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High resolution mapping and identification of candidate genes for the BaMMV/BaYMV-resistance gene *rym13* and *Ryd3* involved in BYDV-tolerance of barley

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High resolution mapping and identification of candidate genes for the BaMMV/BaYMV-resistance gene *rym13* and *Ryd3* involved in BYDV-tolerance of barley

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for a Doctoral Degree (Dr. agr.)

Faculty of Agricultural Sciences, Nutritional Sciences,
and Environmental Management

Submitted by

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Table of contents

List of Figures

List of Tables

Supplemental data

Abbreviations

AFLP	-	Amplified Fragment Length Polymorphism
BAC	-	Bacterial Artificial Chromosome
BaYMV / BaMMV	-	*Barley Yellow Mosaic Virus / Barley Mild Mosaic Virus*
BYDV	-	*Barley Yellow Dwarf Virus*
BC	-	Before Christ
BLAST	-	Basic Local Alignment Search Tool
bp	-	Basepairs
CAPS	-	Cleaved Amplified Polymorphic Sequence
cM	-	Centi Morgan
CRISPR	-	Clustered Regularly Interspaced Short Palindromic Repeats
DAS-ELISA	-	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DH	-	Doubled Haploid
DNA	-	Desoxyribonucleic acid
dNTP	-	Deoxynucleotide
eEF	-	Eucaryotic elongation Factor
e.g.	-	Exempli gratia (for example)
eiF	-	Eukaryotic Translation Initiation Factor
EMS	-	Ethyl Methansulfonate
ER	-	Endoplasmatic Reticulum
EST	-	Expressed Sequence Tag
Fig	-	Figure
Gb	-	Gigabases
GBS	-	Genotyping-By-Sequencing
GZ	-	Genome Zipper
h	-	Hour(s)
i.e.	-	Id est (meaning)
InDel	-	Insertion/Deletion
KASP	-	Kompetitive Allele Specific PCR

kDa	-	Kilo Dalton
min	-	Minutes
mio	-	Million
nm	-	Nanometer
PCR	-	Polymerase Chain Reaction
QTL	-	Quantitative Trait Locus
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
R-genes	-	Resistance Genes
RIL	-	Recombinant Inbred Line
SNP	-	Single Nucleotide Polymorphism
SSR	-	Simple Sequence Repeat
ssRNA	-	Single Stranded Ribonucleic Acid
Tab	-	Table
TILLING	-	Targeting-Induced Local Lesions In Genomes
WGS	-	Whole Genome Sequencing

Abstract

Barley yellow mosaic disease, caused by *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV), and barley yellow dwarf, caused by *Barley yellow dwarf virus* (BYDV), are important diseases of barley causing high yield losses. As BaMMV and BaYMV are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* and BYDV by aphids, the only environmently sound way to prevent these yield losses is breeding for resistant/tolerant cultivars.

In this thesis, high resolution mapping and candidate gene analysis for the BaMMV/BaYMV resistance gene *rym13* and the BYDV tolerance conferring gene *Ryd3* was conducted. For *rym13,* a high resolution mapping population based on 5,191 F_2 plants was established and marker saturation was conducted by mapping the flanking markers to published genomic resources of barley. Using these genomic resources in combination with next-generation-sequencing-techniques led to a fast saturation of the resistance harbouring interval and identification of candidate genes. Analysing phenotypic and genotypic data revealed an independent inheritance of the BaMMV and BaYMV resistance in the cultivar 'Taihoku A'. Two candidate genes for *rym13,* co-segregating with BaMMV resistance in an independent population, were identified.

Regarding *Ryd3*, the former mapping population established by Lüpken et al. (2014) was extended to 7,427 F_2 plants. After mapping the co-segregating markers to the published reference genome of barley, the interval related to tolerance was shortened to 104.14 mio bp. This interval harbours a number of candidate genes. For further investigations to unravel the mechanism of tolerance, a TILLING population was established in the course of this thesis.

This study lays the foundation for subsequent isolation and validation of *rym13* and *Ryd3*.

