört ein in Deutschland erst kürzlich entdeckter neuer BaMMV-Stamm dazu, der dem französischen BaMMV-SIL Stamm sehr ähnlich ist (HABEKUSS et al. 2006). Als weitere, bedeutende bodenbürtige Getreideviren sind das *Wheat Spindle Streak Mosaic Virus* (WSSMV) und das *Soil-borne Cereal Mosaic Virus* (SBCMV) zu nennen, für die in den letzten Jahren insbesondere im Winterweizenanbau eine starke Ausbreitung nachgewiesen wurde (HUTH 2002, HUTH & GOETZ 2007). Aufgrund der vektoriellen Übertragung der Viren durch den weit verbreiteten bodenbürtigen Pilz *Polymyxa graminis* (TOYAMA & KUSABA 1970) ist weder eine chemische Bekämpfung dieser Virosen noch eine weite Fruchtfolgestellung der Wintergerste bzw. des Winterweizens effektiv. Die einzige Möglichkeit zur Vermeidung hoher Ertragsverluste liegt somit im Anbau resistenter Sorten. Insgesamt wurden bisher 16 Resistenzgene gegenüber der Gelbmosaikvirose beschrieben, von denen lediglich die Resistenzgene *rym4* und *rym5* im aktuellen Sortenspektrum in Deutschland vorliegen. Mit der Entdeckung neuer Erregerstämme in Deutschland bzw. Europa, gegen welche *rym4/rym5*-Träger keine Resistenz zeigen, nimmt der Bedarf nach neuen Resistenzgenen bzw. der Integration weiterer Gene in das Sortenmaterial deutlich zu.

Wesentliches Ziel der vorliegenden Arbeit war es daher, molekulare Marker für Resistenzgene der Gerste gegen die Gelbmosaikvirose zu identifizieren, indem genetische Ressourcen mit Hilfe PCR-basierter Marker im Hinblick auf bekannte Resistenzgene analysiert sowie segregierende DH-Populationen untersucht wurden. Bezüglich Weizen zielte das Projekt auf die Identifikation von resistenten bzw. toleranten Sorten gegenüber SBCMV ab, gefolgt von einer molekularen Genotypisierung des bearbeiteten Weizenmaterials als Beginn eines zielgerichteten Resistenzzüchtungsprogramms.

Um neue Resistenzdonoren zu identifizieren, wurden 120 exotische Gerstenherkünfte aus der Genbank in Okayama, Japan, untersucht. Hierzu wurden

die Genotypen, die Resistenz gegen japanische BaYMV-Isolate zeigten, mit Hilfe des codominanten SSR-Markers Bmac0029 (GRANER et al. 1999a) im Hinblick auf die Resistenzgene *rym4* und *rym5* analysiert. Bei 44 Genotypen zeigte sich das für *rym5* spezifische Allel (148 bp) und in weiteren 12 Herkünften ein Fragment von 145 bp, welches für *rym4* spezifisch ist. Die sieben exotischen Gersten ‚Chikurin Ibaraki 3', ‚Hakusanmugi', ‚Hongcheon Anjeunbaengi 2', ‚Ramsar', ‚Sekitori 2', ‚Turkey 3' and ‚Turkey 179' (Heidi Jaiser, pers. Mitt.), die nach diesen Untersuchungen weder *rym4* oder *rym5* trugen und Resistenz in Feldversuchen gegen BaMMV, BaYMV-1 und BaYMV-2 Isolate zeigten, stellen nach weiteren Allelietests mit Resistenzdonoren wertvolles Ausgangsmaterial für eine Erweiterung der genetischen Basis gegenüber BaYMV/BaMMV dar.

Um für eine zielgerichtete Selektion molekulare Marker für Resistenzgene gegen die Gelbmosaikvirose zu entwickeln, wurden sieben verschiedene DH-Populationen genotypisiert. Zusätzlich zu den molekularen Analysen wurden die DH-Populationen anhand von Resistenztests gegenüber BaMMV mittels mechanischer Inokulation in Anlehnung an FRIEDT (1983) phänotypisiert und anschließend DAS-ELISA Tests im Gewächshaus durchgeführt. Zuerst erfolgte bei den molekularen Analysen ein Screening der Kreuzungen zwischen jeweils einem neuen Resistenzdonor und einer anfälligen Varietät mit dem SSR-Marker Bmac0029, um auszuschließen, dass in diesen trotz anderslautender Angaben *rym4* bzw. *rym5* vorhanden sind. Anhand dieser Vorgehensweise wurden in den Kreuzungen ‚Cebada' x ‚Cleopatra' und ‚Belts 1823' x ‚Franziska' die Resistenzgene *rym4* und *rym5* identifiziert. Da diese beiden Resistenzgene in vorherigen Arbeiten kartiert (SCHIEMANN et al. 1997, GRANER et al. 1999a, PELLIO et al. 2005) und isoliert (STEIN et al. 2005) wurden und nicht mehr gegen alle europäischen BaYMV-Stämme eine Resistenz zeigen, wurden keine weiteren Kartierungsarbeiten durchgeführt. Mittels der ‚bulked segregant analysis' (BSA) wurden in den verbleibenden fünf DH-Populationen Resistenzgene gegen die Gelbmosaikvirose mittels molekularer Marker lokalisiert. Dadurch konnte der BaMMV Resistenzlocus von ‚CI 3517', aus der 65 DH-Linien umfassenden Kreuzung ‚CI 3517' x ‚Reni', auf Chromosom 4H kartiert werden. Die aktuelle Kopplungskarte umfasst sieben SSR-Marker, wobei die am engsten gekoppelten Marker Bmac0181, Bmac0384, Ebmac0906 und HVM03 ein Cluster bilden und eine Distanz von 8,4 cM zu dem Resistenzlocus aufweisen. Des Weiteren konnte das Resistenzgen *rym13*

aus ‚Taihoku A', welches eine vollständige Resistenz gegen alle bisher in Europa auftretenden Gelbmosaikvirus-Stämme aufweist, auf dem langen Arm von Chromosom 4H lokalisiert werden. Dort wurden bereits aus vorherigen Arbeiten die Resistenzgene *rym1* (OKADA et al. 2004), *rym8* (BAUER et al. 1997), *rym9* (WERNER et al. 2000a), *rym11* (BAUER et al. 1997, NISSAN-AZZOUZ et al. 2005) und *rym12* (GRANER et al. 1996) lokalisiert. Die betreffende Kopplungsgruppe der Kreuzung ‚Taihoku A' x ‚Plaisant' (154 DH-Linien) besteht aus sieben AFLP-, drei SSR- und zwei RAPD-Markern und besitzt eine Länge von 39,1 cM. Der AFLP-Marker E53M36 ist bei einem Abstand von 1,0 cM mit *rym13* am engsten gekoppelt. Proximal zu dem Resistenzlocus konnte der SSR-Marker HVM67 in einer Distanz von 4,3 cM zu *rym13* kartiert werden. Aufgrund der in dieser Population kartierten SSR-Marker HVM67 und WMS06 kann davon ausgegangen werden, dass *rym13* in dem gleichen Chromosomenabschnitt wie *rym9* und *rym8* lokalisiert ist. Mittels einer SSR-Analyse konnte das Resistenzgen *rym12* von ‚Muju covered 2' aus der Kreuzung ‚Muju covered 2' x ‚Spirit', bestehend aus 151 DH-Linien, ebenfalls auf dem langen Arm von Chromosom 4H kartiert werden. Aufgrund der geringen Markerabsättigung in dieser Region umfasst die genetische Karte mit einer Länge von 38,7 cM lediglich drei SSR-Marker. Dabei zeigt der Marker Ebmac0877 mit einer Distanz von 7,8 cM den geringsten Abstand zu *rym12*. Um Aussagen darüber treffen zu können, ob *rym12* in der gleichen Region wie *rym8, rym9* und *rym13* lokalisiert ist, müssen weitere Marker in dieser Region kartiert werden.