Zusammenfassung

Die Gelbmosaikvirose der Gerste, verursacht durch *Barley yellow mosaic virus* (BaYMV) und *Barley mild mosaic virus* (BaMMV) und die Gelbverzwergung der Gerste, verursacht durch *Barley yellow dwarf virus* (BYDV) führen zu erheblichen Ertragsverlusten. Aufgrund der Übertragung durch den bodenbürdigen Plasmodiophorid *Polymyxa graminis* im Falle der Gelbmosaikvirose bzw. durch Blattläuse (Gelbverzwergung) ist die Züchtung resistenter bzw. toleranter Sorten die einzige ökologisch und ökonomisch sinnvolle Möglichkeit hohe Ernteverluste zu verhindern.

Im Rahmen dieser Arbeit wurde die hochauflösende Kartierung und die Identifikation von Kandidatengenen für das BaMMV Resistenzgen *rym13* sowie das BYDV Toleranzgen *Ryd3* durchgeführt.

Für die Kartierung von *rym13* wurde eine hochauflösende Kartierungspopulation, bestehend aus 5.191 F_2 Pflanzen, erstellt. Durch Verankerung der flankierenden Marker in bereits veröffentlichten genomischen Ressourcen für Gerste in Kombination mit der Verwendung von next-generation-sequencing Techniken (GBS, WGS) konnte das resistenzgentragende Intervall verkürzt und co-segregierende Kandidatengene identifiziert werden. Die Analyse der phänotypischen und genotypischen Daten ergab außerdem eine unabhängige Vererbung der BaMMV und BaYMV- Resistenz in der widerstandsfähigen Elternlinie 'Taihoku A'. Nach Kartierung in einer unabhängigen Validierungspopulation stellten sich zwei Gene als vielversprechende Kandidaten für *rym13* heraus.

Zur Feinkartierung von *Ryd3* wurde die Anzahl der F_2 Pflanzen der Kartierungspopulation von Lüpken et al. (2014) auf 7.427 Pflanzen erhöht. Nach Verankerung der Marker in der Referenzsequenz der Gerste (Mascher et al. 2017) konnte das toleranzgentragende Intervall auf 104,14 mio bp eingeschränkt werden. Auf Grund der großen Anzahl an Genen in diesem Intervall sind hier weiterführende Analysen notwendig, um *Ryd3* zu isolieren. Zu diesem Zweck wurde im Rahmen der Arbeit eine TILLING-Population erstellt und steht nun für weitere Experimente zur Verfügung.

Zusammenfassend stellt diese Arbeit eine essenzielle Grundlage für die Isolation von *rym13* und *Ryd3* dar.

1 General Introduction

1.1 Barley (*Hordeum vulgare* L.)

Barley belongs to one of the agriculturally most important plant families, the *Poaceae* and to the tribe of the *Triticeae*, which includes the major temperate cereals wheat (*Triticum aestivum*), rye (*Secale cereale*), barley (*Hordeum vulgare*) and triticale (*Triticosecale*) (Gaut 2002). *Hordeum* is a monophyletic group, which separated about thirteen million years ago from wheat (Bothmer and Komatsuda 2011). The first domestication of barley may have taken place in two areas in the Israel-Jordan region, which is known as the "fertile crescent" (Figure 1).