Der BaMMV-Resistenzlocus von ‚Shimane Omugi' konnte auf dem langen Arm von Chromosom 6H lokalisiert werden. Hierzu wurden die zwei Kreuzungen ‚Shimane Omugi' x ‚Gilberta' und ‚Shimane Omugi' x ‚Sumo' zu einer Kartierungspopulation von 97 DH-Linien zusammengefasst. Die Kopplungsgruppe umfasst eine Länge von 13,5 cM mit insgesamt sechs AFLP-Markern und sechs Mikrosatellitenmarkern. Der Marker mit der geringsten Kopplung zu dem Resistenzlocus ist der AFLP-Marker E40M54, welcher in einer Distanz von 2,2 cM kartiert werden konnte. Des Weiteren konnten drei AFLP-Marker, welche co-segregieren, proximal mit einem Abstand von 3,3 cM zu dem Resistenzgen kartiert werden. In der 163 DH-Linien umfassenden Population ‚Chikurin Ibaraki 1' x ‚Igri' konnte das Resistenzgen *rym15* in der centromeren Region von Chromosom 6H lokalisiert werden. Die aktuelle Kopplungsgruppe, die 30 cM umfasst, besteht aus sechs Mikrosatellitenmarkern, wobei drei SSR-Marker das BaMMV-Resistenzgen *rym15* flankieren. Die beiden am

engsten gekoppelten SSR-Marker sind Bmac0018 und Bmac0127, welche proximal einen Abstand von 1,0 cM zu dem Resistenzlocus aufweisen. Distal konnte der Mikrosatellit Ebmac0874 in einer Distanz von 6,0 cM zu dem Resistenzgen *rym15* lokalisiert werden. Die beiden Marker Bmac0018 und Bmac0127 sind aufgrund ihrer geringen genetischen Distanz zu dem BaMMV-Resistenzlocus sehr gut für eine markergestützte Selektion geeignet. Im Hinblick auf die Resistenz von ‚Shimane Omugi' und dessen Lokalisation auf Chromosom 6H kann durchaus vermutet werden, dass es sich um identische Resistenzloci handelt. Um dies bestätigen zu können, müssen weitere Allelietests durchgeführt werden.

Zur Identifikation resistenter Weizengenotypen gegenüber *Soil-borne cereal mosaic virus* (SBCMV) wurden 1146 Sorten und Genotypen in Feldversuchen in Frankreich von Züchtern getestet, von denen 64 potentielle Kreuzungspartner auf molekularer Ebene unter Verwendung von 40 Mikrosatelliten und 30 *EcoR*I+3/*Mse*I+3 AFLP-Primerkombinationen charakterisiert wurden. Basierend auf der Auswertung der Fragmentmuster und der Erstellung einer 0/1 Matrix wurde die genetische Ähnlichkeit nach NEI und LI (1979) errechnet sowie die genetische Diversität nach Shannon-Weaver (1949). Im Rahmen der SSR-Analysen wurden insgesamt 305 Fragmente detektiert, wobei 1 bis 17 Allelen pro Locus entsprechend durchschnittlich 7,65 Allelen pro Locus, identifiziert werden konnten. Die ermittelten Polymorphic Information Content (PIC) – Werte lagen zwischen 0,00 (wmc41) und 0,89 (wmc276). Innerhalb des Sortimentes wurde anhand der Daten eine genetische Diversität (DI) von DI=0,57 ermittelt und die genetische Ähnlichkeit (GS) umfasste einen Bereich von GS=0,19-0,86 (Mittelwert GS=0,49), wobei der größte Wert der genetischen Ähnlichkeit zwischen den dänischen Züchtungslinien '701-176c' und '701-177c' und die geringste Ähnlichkeit zwischen den Varietäten ‚Sponsor' und ‚Enesco' auftrat. Ähnliche Ergebnisse zeigten die AFLP-Analysen. Basierend auf der Untersuchung von 1847 Fragmenten wurde eine genetische Diversität von H'=0,52 ermittelt, wobei der Prozentanteil der polymorphen Loci bei 88,2 % lag. Die genetische Ähnlichkeit wurde anhand der Analysen mit GS=0,50-0,97 (Mittel GS=0,74) bestimmt. Die größte genetische Ähnlichkeit konnte zwischen den Varietäten ‚Tremie' und ‚Taldor' und die geringste wiederum zwischen ‚Sponsor' und ‚Enesco' ermittelt werden. Die Clusteranalysen, die auf den genetischen Ähnlichkeitskoeffizienten basieren, zeigten bei den SSR- sowie den AFLP-Analysen eine deutliche Gruppierung von Genotypen

gleicher geographischer Abstammung, welche sich weiter entsprechend der Herkunft der untersuchten Sorten (Züchterhäuser) untergliedert. Im Hinblick auf die genetische Diversität differenziert nach den jeweiligen Gruppen, die sich nach den drei verschiedenen Züchtungshäusern richten, konnte für das dänische (H'=0,443) und deutsche Sortiment (H'=0,439) eine ähnlich große genetische Diversität beobachtet werden. Dagegen war die Diversität zwischen den Varietäten der französischen Gruppe mit H'=0,524 deutlich größer. Aufgrund dieser Ergebnisse ist von einer ausreichenden genetischen Variabilität zwischen den resistenten Linien und im Vergleich zu anfälligen Sorten auszugehen, so dass eine gute Basis für eine effektive Resistenzzüchtung von Weizen gegen SBCMV gegeben ist.

Die hier präsentierten Ergebnisse der genetischen Diversität von Zuchtmaterial der Gerste und des Weizens verschiedener europäischer Züchter verdeutlichen das große Potenzial für zukünftige Züchtungsprogramme. Des Weiteren ermöglicht die Entwicklung von molekularen Markern für verschiedene Virusresistenzgene die Identifizierung und Bestätigung der chromosomalen Lokalisation und die indirekte marker-gestützte Selektion auf diese Resistenzen („Smart Breeding").

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9 Appendix

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Table A1: PCR recipes and PCR program of the 26 microsatellites used for bulked segregant analysis.

SSR	forward primer	reverse primer	repeat motif	labelling	fragment size	PCR recipe	PCR program	Chromosome
Bmac0399	CGATGCTTTACTATGAGAGGT	GGGTCTGAAGCCTGAAC	(AC)21	700	145	1	D	1H
Bmag0211	ATTCATCGATCTTGTATTAGTCC	ACATCATGTCGATCAAAGC	(CT)16	700	174	2	F	1H
WMC1E8	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	(AC)24	700	197	3	E	1H
HVM36	TCCAGCCGACAATTTCTTG	AGTACTCCGACACCACGTCC	(GA)13	800	114	2	A	2H
Bmac0093	CGTTTGGGACGTATCAAT	GGGAGTCTTGAGCCTACTG	(AC)24	700	151	1	E	2H
EBmac0415	GAAACCCATCATAGCAGC	AAACAGCAGCAAGAGGAG	(AC)17	700	247	1	E	2H
HvLTPPB	AGACGCTGAGTACGTTGAG	CAAAGTACAACAAACTCACGA	(AC)10(AT)5	800	221	4	E	3H
Bmag0136	GTACGCTTTCAAACCTGG	GTAGGAGGAAGAATAAGGAGG	(AG)6-(AG)10-(AG)6	800	197	2	F	3H
Bmag0013	AAGGGGAATCAAAATGGGAG	TCGAATAGGTCTCCGAAGAAA	(CT)21	700	155	1	F	3H
Bmag0225	AACACACCAAAAATATTACATCA	CGAGTAGTTCCCATGTGAC	(AG)26	700	162	1	F	3H
HVM40	CGATTCCCCTTTTCCCAC	ATTCTCCGCCGTCCACTC	(GA)6(GT)4(GA)7	800	160	6	A	4H
Bmag0353	ACTAGTACCCACTATGCACGA	ACGTTCATTAAAATCACAACTG	(AG)21	800	119	1	F	4H
HVM67	GTCGGGCTCCATTGCTCT	CCGGTACCCAGTGACGAC	(GA)11	700	116	5	A	4H
HVMLO3	CTTCCATGTCACCTACAG	CGAACTGGTATTCCAAGG	/	700	88	1	D	4H
EBmac0684	TTCCGTTGAGCTTTCATACAC	ATTGAATCCCAACAGACACAA	(TA)7(TG)11,(TG)11(TTTG)5	800	172	1	E	5H
Bmag0337	ACAAAGAGGGAGTAGTACGC	GACCCATGATATATGAAGATCA	(AG)22	800	145	1	E	5H
HvLOX	CAGCATATCCATCTGATCTG	CACCCTTATTTATTGCCTTAA	(AG)9	800	150	7	F	5H
EBmac0970	ACATGTGATACCAAGGCAC	TGCATAGATGATGTGCTTG	(AC)8	700	112	1	E	5H
Bmac0316	ATGGTAGAGGTCCCAACTG	ATCACTGCTGTGCCTAGC	(AC)19	800	135	1	E	6H
Bmac0018	GTCCTTTACGCATGAACCGT	ACATACGCCAGACTCGTGTG	(AC)11	800	138	2	F	6H
Bmac0040	AGCCCGATCAGATTTACG	TTCTCCCCTTTGGTCCTTG	(AC)20	800	236	6	F	6H
EBmac0806	ACTAAGTCCTTTCACGAGGA	GTGTGTAGTAGGTGGGTACTTG	(CA)4GA(CA)8,(CA)5	800	168	1	E	6H
Bmag0021	ATTTTTATCAGAACGTCTCTCTC	CTAACTTCTCTCTCCCTCTCC	(CA)10AA(GA)28	800	143	2	F	7H
HVCMA	GCCTCGGTTTGGACATATAAAG	GTAAAGCAAATGTTGAGCAACG	(AT)9	800	141	2	D	7H
Bmag0120	ATTTCATCCCCAAAGGAGAC	GTCACATAGACAGTTGTCTTCC	(AG)15	700	230	1	F	7H
Bmag0007	TGAAGGAAGAATAAACAACCAACA	TCCCCTATTATAGTGACGGTGTG	(AG)16(AC)16	800	185	1	F	7H

Table A2: PCR recipes in µl for the SSR markers used in Table A1.

No.	1	2	3	4	5	6	7
DNA	2.0	2.0	2.0	2.0	2.0	2.0	2.0
H_20	6.7	10.7	13.2	13.1	5.7	13.0	11.9
PCR-Buffer	2.0	2.0	2.0	2.0	2.0	2.0	2.0
dNTPs	0.4	0.4	0.2	0.4	0.4	0.4	0.4
$MgCl_2$	0.8	0.8	0.4	0.4	1.8	0.5	1.6
R-primer (2pmol/µl)	4.0	2.0	1.0	1.0	4.0	1.0 (5pmol/µl)	1.0 (5pmol/µl)
F-primer (2pmol/µl)	4.0	2.0	1.0	1.0	4.0	1.0 (5pmol/µl)	1.0 (5pmol/µl)
Taq-polymerase	0.1	0.1	0.2	0.1	0.1	0.1	0.1

Table A3: PCR program for the SSR-analysis.

PCR program	Phases
A	18 cycles of 1 min at 94°C, 1 min at 64°C (0.5°C/cycle), 1 min at 72°C 30 cycles of 1 min at 94°C, 1min at 55°C, 1 min at 72°C 1 cycle of 5 min at 72°C
B	18 cycles of 1 min at 94°C, 1 min at 69°C (0.5°C/cycle), 1 min at 72°C 30 cycles of 1 min at 94°C, 1min at 55°C, 1 min at 72°C 1 cycle of 5 min at 72°C
C	1 cycle of 3 min at 94°C, 2 min at 55°C, 1 min 30 s at 72°C 30 cycles of 1 min at 94°C, 1min at 55°C, 1 min 30 s at 72°C 1 cycle of 5 min at 72°C
D	1 cycle of 3 min at 94°C, 1 min at 66°C, 1 min at 72°C 6 cycles of 30 s at 94°C, 30 s at 65°C 24 cycles of 30 s at 72°C, 30 s at 94°C, 30 s 60°C 1 cycle of 5 min at 72°C
E	1 cycle of 3 min at 94°C, 1 min at 55°C, 1 min at 72°C 30 cycles of 1 min at 94°C, 1min at 55°C, 1 min at 72°C 1 cycle of 5 min at 72°C
F	1 cycle of 3 min at 94°C, 1 min at 58°C, 1 min at 72°C 30 cycles of 1 min at 94°C, 1min at 58°C, 1 min at 72°C 1 cycle of 5 min at 72°C
Ebmac906	1 cycle of 3 min at 94°C, 30 s at 52°C, 30 s at 72°C 25 cycles of 30 s at 94°C, 30 s at 52°C, 30 s at 72°C 1 cycle of 5 min at 72°C
GBM	1 cycle of 3 min at 94°C 10 cycles of 30 s at 94°C, 30 s 60°C (-0.5°C/cycle), 15 s 72°C 30 cycles of 30 s at 94°C, 30 s 55°C, 15 s 72°C 1 cycle of 5 min at 72°C

PCR program	Phases
HVM03	1 cycle of 3 min at 94°C, 2 min at 55°C, 1 min 30 s at 72°C 30 cycles of 1 min at 94°C, 2 min at 55°C, 1 min 30 s at 72°C 1 cycle of 5 min at 72°C
HVM15	18 cycles of 3 min at 94°C, 1 min at 94°C, 30 s 64°C (-0.5°C/cycle), 1 min at 72°C 30 cycles of 1 min at 94°C, 1min at 55°C, 1 min at 72°C

Table A4: PCR recipes of the wheat SSRs.

	I	II
DNA	2.0 µl	2.0 µl
10x PCR-buffer	2.5 µl	1.5 µl
dNTPs	0.4 µl	0.3 µl
$MgCl_2$	/	0.4 µl
R-primer	1.0 ml	0.15 µl
F-primer	1.0 µl	0.15 µl
M13-primer	/	0.15 µl
H_20	18.0 µl	10.25 µl
Taq- polymerase	1.0 µl	1.0 µl

Table A5: PCR programs for the wheat SSR reactions.

PCR program	Phases
I	1 cycle of 3 min at 94°C 45 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C 1 cycle of 10 min at 72°C
II	1 cycle of 3 min at 95°C 35 cycles of 20 s at 95°C, 20 s at 55°C, 30 s at 72°C 1 cycle at 5 min at 72°C

Table A6: Genetic similarity coefficient (DICE) using SSRs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 BE01	1.00																										
2 BE02	0.51	1.00																									
3 BE03	0.51	0.37	1.00																								
4 BE04	0.56	0.48	0.54	1.00																							
5 BE05	0.56	0.42	0.61	0.64	1.00																						
6 BE06	0.61	0.47	0.58	0.64	0.78	1.00																					
7 BE07	0.46	0.40	0.53	0.50	0.62	0.67	1.00																				
8 BE08	0.53	0.37	0.56	0.59	0.70	0.77	0.62	1.00																			
9 BE09	0.47	0.38	0.61	0.61	0.59	0.56	0.62	0.54	1.00																		
10 BE10	0.48	0.41	0.49	0.48	0.58	0.59	0.58	0.51	0.52	1.00																	
11 Asperge	0.58	0.48	0.57	0.51	0.53	0.60	0.58	0.54	0.57	0.59	1.00																
12 BE12	0.53	0.40	0.50	0.42	0.52	0.58	0.60	0.58	0.50	0.60	0.59	1.00															
13 BE13	0.48	0.31	0.44	0.43	0.58	0.61	0.53	0.50	0.55	0.55	0.54	0.55	1.00														
14 BE14	0.46	0.45	0.44	0.46	0.57	0.58	0.62	0.53	0.51	0.42	0.53	0.61	0.46	1.00													
15 BE15	0.58	0.44	0.54	0.61	0.71	0.73	0.67	0.70	0.61	0.61	0.62	0.61	0.62	0.59	1.00												
16 BE16	0.51	0.43	0.53	0.56	0.65	0.67	0.61	0.53	0.67	0.58	0.54	0.54	0.57	0.62	0.60	1.00											
17 BE17	0.56	0.44	0.59	0.59	0.61	0.65	0.56	0.63	0.59	0.58	0.62	0.54	0.49	0.51	0.63	0.56	1.00										
18 BE18	0.48	0.40	0.49	0.52	0.62	0.61	0.55	0.64	0.50	0.49	0.62	0.58	0.55	0.52	0.60	0.53	0.56	1.00									
19 BE19	0.53	0.46	0.43	0.48	0.48	0.58	0.52	0.58	0.52	0.52	0.61	0.56	0.53	0.55	0.59	0.54	0.61	0.50	1.00								
20 BE20	0.51	0.42	0.41	0.44	0.50	0.58	0.52	0.56	0.50	0.54	0.57	0.69	0.55	0.51	0.57	0.54	0.50	0.58	0.65	1.00							
21 BE21	0.50	0.46	0.52	0.49	0.53	0.58	0.52	0.52	0.57	0.48	0.61	0.51	0.56	0.51	0.61	0.50	0.59	0.62	0.59	0.65	1.00						
22 BE22	0.48	0.41	0.51	0.56	0.62	0.61	0.61	0.64	0.62	0.61	0.62	0.60	0.56	0.54	0.67	0.50	0.60	0.63	0.57	0.60	0.62	1.00					
23 BE23	0.44	0.42	0.41	0.46	0.44	0.47	0.49	0.47	0.53	0.47	0.46	0.53	0.41	0.47	0.46	0.51	0.48	0.40	0.46	0.48	0.43	0.52	1.00				
24 BE24	0.48	0.32	0.44	0.47	0.53	0.52	0.40	0.48	0.53	0.48	0.52	0.53	0.69	0.38	0.55	0.53	0.47	0.51	0.47	0.55	0.57	0.54	0.54	1.00			
25 BE25	0.49	0.44	0.46	0.47	0.53	0.54	0.48	0.52	0.50	0.46	0.53	0.46	0.49	0.46	0.55	0.56	0.59	0.46	0.65	0.57	0.59	0.52	0.42	0.50	1.00		
26 BE26	0.52	0.43	0.48	0.47	0.53	0.59	0.53	0.59	0.53	0.55	0.52	0.57	0.50	0.50	0.65	0.53	0.58	0.53	0.58	0.58	0.62	0.59	0.52	0.54	0.64	1.00	
27 BE27	0.46	0.35	0.53	0.54	0.59	0.62	0.53	0.62	0.59	0.53	0.56	0.54	0.66	0.48	0.67	0.55	0.59	0.57	0.49	0.58	0.63	0.62	0.45	0.61	0.50	0.61	1.00
28 BE28	0.54	0.47	0.42	0.50	0.53	0.62	0.51	0.57	0.55	0.50	0.56	0.53	0.54	0.60	0.64	0.59	0.55	0.55	0.59	0.55	0.59	0.58	0.43	0.56	0.52	0.54	0.55
29 BE29	0.55	0.49	0.51	0.52	0.58	0.63	0.53	0.55	0.58	0.55	0.58	0.50	0.50	0.47	0.64	0.55	0.60	0.47	0.60	0.64	0.64	0.57	0.55	0.53	0.67	0.67	0.55