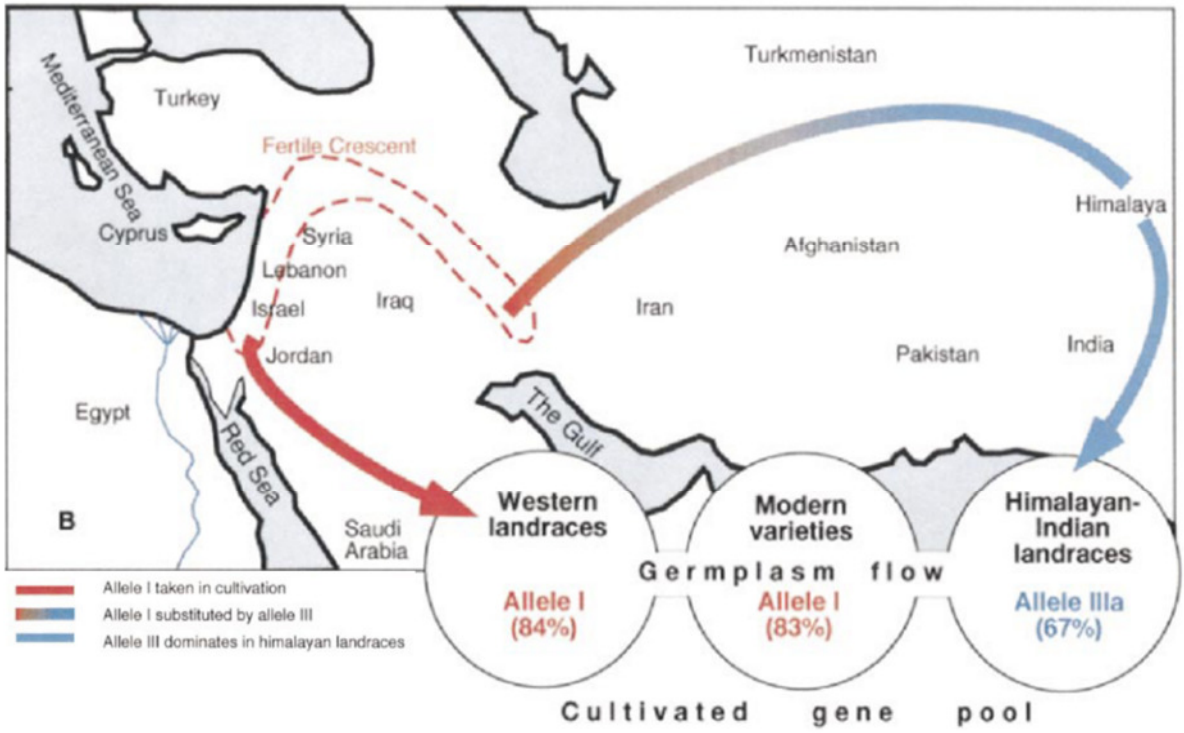

Figure 1: Origin and distribution of barley (Badr et al. 2000).

The oldest barley records are dated to 15,000 years ago and selection for better landraces started around 8,000 BC (Badr et al. 2000). From the 'fertile crescent', barley spread to temperate areas all over the world. With a harvested area of 46,923,218 ha in 2016, barley is the fourth most important cereal worldwide next to wheat (220,107,551 ha), maize (187,959,116 ha) and rice (159,807,722 ha). In 2016 in Germany, barley was harvested on an area of 1,605,000 ha (FAO 2018). More than half of the produced barley is used for feed (55-60%), 30 to 40 % are used for malting and only two to three percent are used in direct

human nutrition (Ullrich 2011). Besides this, barley has a rather high beta glucan content (Ahlemeyer et al. 2009; Sullivana et al. 2013) ranging from 5 to 11%. Beta glucan has a cholesterol decreasing effect (Wang et al. 2013) resulting in a lower risk for chronic heart diseases and type-2 diabetes. Because of this, the importance of barley for human nutrition may increase in the future.

The primary genepool of barley consists of breeding lines, landraces and the ancestral barley *Hordeum spontaneum*. The secondary genepool consists of the species *Hordeum bulbosum*. Based on crossing to this species, doubled haploid production was established by Kasha and Kao (1970). Additionally, *H. bulbosum* is also an important donor for resistance genes (Czembor and Czembor 2008; Fetch et al. 2009; Johnston et al. 2013; Pickering and Johnston 2005; Pickering et al. 2000; Ruge-Wehling et al. 2006; Ruge et al. 2003; Ruge-Wehling et al. 2013; Walther et al. 2000). The tertiary genepool of barley comprises all the other wild *Hordeum* species like *Hordeum murinum* or *Hordeum capense*. Different wild species carry important characteristics like resistance genes or they show increased tolerance to abiotic stress or better adaption to poor soils (Ullrich 2011). These genes are of special interest for breeding programs challenging for example the climate change and e.g. *Hordeum chilense* shows resistances to several diseases affecting barley and wheat (Rubiales and Moral 2011; 2012).

Barley has a diploid genome with 2n=2x=14 chromosomes, which are named according to their homologous relationship to chromosomes of other *Triticeae* species (Graner et al. 1996). In barley, many high-density marker maps, originating from different populations analyzed with different marker types are available (Li et al. 2003; Li et al. 2010; Sato et al. 2009; 2007; Varshney et al. 2007). Additionally, a first draft of the physical map with 4.98 Gb was developed by the International Barley Genome Sequencing Consortium (Mayer et al. 2012), comprising 95% of the whole barley genome which consists of 5.1 Gb. The genome includes 26,159 high-confidence (HC; genes with homology to at least one reference genome) and 53,220 low-confidence (LC) genes. In 2017, the reference sequence of barley was published (Mascher et al. 2017). The reference sequence consists of 6,347 ordered super-scaffolds, which represent 4.79 Gb of the barley genome whereof 4.54 Gb were assigned to distinct chromosomal regions in the Hi-C map (obtained by chromosome conformation capture sequencing). This information, in combination with the Genome

Zipper (Mayer et al. 2011) and the POPSEQ map (Mascher et al. 2013a), is a very useful tool for fast candidate gene identification as already shown for the isolation or fine mapping of the genes *mnd*, *lax-a*, *spt1* and *Rpg1* (Jost et al. 2016; Mascher et al. 2014; Richards et al. 2016; Wendler et al. 2014).

1.2 Viral diseases of barley

1.2.1 Barley yellow mosaic disease

A complex of two viruses causes barley yellow mosaic disease, i.e. *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV). They both belong to the family of the *Potyviridae* and to the genus *Bymovirus* (reviewed in Lopez-Moya and Garcia 2008). The virions are flexuous filamentous particles with a size of 12-13 nm in diameter which form pinwheel shaped inclusion bodies in infected cells. Both viruses are positive-sense single stranded RNA-viruses with a bipartite genome comprising RNA1 (approximately 7.7 kb) and RNA2 (3.6 kb, Davidson et al. 1991). RNA 1 encodes for nine proteins (P3, 7K1, CI, 7K2, VPg, NIa, Nib and CP; Kashiwazaki et al. 1990) whereas RNA 2 only encodes for two proteins (P1 and P2, Davidson et al. 1991). Both carry a 5' covalently linked viral genome-linked protein (Figure 2, VPg). Comparing RNA 1 and 2 of BaYMV and BaMMV revealed that they have a similar genetic organization but a low level of sequence identity (Meyer and Dessens 1996). All potyviral proteins, except P1, are produced in an equimolar ratio (Ivanov et al. 2014).

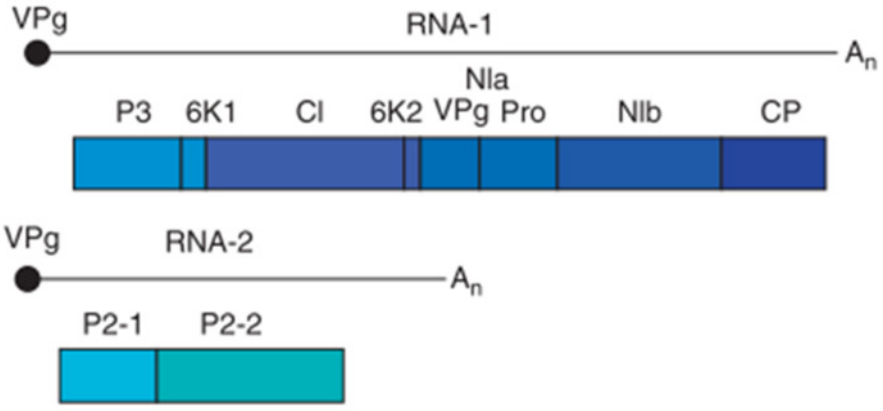

Figure 2: Schematic order of potyviral RNA and encoded proteins (Lopez-Moya and Garcia, 2008).