Table A6 Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
30 BE30	0.53	0.42	0.52	0.55	0.59	0.66	0.60	0.58	0.61	0.54	0.57	0.58	0.58	0.57	0.67	0.60	0.59	0.54	0.58	0.58	0.65	0.64	0.44	0.53	0.55	0.57	0.69
31 701-37c	0.45	0.38	0.61	0.55	0.61	0.56	0.52	0.54	0.62	0.52	0.55	0.55	0.54	0.44	0.55	0.54	0.57	0.58	0.50	0.53	0.59	0.58	0.48	0.54	0.51	0.52	0.59
32 701-42c	0.50	0.47	0.55	0.62	0.62	0.63	0.51	0.53	0.64	0.50	0.52	0.51	0.57	0.48	0.64	0.61	0.60	0.53	0.53	0.55	0.58	0.61	0.43	0.58	0.54	0.54	0.68
33 701-176a	0.47	0.50	0.50	0.61	0.57	0.54	0.50	0.48	0.65	0.44	0.53	0.42	0.52	0.51	0.55	0.56	0.61	0.52	0.55	0.46	0.57	0.56	0.54	0.53	0.59	0.52	0.60
34 701-176c	0.49	0.48	0.52	0.64	0.61	0.54	0.46	0.56	0.66	0.46	0.49	0.44	0.50	0.53	0.59	0.56	0.62	0.58	0.53	0.48	0.59	0.62	0.50	0.54	0.62	0.58	0.59
35 701-177a	0.54	0.47	0.53	0.67	0.62	0.57	0.51	0.51	0.65	0.46	0.54	0.43	0.54	0.50	0.62	0.59	0.65	0.53	0.53	0.47	0.54	0.59	0.52	0.54	0.60	0.56	0.62
36 701-177c	0.44	0.40	0.43	0.55	0.50	0.49	0.43	0.47	0.59	0.39	0.42	0.40	0.53	0.49	0.54	0.52	0.50	0.45	0.54	0.44	0.51	0.43	0.48	0.47	0.53	0.51	0.52
37 701-191a	0.50	0.37	0.47	0.48	0.54	0.57	0.58	0.58	0.54	0.49	0.50	0.58	0.57	0.57	0.56	0.51	0.54	0.49	0.54	0.64	0.60	0.62	0.45	0.48	0.52	0.55	0.58
38 701-210a	0.53	0.40	0.50	0.48	0.56	0.58	0.56	0.60	0.54	0.50	0.55	0.67	0.51	0.59	0.59	0.50	0.57	0.49	0.60	0.65	0.61	0.62	0.53	0.45	0.51	0.58	0.60
39 701-210b	0.49	0.44	0.50	0.51	0.57	0.62	0.58	0.59	0.53	0.61	0.58	0.63	0.60	0.53	0.59	0.58	0.61	0.60	0.57	0.61	0.65	0.65	0.54	0.64	0.53	0.62	0.63
40 701-244c	0.44	0.40	0.40	0.48	0.47	0.51	0.49	0.47	0.49	0.51	0.48	0.46	0.51	0.45	0.49	0.51	0.45	0.47	0.48	0.48	0.48	0.55	0.44	0.45	0.52	0.46	0.49
41 701-256b	0.55	0.42	0.58	0.53	0.59	0.57	0.49	0.52	0.56	0.45	0.50	0.48	0.55	0.49	0.56	0.56	0.59	0.58	0.50	0.48	0.63	0.51	0.46	0.57	0.59	0.51	0.54
42 701-372c	0.51	0.43	0.55	0.56	0.62	0.61	0.59	0.57	0.60	0.49	0.50	0.50	0.57	0.49	0.65	0.59	0.56	0.57	0.50	0.58	0.68	0.61	0.45	0.55	0.63	0.61	0.58
43 701-422b	0.49	0.40	0.57	0.57	0.64	0.65	0.54	0.59	0.61	0.58	0.66	0.52	0.64	0.47	0.75	0.56	0.57	0.64	0.59	0.56	0.63	0.65	0.42	0.57	0.55	0.58	0.68
44 701-477b	0.46	0.39	0.54	0.48	0.56	0.59	0.58	0.55	0.56	0.49	0.59	0.52	0.55	0.48	0.61	0.55	0.54	0.55	0.62	0.58	0.62	0.59	0.45	0.46	0.54	0.50	0.60
45 701-477c	0.48	0.34	0.49	0.52	0.52	0.57	0.55	0.58	0.56	0.45	0.54	0.49	0.48	0.54	0.58	0.53	0.58	0.45	0.60	0.54	0.54	0.59	0.55	0.50	0.58	0.51	0.57
46 701-481a	0.41	0.46	0.46	0.41	0.50	0.47	0.48	0.40	0.55	0.50	0.53	0.46	0.49	0.38	0.50	0.54	0.46	0.46	0.44	0.48	0.47	0.50	0.43	0.44	0.57	0.47	0.52
47 798-398b	0.36	0.47	0.40	0.44	0.50	0.51	0.51	0.43	0.45	0.39	0.46	0.42	0.50	0.49	0.50	0.45	0.45	0.55	0.55	0.53	0.58	0.51	0.38	0.43	0.52	0.44	0.49
48 Autan	0.47	0.38	0.48	0.53	0.59	0.62	0.58	0.54	0.64	0.56	0.47	0.51	0.58	0.48	0.66	0.63	0.59	0.48	0.57	0.57	0.61	0.62	0.50	0.58	0.49	0.60	0.65
49 Bobino	0.52	0.48	0.52	0.51	0.57	0.64	0.54	0.58	0.55	0.52	0.59	0.55	0.52	0.53	0.63	0.52	0.57	0.56	0.55	0.61	0.71	0.60	0.52	0.48	0.57	0.54	0.60
50 Brando	0.43	0.26	0.46	0.49	0.47	0.48	0.42	0.51	0.55	0.46	0.49	0.43	0.44	0.42	0.55	0.50	0.49	0.46	0.41	0.45	0.46	0.56	0.43	0.50	0.47	0.52	0.50
51 Cadenza	0.52	0.44	0.52	0.53	0.55	0.54	0.40	0.46	0.51	0.48	0.55	0.48	0.43	0.40	0.51	0.54	0.53	0.52	0.53	0.63	0.61	0.50	0.44	0.49	0.60	0.52	0.52
52 Charger	0.54	0.49	0.55	0.56	0.65	0.63	0.59	0.57	0.62	0.53	0.67	0.60	0.57	0.60	0.65	0.59	0.62	0.69	0.60	0.66	0.72	0.63	0.49	0.50	0.56	0.54	0.64
53 Claire	0.39	0.36	0.44	0.44	0.47	0.48	0.51	0.45	0.58	0.47	0.48	0.49	0.48	0.43	0.50	0.51	0.45	0.43	0.50	0.49	0.48	0.50	0.45	0.50	0.38	0.50	0.51
54 Enesco	0.46	0.28	0.51	0.44	0.45	0.42	0.43	0.45	0.54	0.40	0.46	0.43	0.40	0.45	0.47	0.49	0.54	0.35	0.47	0.43	0.44	0.46	0.40	0.44	0.48	0.44	0.40
55 Farandole	0.39	0.37	0.39	0.41	0.50	0.55	0.48	0.46	0.46	0.42	0.56	0.42	0.51	0.44	0.58	0.53	0.42	0.48	0.54	0.54	0.56	0.47	0.46	0.47	0.43	0.47	0.51