The replication of the virus takes place in cytoplasmic membrane-bound viral replication complexes (VRCs) of infected cells. The expression of the potyviral 6K2 protein induces the

formation of VRCs and is essential for viral infection (Wei et al. 2010). The protein P2-2 encodes for a cystein protease, which is related to the potyviral helper component HC-Pro. This protease can interact with RNA and seems to bind double-stranded small interfering RNA (siRNA) molecules. The CI protein, with a size of 70 kDa, is responsible for the formation of the pinwheel-shaped cylindrical inclusion bodys in the cytoplasma of infected cells and has RNA helicase activity indicating a function in RNA replication (Lopez-Moya and Garcia 2008).

The barley yellow mosaic disease was first detected in Japan (Ikata and Kawai 1940), where at least seven strains of BaYMV and two of BaMMV are currently known (Nomura et al. 1996; Sotome et al. 2010). In Europe, the disease was first detected in Germany (Huth and Lesemann 1978) and subsequently in many other countries like France (Signoret and Huth 1993), the Netherlands (Langenberg and Wal 1986), Great Britain (Hill and Evans 1980), Ukrain (Fantakhun et al. 1987) and Spain (Achon et al. 2005). In Europe, two strains of BaYMV (BaYMV, BaYMV-2) and three of BaMMV are known (BaMMV, BaMMV-Sil, BaMMV-Teik; Hariri et al. 2003; Habekuss et al. 2008b).

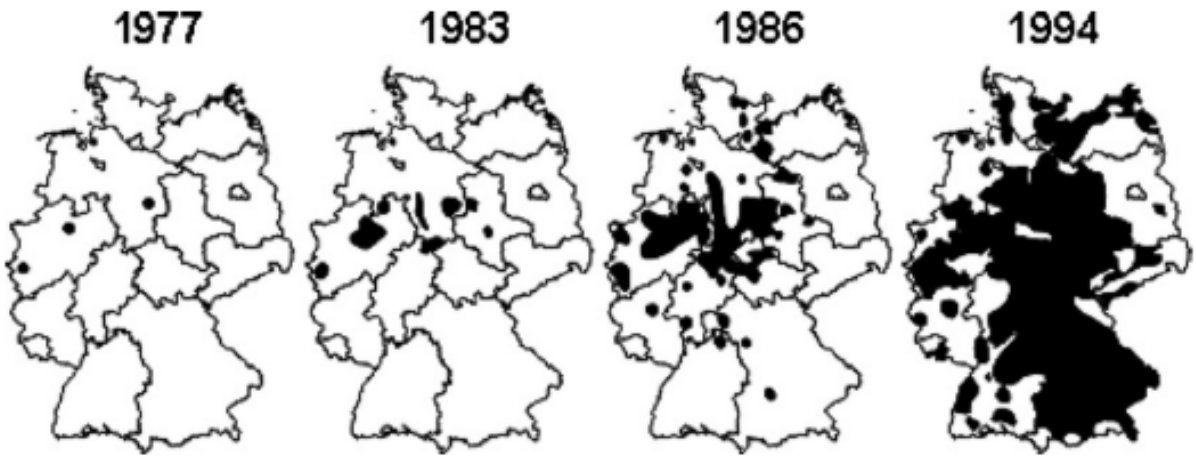

Figure 3: Distribution of yellow mosaic virus infection in Germany over the time (Kuehne 2009).

Figure 3 shows the distribution of BaYMV/BaMMV in the period 1977 to 1994 in Germany. BaYMV and BaMMV are transmitted by the soilborne plasmodiophorid *Polymyxa graminis* (Adams et al. 1988; Kusaba and Toyama 1970; Toyama and Kusaba 1970), which is an

obligate parasite of the roots and is able to transmit a set of viruses, which are listed in table 1.

Table 1: Different viruses transmitted by *Polymyxa graminis* (modified after Kanyuka et al. 2003).

Virus	Genus	Hosts	Reference
Aubian wheat mosaic virus (AWMV)	unassigned	wheat	Hariri et al. (2001)
Barley mild mosaic virus (BaMMV)	Bymovirus	barley	Plumb et al. (1986)
Barley yellow mosaic virus (BaYMV)			
Chinese wheat mosaic virus (CWMV)	Furovirus	wheat	Diao et al. (1999)
Oat golden stripe virus (OGSV)	Furovirus	oats	Adams et al. (1988)
Oat mosaic virus (OMV)	Bymovirus	oats	Walker et al. (1998)
			Monger et al. (2001)
Peanut clump virus (IPCV)	Pecluvirus	peanut, sorghum	Dieryck et al. (2011)
Rice necrosis mosaic virus (RNMV)	Bymovirus	rice	Hibino (1996)
Rice stripe necrosis virus (RSNV)	Benyvirus	rice	Lozano and Morales (2009)
Soil-borne wheat mosaic virus (SBWMV)	Furovirus	wheat, barley, rye, triticale	Rao and Brakke (1969)
Sorghum chlorotic spot virus (SCSV)	Furovirus	sorghum	Kanyuka et al. (2003)
Wheat spindle streak mosaic virus (WSSMV)	Bymovirus	wheat, rye, triticale	Slykhuis (1978)
Wheat yellow mosaic virus (WYMV)	Bymovirus	wheat	Ohki et al. (2014)

The lifetime of *Polymyxa graminis* consists of two phases: the sporangial and the sporogenic phase (Figure 4). The sporangial phase starts with the penetration of primary zoospores to the epidermal or root hairy cells of the host plant. The zoospore encysts at the surface of the host cell wall and injects its content to the cytoplasm of the host cell (Aist and Williams 1971; Kanyuka et al. 2003). After several cycles of cruciform and non-cruciform divisions, mature secondary zoospores are released. These secondary zoospores may enter in a new sporangial or a sporogenic phase by penetrating a host cell. The sporogenic phase also starts by encystment of the zoospore and during this phase a cluster of resting spores develops within the host cell and can survive for decades in the soil. When environmental conditions are suitable and an appropriate host plant is available, the

resting spores release a primary zoospore and the life cycle again starts from the beginning (Kanyuka et al. 2003).