Table A6 Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
56 Gaspard	0.30	0.35	0.33	0.42	0.36	0.39	0.36	0.35	0.46	0.27	0.38	0.32	0.33	0.36	0.42	0.40	0.32	0.32	0.34	0.40	0.39	0.39	0.44	0.41	0.33	0.39	0.41
57 Gascogne	0.31	0.39	0.35	0.33	0.48	0.41	0.40	0.36	0.40	0.36	0.33	0.40	0.43	0.42	0.48	0.38	0.38	0.38	0.38	0.42	0.47	0.47	0.41	0.45	0.35	0.53	0.44
58 Igor	0.30	0.40	0.35	0.42	0.40	0.37	0.38	0.35	0.42	0.29	0.38	0.36	0.33	0.36	0.42	0.47	0.36	0.40	0.36	0.44	0.43	0.43	0.42	0.37	0.38	0.37	0.41
59 Intense	0.33	0.34	0.37	0.41	0.38	0.40	0.46	0.44	0.44	0.26	0.39	0.39	0.36	0.42	0.46	0.42	0.42	0.46	0.45	0.37	0.43	0.46	0.36	0.32	0.41	0.34	0.40
60 Levis	0.31	0.32	0.45	0.41	0.44	0.37	0.46	0.32	0.48	0.34	0.37	0.46	0.37	0.47	0.40	0.46	0.34	0.34	0.38	0.33	0.34	0.47	0.44	0.36	0.33	0.39	0.36
61 Rubens	0.33	0.37	0.45	0.41	0.38	0.37	0.38	0.36	0.46	0.28	0.35	0.35	0.31	0.33	0.36	0.36	0.38	0.38	0.33	0.46	0.41	0.45	0.41	0.34	0.39	0.35	0.38
62 Sponsor	0.30	0.37	0.35	0.38	0.42	0.37	0.36	0.33	0.40	0.29	0.33	0.32	0.37	0.34	0.36	0.38	0.38	0.38	0.34	0.36	0.37	0.41	0.33	0.30	0.33	0.31	0.47
63 Taldor	0.30	0.40	0.24	0.26	0.31	0.34	0.28	0.27	0.31	0.27	0.32	0.34	0.27	0.34	0.29	0.37	0.35	0.30	0.39	0.43	0.38	0.34	0.31	0.31	0.43	0.34	0.29
64 Tremie	0.31	0.35	0.37	0.40	0.47	0.39	0.38	0.41	0.40	0.30	0.33	0.31	0.30	0.38	0.43	0.40	0.40	0.40	0.31	0.24	0.37	0.39	0.35	0.32	0.31	0.28	0.34

Table A6 Continued

	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
28 BE28	1.00																				
29 BE29	0.57	1.00																			
30 BE30	0.61	0.64	1.00																		
31 701-37c	0.47	0.58	0.53	1.00																	
32 701-42c	0.54	0.59	0.64	0.64	1.00																
33 701-176a	0.55	0.60	0.53	0.61	0.74	1.00															
34 701-176c	0.58	0.62	0.53	0.58	0.69	0.82	1.00														
35 701-177a	0.56	0.63	0.57	0.56	0.76	0.85	0.86	1.00													
36 701-177c	0.51	0.56	0.54	0.53	0.55	0.63	0.67	0.66	1.00												
37 701-191a	0.53	0.60	0.65	0.52	0.51	0.44	0.56	0.55	0.56	1.00											
38 701-210a	0.55	0.64	0.67	0.55	0.55	0.50	0.51	0.53	0.60	0.79	1.00										
39 701-210b	0.60	0.58	0.57	0.58	0.60	0.55	0.58	0.56	0.46	0.56	0.67	1.00									
40 701-244c	0.52	0.45	0.48	0.46	0.53	0.63	0.60	0.57	0.42	0.45	0.46	0.62	1.00								
41 701-256b	0.59	0.54	0.56	0.65	0.58	0.65	0.67	0.64	0.56	0.52	0.52	0.57	0.57	1.00							
42 701-372c	0.55	0.67	0.60	0.60	0.69	0.60	0.63	0.65	0.56	0.55	0.56	0.56	0.45	0.66	1.00						
43 701-422b	0.58	0.60	0.65	0.61	0.62	0.59	0.64	0.64	0.57	0.52	0.56	0.59	0.49	0.59	0.60	1.00					
44 701-477b	0.53	0.58	0.58	0.56	0.55	0.58	0.54	0.57	0.50	0.51	0.54	0.56	0.41	0.56	0.62	0.70	1.00				
45 701-477c	0.61	0.63	0.60	0.52	0.59	0.54	0.58	0.57	0.49	0.60	0.60	0.60	0.51	0.52	0.53	0.56	0.57	1.00			
46 701-481a	0.44	0.56	0.53	0.51	0.45	0.45	0.49	0.49	0.42	0.42	0.48	0.49	0.40	0.48	0.50	0.51	0.46	0.44	1.00		
47 798-398b	0.47	0.47	0.48	0.56	0.59	0.52	0.46	0.46	0.48	0.45	0.48	0.56	0.45	0.57	0.55	0.50	0.53	0.51	0.40	1.00	
48 Autan	0.54	0.63	0.61	0.58	0.65	0.65	0.60	0.64	0.57	0.52	0.53	0.60	0.44	0.57	0.65	0.61	0.67	0.54	0.39	0.50	1.00

Table A6 Continued

	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
49 Bobino	0.57	0.70	0.67	0.61	0.58	0.57	0.55	0.52	0.53	0.58	0.65	0.57	0.50	0.61	0.66	0.59	0.63	0.60	0.45	0.58	0.61	1.00					
50 Brando	0.50	0.55	0.45	0.45	0.48	0.48	0.52	0.52	0.49	0.44	0.53	0.47	0.45	0.43	0.55	0.53	0.46	0.46	0.48	0.31	0.50	0.51	1.00				
51 Cadenza	0.49	0.69	0.55	0.55	0.54	0.51	0.53	0.56	0.51	0.52	0.61	0.57	0.44	0.57	0.58	0.59	0.54	0.56	0.57	0.52	0.53	0.67	0.58	1.00			
52 Charger	0.58	0.61	0.68	0.65	0.67	0.62	0.64	0.65	0.53	0.62	0.66	0.64	0.51	0.60	0.61	0.65	0.64	0.59	0.54	0.61	0.58	0.70	0.44	0.60	1.00		
53 Claire	0.40	0.47	0.49	0.50	0.48	0.48	0.42	0.46	0.49	0.49	0.50	0.48	0.39	0.41	0.49	0.47	0.47	0.41	0.38	0.39	0.56	0.44	0.42	0.40	0.53	1.00	
54 Enesco	0.50	0.47	0.47	0.42	0.46	0.50	0.52	0.51	0.45	0.45	0.52	0.48	0.43	0.50	0.49	0.41	0.45	0.55	0.38	0.37	0.48	0.46	0.46	0.46	0.50	0.45	1.00
55 Farandole	0.49	0.51	0.52	0.43	0.51	0.52	0.52	0.47	0.42	0.42	0.50	0.52	0.42	0.46	0.55	0.64	0.67	0.53	0.45	0.48	0.56	0.52	0.43	0.45	0.59	0.42	0.44
56 Gaspard	0.43	0.47	0.36	0.38	0.54	0.52	0.44	0.52	0.38	0.31	0.38	0.42	0.33	0.40	0.49	0.40	0.47	0.45	0.30	0.33	0.48	0.37	0.39	0.35	0.43	0.38	0.38
57 Gascogn	0.43	0.48	0.44	0.39	0.45	0.41	0.39	0.41	0.33	0.36	0.42	0.43	0.29	0.42	0.51	0.44	0.46	0.34	0.37	0.40	0.49	0.41	0.32	0.35	0.47	0.40	0.32
58 Igor	0.37	0.43	0.40	0.44	0.47	0.43	0.40	0.45	0.38	0.35	0.40	0.46	0.36	0.44	0.49	0.42	0.47	0.43	0.39	0.49	0.46	0.43	0.32	0.44	0.45	0.34	0.28
59 Intense	0.46	0.33	0.41	0.39	0.42	0.51	0.49	0.51	0.37	0.36	0.37	0.37	0.43	0.52	0.42	0.46	0.54	0.42	0.36	0.41	0.41	0.47	0.32	0.39	0.46	0.27	0.29
60 Levis	0.38	0.29	0.35	0.41	0.47	0.45	0.41	0.47	0.29	0.38	0.38	0.39	0.42	0.42	0.40	0.34	0.40	0.34	0.30	0.35	0.39	0.32	0.28	0.27	0.45	0.38	0.38
61 Rubens	0.32	0.42	0.44	0.45	0.49	0.43	0.39	0.43	0.35	0.36	0.42	0.39	0.37	0.42	0.46	0.36	0.42	0.38	0.34	0.44	0.39	0.45	0.34	0.45	0.47	0.34	0.32
62 Sponsor	0.34	0.36	0.44	0.40	0.47	0.39	0.35	0.39	0.36	0.35	0.36	0.31	0.27	0.40	0.38	0.36	0.37	0.32	0.35	0.42	0.40	0.37	0.24	0.33	0.45	0.30	0.19
63 Taldor	0.35	0.48	0.32	0.34	0.46	0.47	0.43	0.42	0.32	0.31	0.39	0.32	0.32	0.32	0.39	0.29	0.35	0.33	0.31	0.30	0.40	0.36	0.33	0.38	0.42	0.28	0.28
64 Tremie	0.30	0.31	0.40	0.37	0.41	0.41	0.40	0.39	0.31	0.26	0.29	0.31	0.28	0.40	0.40	0.36	0.37	0.29	0.32	0.31	0.35	0.37	0.27	0.24	0.41	0.31	0.31

Table A6 Continued

	55	56	57	58	59	60	61	62	63	64
55 Farandole	1.00									
56 Gaspard	0.48	1.00								
57 Gascogne	0.45	0.60	1.00							
58 Igor	0.46	0.58	0.55	1.00						
59 Intense	0.43	0.45	0.47	0.57	1.00					
60 Levis	0.32	0.51	0.50	0.46	0.54	1.00				
61 Rubens	0.39	0.51	0.48	0.67	0.52	0.41	1.00			
62 Sponsor	0.32	0.42	0.48	0.44	0.52	0.53	0.48	1.00		
63 Taldor	0.38	0.48	0.45	0.36	0.37	0.35	0.42	0.45	1.00	
64 Tremie	0.29	0.30	0.37	0.35	0.41	0.39	0.37	0.40	0.25	1.00

Table A7: Genetic similarity coefficient (DICE) using AFLPs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 BE01	1.00																										
2 BE02	0.88	1.00																									
3 BE03	0.86	0.89	1.00																								
4 BE04	0.82	0.86	0.86	1.00																							
5 BE05	0.82	0.86	0.85	0.84	1.00																						
6 BE06	0.82	0.85	0.85	0.84	0.86	1.00																					
7 BE07	0.80	0.82	0.83	0.81	0.83	0.84	1.00																				
8 BE08	0.83	0.85	0.85	0.84	0.83	0.85	0.84	1.00																			
9 BE09	0.81	0.84	0.84	0.82	0.82	0.83	0.81	0.83	1.00																		
10 BE10	0.80	0.84	0.83	0.82	0.81	0.84	0.82	0.84	0.83	1.00																	
11 Asperge	0.80	0.83	0.83	0.80	0.80	0.81	0.81	0.82	0.80	0.83	1.00																
12 BE12	0.83	0.85	0.85	0.82	0.82	0.84	0.81	0.84	0.82	0.83	0.84	1.00															
13 BE13	0.79	0.82	0.81	0.80	0.81	0.80	0.81	0.81	0.79	0.80	0.80	0.82	1.00														
14 BE14	0.82	0.85	0.85	0.84	0.82	0.85	0.81	0.83	0.83	0.83	0.82	0.83	0.82	1.00													
15 BE15	0.81	0.84	0.85	0.82	0.82	0.83	0.83	0.83	0.83	0.82	0.81	0.83	0.81	0.83	1.00												
16 BE16	0.81	0.86	0.84	0.83	0.83	0.84	0.83	0.83	0.83	0.84	0.81	0.83	0.81	0.84	0.85	1.00											
17 BE17	0.82	0.85	0.84	0.83	0.81	0.84	0.80	0.82	0.83	0.81	0.80	0.82	0.80	0.83	0.83	0.86	1.00										
18 BE18	0.79	0.81	0.80	0.78	0.78	0.79	0.78	0.80	0.79	0.80	0.78	0.80	0.77	0.80	0.79	0.81	0.81	1.00									
19 BE19	0.81	0.84	0.83	0.82	0.81	0.82	0.81	0.81	0.82	0.81	0.80	0.82	0.79	0.83	0.83	0.83	0.83	0.81	1.00								
20 BE20	0.80	0.83	0.82	0.81	0.81	0.82	0.81	0.81	0.81	0.81	0.79	0.81	0.80	0.82	0.81	0.83	0.81	0.80	0.83	1.00							
21 BE21	0.79	0.83	0.82	0.81	0.81	0.81	0.79	0.80	0.80	0.81	0.80	0.81	0.79	0.82	0.81	0.81	0.81	0.78	0.82	0.81	1.00						
22 BE22	0.78	0.82	0.81	0.80	0.80	0.81	0.78	0.80	0.79	0.79	0.79	0.80	0.77	0.81	0.81	0.81	0.80	0.78	0.80	0.80	0.83	1.00					
23 BE23	0.78	0.80	0.80	0.79	0.78	0.80	0.77	0.79	0.79	0.79	0.77	0.80	0.76	0.79	0.79	0.80	0.79	0.78	0.79	0.78	0.78	0.78	1.00				
24 BE24	0.81	0.85	0.83	0.81	0.81	0.82	0.81	0.83	0.81	0.82	0.81	0.81	0.80	0.82	0.82	0.83	0.83	0.79	0.81	0.81	0.80	0.81	0.82	1.00			
25 BE25	0.77	0.79	0.80	0.79	0.78	0.80	0.77	0.78	0.79	0.78	0.76	0.79	0.76	0.80	0.79	0.79	0.79	0.77	0.79	0.77	0.76	0.76	0.77	0.80	1.00		
26 BE26	0.77	0.81	0.80	0.78	0.78	0.79	0.78	0.79	0.78	0.78	0.77	0.78	0.77	0.80	0.79	0.80	0.80	0.78	0.80	0.78	0.79	0.76	0.77	0.80	0.80	1.00	
27 BE27	0.80	0.83	0.83	0.82	0.82	0.82	0.80	0.81	0.81	0.81	0.80	0.81	0.80	0.82	0.82	0.83	0.81	0.80	0.81	0.81	0.81	0.82	0.80	0.82	0.80	0.80	1.00
28 BE28	0.80	0.83	0.83	0.80	0.81	0.82	0.78	0.80	0.81	0.79	0.79	0.81	0.80	0.82	0.81	0.83	0.82	0.79	0.81	0.80	0.80	0.79	0.79	0.81	0.80	0.79	0.83
29 BE29	0.79	0.80	0.80	0.79	0.79	0.80	0.78	0.79	0.79	0.79	0.77	0.80	0.78	0.79	0.80	0.80	0.78	0.76	0.79	0.77	0.78	0.76	0.77	0.80	0.78	0.78	0.81