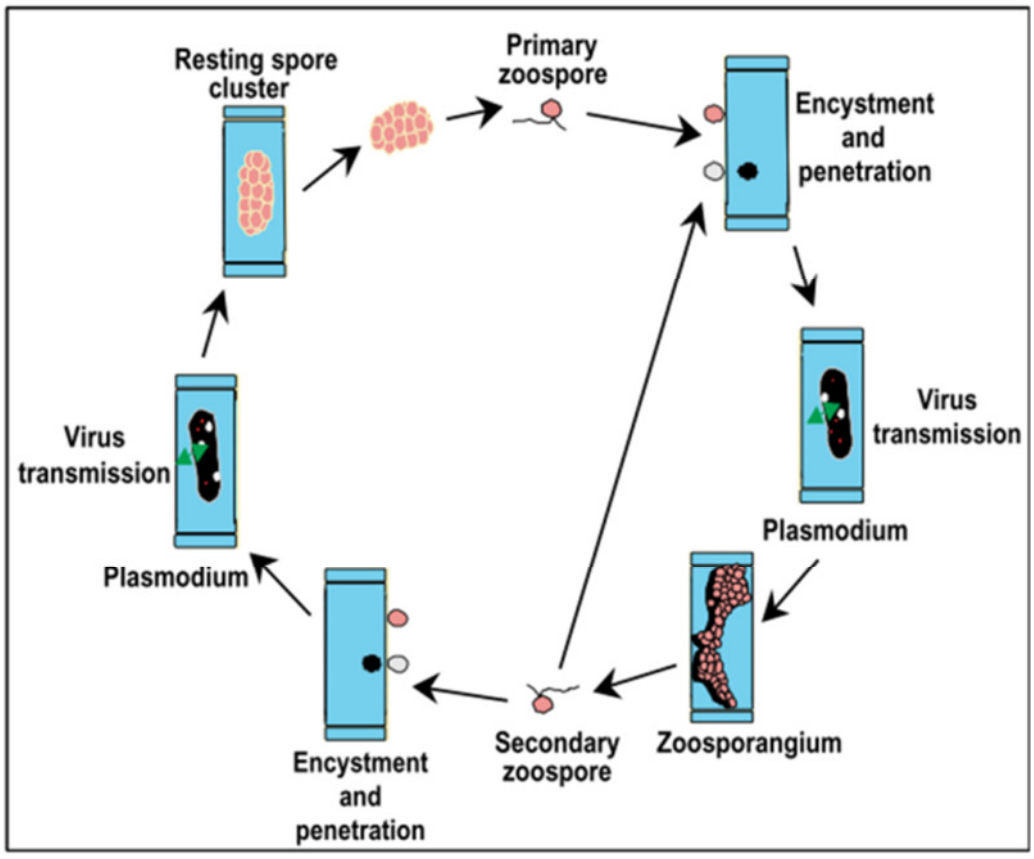

Figure 4: Life cycle of *Polymyxa graminis* (Kanyuka et al. 2003).

The entrance of the virus into the cytoplasm of plant cells has not been studied in detail yet, but if the vector carries the virus, it cannot be removed from the zoospores (Kanyuka et al. 2003). The resting spores also stay viruliferous after treatment with sodium hydroxide and hydrochloric acid (Rao and Brakke 1969).

In infested fields, typical yellow patches can be observed when susceptible plants are grown (Figure 5a). On the single plant level, virus infection leads to yellow stripes on the youngest leaves (Figure 5b). The yellowish coloring begins from the leaf apex and leads to mosaic symptoms and necrosis. Temperatures above 20°C inhibit symptom expression on newly developing leaves. Infected plants show reduced growth and winter hardiness resulting in a reduced yield of up to 50% (Plumb et al. 1986). Genes conferring resistance against BaMMV and BaYMV are quite frequent in barley (*Hordeum* sp.). As shown in figure 6, 18 different resistance genes corresponding to at least nine loci were identified in barley

(Ordon and Perovic 2013). Introgressed from *Hordeum bulbosum*, *Rym14*^{Hb} and *Rym16*^{Hb} (Ruge et al. 2003; Ruge-Wehling et al. 2006) are inherited in dominant manner as well as

Figure 5: Symptoms of barley yellow mosaic disease.
A shows typical symptoms on an infested field (foto by A. Habekuss). B shows typical yellow stripes on infected leaves of a susceptible barley plant.

Rym17 derived from a barley accession from Pakistan (Kai et al. 2012) and *Rym2*. The other known resistance genes against BaMMV/BaYMV are recessive. Resistance against all currently known BaYMV/BaMMV strains in Europe is confered by the genes *rym1, Rym2, rym11, rym12, Rym14*^{Hb} *or Rym16*^{Hb} (Lüpken 2015; Kanyuka et al. 2004). Complete BaMMV resistance is inherited by the genes *rym4*, *rym7*, *rym9, rym13* and *rym15* (Le Gouis et al. 2004). *rym5* and *rym8* confer resistance against BaMMV, except the resistance breaking strains BaMMV-Teik and BaMMV-Sil (Habekuss et al. 2008b). *rym4* and *rym8* confer resistance to BaYMV, except to BaYMV-2 as well as resistance to BaMMV. The genes *rym3*, *rym5* and *rym10* confer resistance to both BaYMV strains (Ordon et al. 1993; McGrann and Adams 2003). *Rym6* seems to be not effective against European virus strains (Iida et al. 1999) and the genes *Rym17* and *rym18* were tested for their resistance only with respect to Japanese strains, until now (Kai et al. 2012).