Table A7 Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
30 BE30	0.80	0.82	0.81	0.80	0.79	0.81	0.77	0.81	0.80	0.79	0.78	0.80	0.78	0.81	0.80	0.81	0.81	0.78	0.79	0.79	0.78	0.78	0.78	0.80	0.79	0.78	0.80
31 701-37c	0.81	0.84	0.83	0.80	0.81	0.82	0.81	0.81	0.81	0.80	0.79	0.81	0.80	0.82	0.83	0.82	0.82	0.78	0.83	0.80	0.79	0.79	0.78	0.82	0.80	0.80	0.82
32 701-42c	0.78	0.81	0.80	0.78	0.78	0.80	0.78	0.79	0.80	0.78	0.76	0.78	0.77	0.79	0.79	0.79	0.79	0.76	0.80	0.80	0.78	0.77	0.77	0.79	0.79	0.77	0.79
33 701-176a	0.80	0.84	0.83	0.83	0.81	0.82	0.80	0.81	0.82	0.81	0.79	0.82	0.78	0.82	0.82	0.83	0.83	0.79	0.83	0.81	0.80	0.80	0.80	0.82	0.79	0.80	0.83
34 701-176c	0.81	0.85	0.84	0.83	0.83	0.83	0.81	0.83	0.82	0.81	0.81	0.83	0.80	0.83	0.82	0.84	0.83	0.79	0.83	0.81	0.81	0.80	0.79	0.83	0.80	0.79	0.82
35 701-177a	0.80	0.84	0.82	0.83	0.82	0.83	0.79	0.82	0.82	0.81	0.80	0.82	0.79	0.82	0.83	0.83	0.83	0.79	0.81	0.80	0.81	0.80	0.79	0.81	0.78	0.78	0.82
36 701-177c	0.79	0.82	0.82	0.81	0.81	0.83	0.78	0.80	0.81	0.81	0.79	0.81	0.79	0.83	0.81	0.82	0.81	0.78	0.81	0.80	0.80	0.80	0.79	0.82	0.79	0.79	0.82
37 701-191a	0.80	0.83	0.82	0.81	0.80	0.81	0.78	0.80	0.80	0.80	0.78	0.81	0.78	0.81	0.81	0.81	0.81	0.78	0.80	0.79	0.79	0.80	0.79	0.82	0.78	0.78	0.80
38 701-210a	0.78	0.80	0.80	0.79	0.78	0.79	0.76	0.78	0.79	0.79	0.79	0.79	0.77	0.80	0.79	0.80	0.79	0.78	0.79	0.79	0.78	0.79	0.78	0.81	0.78	0.78	0.81
39 701-210b	0.80	0.82	0.81	0.80	0.81	0.81	0.79	0.81	0.79	0.80	0.79	0.81	0.79	0.81	0.81	0.81	0.81	0.79	0.81	0.79	0.80	0.78	0.78	0.82	0.79	0.79	0.82
40 701-244c	0.78	0.82	0.81	0.80	0.81	0.81	0.79	0.80	0.79	0.80	0.79	0.80	0.78	0.81	0.80	0.82	0.81	0.79	0.81	0.80	0.79	0.80	0.78	0.81	0.78	0.78	0.81
41 701-256b	0.77	0.80	0.80	0.78	0.80	0.80	0.78	0.78	0.78	0.77	0.78	0.78	0.77	0.79	0.79	0.81	0.79	0.77	0.80	0.78	0.79	0.79	0.77	0.80	0.79	0.77	0.81
42 701-372c	0.78	0.81	0.80	0.78	0.79	0.80	0.77	0.79	0.78	0.79	0.77	0.79	0.77	0.80	0.78	0.80	0.79	0.78	0.79	0.77	0.78	0.79	0.76	0.79	0.77	0.78	0.80
43 701-422b	0.75	0.77	0.79	0.77	0.76	0.77	0.76	0.77	0.76	0.77	0.76	0.76	0.74	0.76	0.77	0.77	0.76	0.75	0.76	0.77	0.76	0.74	0.75	0.77	0.76	0.76	0.77
44 701-477b	0.77	0.81	0.80	0.77	0.77	0.77	0.77	0.80	0.76	0.77	0.77	0.78	0.77	0.77	0.78	0.78	0.78	0.76	0.76	0.76	0.76	0.76	0.75	0.78	0.74	0.76	0.79
45 701-477c	0.79	0.82	0.82	0.79	0.78	0.81	0.79	0.80	0.78	0.78	0.78	0.80	0.77	0.80	0.80	0.80	0.79	0.76	0.78	0.78	0.78	0.78	0.76	0.80	0.75	0.78	0.78
46 701-481a	0.79	0.82	0.81	0.80	0.80	0.80	0.80	0.81	0.78	0.79	0.79	0.80	0.77	0.81	0.80	0.81	0.79	0.77	0.80	0.78	0.79	0.78	0.76	0.80	0.76	0.77	0.80
47 798-398b	0.78	0.81	0.80	0.77	0.79	0.77	0.77	0.79	0.77	0.77	0.76	0.78	0.77	0.78	0.78	0.79	0.77	0.77	0.77	0.76	0.77	0.74	0.75	0.78	0.75	0.75	0.77
48 Autan	0.75	0.78	0.78	0.77	0.77	0.78	0.75	0.77	0.75	0.76	0.75	0.77	0.76	0.77	0.76	0.76	0.75	0.73	0.74	0.75	0.75	0.75	0.75	0.76	0.74	0.75	0.77
49 Bobino	0.76	0.78	0.78	0.76	0.77	0.75	0.75	0.76	0.75	0.74	0.76	0.77	0.76	0.77	0.76	0.76	0.77	0.75	0.77	0.75	0.76	0.74	0.73	0.76	0.72	0.74	0.76
50 Brando	0.75	0.76	0.76	0.75	0.75	0.75	0.74	0.75	0.74	0.74	0.74	0.76	0.73	0.75	0.74	0.76	0.74	0.74	0.75	0.73	0.73	0.73	0.74	0.75	0.73	0.72	0.75
51 Cadenza	0.78	0.80	0.79	0.78	0.78	0.78	0.76	0.77	0.77	0.77	0.76	0.77	0.75	0.79	0.77	0.78	0.78	0.74	0.77	0.75	0.77	0.76	0.74	0.78	0.73	0.74	0.77
52 Charger	0.74	0.76	0.76	0.75	0.75	0.76	0.74	0.75	0.74	0.74	0.74	0.75	0.75	0.76	0.75	0.76	0.74	0.73	0.76	0.75	0.74	0.73	0.71	0.74	0.72	0.74	0.76
53 Claire	0.76	0.79	0.79	0.78	0.77	0.76	0.76	0.77	0.76	0.75	0.76	0.77	0.75	0.77	0.76	0.77	0.76	0.75	0.77	0.76	0.75	0.75	0.73	0.76	0.74	0.75	0.77
54 Enesco	0.76	0.77	0.76	0.75	0.77	0.76	0.76	0.76	0.75	0.74	0.76	0.77	0.75	0.75	0.76	0.77	0.76	0.73	0.75	0.76	0.75	0.74	0.73	0.76	0.73	0.72	0.76
55 Farandole	0.76	0.79	0.78	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.75	0.77	0.74	0.78	0.76	0.78	0.78	0.75	0.77	0.76	0.76	0.75	0.73	0.77	0.74	0.74	0.76

Table A7 Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
56 Gaspard	0.58	0.60	0.60	0.59	0.59	0.59	0.58	0.57	0.59	0.58	0.59	0.59	0.57	0.60	0.58	0.60	0.59	0.59	0.61	0.59	0.60	0.58	0.59	0.59	0.61	0.58	0.61
57 Gascogne	0.57	0.58	0.59	0.56	0.58	0.58	0.57	0.57	0.58	0.57	0.58	0.58	0.56	0.59	0.57	0.59	0.57	0.57	0.59	0.58	0.58	0.58	0.58	0.59	0.59	0.58	0.59
58 Igor	0.56	0.57	0.57	0.55	0.57	0.56	0.56	0.55	0.56	0.54	0.57	0.56	0.55	0.56	0.56	0.57	0.55	0.56	0.58	0.57	0.57	0.57	0.57	0.57	0.59	0.57	0.58
59 Intense	0.55	0.56	0.57	0.56	0.57	0.56	0.56	0.55	0.57	0.54	0.56	0.56	0.55	0.57	0.56	0.57	0.55	0.56	0.58	0.56	0.57	0.56	0.56	0.57	0.59	0.57	0.58
60 Levis	0.55	0.56	0.56	0.55	0.55	0.55	0.55	0.54	0.56	0.53	0.56	0.55	0.54	0.56	0.55	0.56	0.55	0.56	0.57	0.56	0.57	0.56	0.56	0.56	0.58	0.56	0.57
61 Rubens	0.55	0.57	0.57	0.56	0.56	0.57	0.56	0.56	0.57	0.55	0.56	0.56	0.55	0.57	0.56	0.57	0.56	0.56	0.57	0.56	0.57	0.56	0.57	0.56	0.60	0.57	0.57
62 Sponsor	0.54	0.55	0.56	0.54	0.56	0.55	0.55	0.54	0.56	0.53	0.55	0.55	0.54	0.56	0.54	0.56	0.54	0.55	0.56	0.55	0.56	0.56	0.56	0.55	0.58	0.56	0.57
63 Taldor	0.68	0.71	0.71	0.68	0.69	0.69	0.68	0.67	0.68	0.67	0.67	0.68	0.67	0.69	0.70	0.70	0.69	0.68	0.70	0.68	0.69	0.67	0.66	0.70	0.69	0.68	0.69
64 Tremie	0.68	0.72	0.72	0.69	0.70	0.70	0.69	0.68	0.69	0.68	0.69	0.69	0.69	0.70	0.71	0.71	0.70	0.68	0.71	0.69	0.70	0.68	0.67	0.70	0.69	0.69	0.70

Table A7 Continued

	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
28 BE28	1.00																				
29 BE29	0.81	1.00																			
30 BE30	0.80	0.80	1.00																		
31 701-37c	0.82	0.80	0.81	1.00																	
32 701-42c	0.79	0.78	0.79	0.81	1.00																
33 701-176a	0.81	0.80	0.80	0.82	0.81	1.00															
34 701-176c	0.82	0.80	0.81	0.81	0.80	0.85	1.00														
35 701-177a	0.81	0.80	0.80	0.81	0.79	0.84	0.87	1.00													
36 701-177c	0.81	0.80	0.80	0.81	0.80	0.84	0.84	0.85	1.00												
37 701-191a	0.80	0.77	0.80	0.81	0.79	0.80	0.82	0.81	0.81	1.00											
38 701-210a	0.79	0.77	0.78	0.80	0.78	0.80	0.80	0.80	0.80	0.82	1.00										
39 701-210b	0.81	0.79	0.79	0.81	0.78	0.81	0.83	0.83	0.81	0.82	0.81	1.00									
40 701-244c	0.81	0.79	0.80	0.81	0.80	0.80	0.81	0.80	0.81	0.80	0.79	0.81	1.00								
41 701-256b	0.78	0.78	0.78	0.80	0.78	0.79	0.79	0.79	0.79	0.78	0.79	0.81	0.80	1.00							
42 701-372c	0.79	0.78	0.78	0.79	0.77	0.79	0.80	0.80	0.80	0.78	0.78	0.79	0.80	0.80	1.00						
43 701-422b	0.77	0.75	0.77	0.76	0.75	0.77	0.77	0.76	0.78	0.76	0.74	0.77	0.76	0.77	0.76	1.00					
44 701-477b	0.77	0.75	0.75	0.76	0.74	0.77	0.79	0.78	0.78	0.77	0.76	0.78	0.76	0.75	0.78	0.74	1.00				
45 701-477c	0.79	0.78	0.79	0.79	0.75	0.79	0.79	0.80	0.80	0.79	0.79	0.78	0.80	0.77	0.79	0.75	0.80	1.00			
46 701-481a	0.79	0.77	0.78	0.79	0.75	0.79	0.81	0.81	0.80	0.80	0.78	0.79	0.79	0.77	0.77	0.76	0.78	0.81	1.00		
47 798-398b	0.78	0.76	0.75	0.78	0.74	0.77	0.79	0.78	0.77	0.77	0.75	0.78	0.78	0.76	0.75	0.73	0.77	0.79	0.81	1.00	
48 Autan	0.75	0.75	0.76	0.75	0.73	0.75	0.77	0.76	0.75	0.76	0.74	0.76	0.77	0.75	0.74	0.74	0.76	0.78	0.77	0.77	1.00

Table A7 Continued

	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
49 Bobino	0.75	0.74	0.75	0.75	0.73	0.75	0.78	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.74	0.73	0.75	0.76	0.77	0.76	0.76	1.00					
50 Brando	0.74	0.74	0.72	0.74	0.73	0.74	0.76	0.75	0.75	0.74	0.73	0.76	0.75	0.74	0.74	0.72	0.75	0.76	0.76	0.76	0.75	0.76	1.00				
51 Cadenza	0.77	0.75	0.76	0.76	0.75	0.78	0.79	0.78	0.78	0.76	0.75	0.78	0.77	0.77	0.76	0.73	0.76	0.79	0.80	0.77	0.75	0.76	0.76	1.00			
52 Charger	0.75	0.72	0.73	0.74	0.72	0.74	0.76	0.75	0.74	0.73	0.74	0.75	0.74	0.75	0.74	0.71	0.75	0.76	0.76	0.74	0.75	0.74	0.74	0.76	1.00		
53 Claire	0.76	0.74	0.74	0.77	0.73	0.77	0.77	0.77	0.75	0.76	0.74	0.75	0.74	0.75	0.74	0.74	0.76	0.78	0.78	0.77	0.75	0.74	0.74	0.77	0.76	1.00	
54 Enesco	0.76	0.74	0.74	0.74	0.73	0.74	0.77	0.77	0.76	0.74	0.75	0.76	0.75	0.75	0.75	0.73	0.75	0.77	0.77	0.77	0.74	0.76	0.76	0.78	0.75	0.77	1.00
55 Farandole	0.75	0.74	0.75	0.76	0.75	0.77	0.79	0.78	0.78	0.76	0.76	0.78	0.76	0.76	0.75	0.74	0.76	0.78	0.78	0.77	0.75	0.75	0.75	0.77	0.76	0.79	0.79
56 Gaspard	0.59	0.60	0.59	0.60	0.62	0.60	0.59	0.58	0.59	0.59	0.60	0.58	0.60	0.61	0.58	0.58	0.56	0.56	0.56	0.57	0.56	0.57	0.54	0.55	0.54	0.57	0.54
57 Gascogne	0.59	0.59	0.57	0.59	0.61	0.59	0.58	0.57	0.58	0.58	0.59	0.57	0.60	0.61	0.57	0.57	0.56	0.55	0.55	0.56	0.55	0.56	0.53	0.54	0.53	0.55	0.54
58 Igor	0.57	0.58	0.56	0.58	0.59	0.57	0.56	0.56	0.57	0.57	0.57	0.56	0.58	0.60	0.55	0.55	0.56	0.54	0.54	0.54	0.53	0.53	0.53	0.53	0.52	0.55	0.53
59 Intense	0.57	0.58	0.55	0.57	0.58	0.58	0.56	0.55	0.56	0.56	0.57	0.56	0.58	0.60	0.55	0.55	0.54	0.53	0.53	0.54	0.53	0.53	0.52	0.53	0.52	0.54	0.51
60 Levis	0.57	0.57	0.55	0.57	0.58	0.57	0.56	0.55	0.56	0.56	0.56	0.55	0.58	0.59	0.54	0.55	0.54	0.53	0.52	0.54	0.52	0.53	0.52	0.52	0.51	0.54	0.52
61 Rubens	0.57	0.58	0.56	0.57	0.60	0.59	0.56	0.56	0.57	0.57	0.57	0.56	0.58	0.59	0.55	0.55	0.54	0.54	0.54	0.54	0.54	0.53	0.52	0.53	0.52	0.55	0.52
62 Sponsor	0.55	0.57	0.54	0.56	0.58	0.57	0.55	0.54	0.55	0.56	0.56	0.55	0.57	0.59	0.55	0.55	0.53	0.53	0.52	0.53	0.53	0.53	0.52	0.52	0.52	0.53	0.50
63 Taldor	0.69	0.68	0.67	0.70	0.70	0.70	0.70	0.68	0.69	0.69	0.68	0.68	0.70	0.70	0.67	0.66	0.67	0.66	0.66	0.66	0.64	0.66	0.63	0.65	0.64	0.66	0.64
64 Tremie	0.70	0.69	0.68	0.71	0.71	0.70	0.71	0.69	0.69	0.70	0.69	0.69	0.71	0.70	0.68	0.66	0.67	0.67	0.67	0.67	0.65	0.66	0.63	0.65	0.64	0.67	0.64

Table A7 Continued

	55	56	57	58	59	60	61	62	63	64
55 Farandole	1.00									
56 Gaspard	0.59	1.00								
57 Gascogne	0.58	0.91	1.00							
58 Igor	0.57	0.90	0.92	1.00						
59 Intense	0.57	0.90	0.91	0.93	1.00					
60 Levis	0.56	0.88	0.90	0.92	0.93	1.00				
61 Rubens	0.56	0.89	0.90	0.90	0.92	0.92	1.00			
62 Sponsor	0.55	0.90	0.91	0.93	0.93	0.93	0.93	1.00		
63 Taldor	0.68	0.82	0.84	0.82	0.82	0.82	0.83	0.82	1.00	
64 Tremie	0.68	0.82	0.83	0.82	0.81	0.81	0.82	0.81	0.97	1.00

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