In Europe, nearly all resistant cultivars carry *rym4* (Bundessortenamt 2017). However, this gene was overcome in the late 1980‘s by BaYMV-2 (Huth 1989). In the following years, *rym5* was introgressed into cultivars but overcome by BaMMV-Teik and BaMMV-Sil

(Habekuss et al. 2008b). However, according to our knowledge the area infested by these BaMMV-strains is still limited (pers. communication A. Habekuß). Genes not incorporated in registered cultivars up to now are resources to improve resistance in future breeding programs or may be used to pyramid genes and to improve the durability of resistance. Two strategies for pyramiding different resistance genes were developed by Werner et al. (2005).

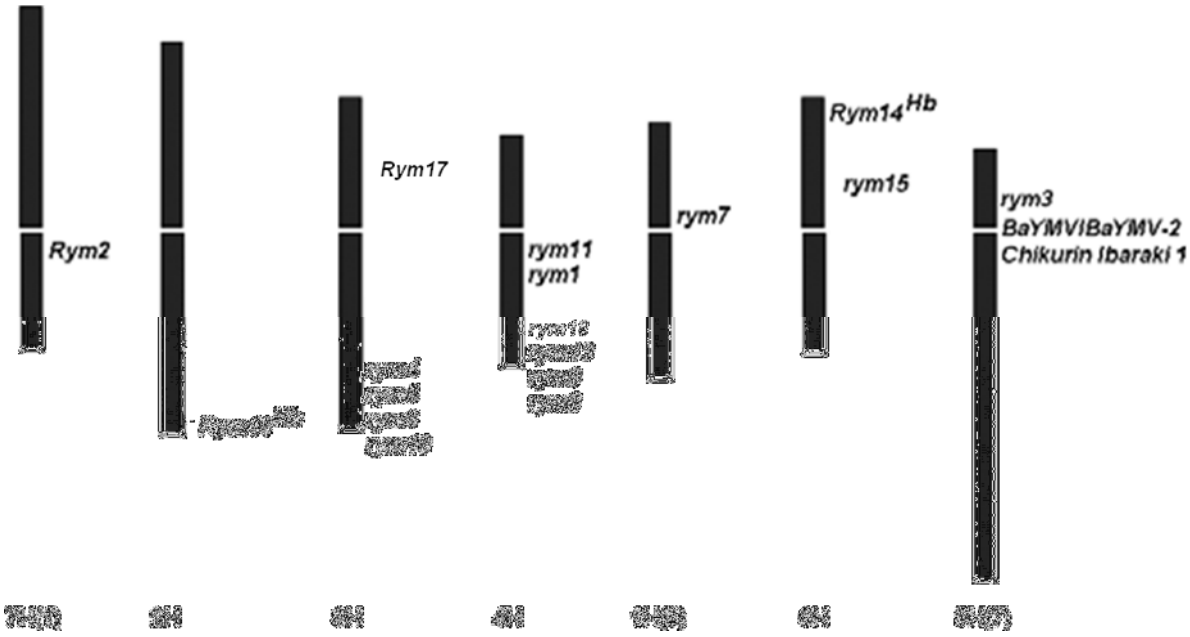

Figure 6: Distribution of known resistance genes against barley yellow mosaic disease (modified according to Ordon et al. 2009).

1.2.2 Barley yellow dwarf disease

The *Barley yellow dwarf virus* belongs to the family of the *Luteoviridae* and was first detected in 1951 in barley in California, USA (Oswald and Houston 1953) followed by reports all over the world in the following years, e.g. 1963 in Australia by Smith (1964,1968), in France by Bogavac et al. (1968). Luteoviruses are positive single-stranded RNA-viruses with a genome size of 5.6 kb to 6.0 kb (Ali et al. 2014). BYDV forms icosahedral virions with a diameter of 20-30 nm enclosed by a capsid composed of 180 subunits of major coat protein (CP) and fewer copies of a readthrough domain (RTD) (Ali et al. 2014). The RNA shows no 5' cap and 3' poly (A) tail (Figure 7; Allen et al. 1999). Therefore, a cap-independent translation is required (Treder et al. 2008).

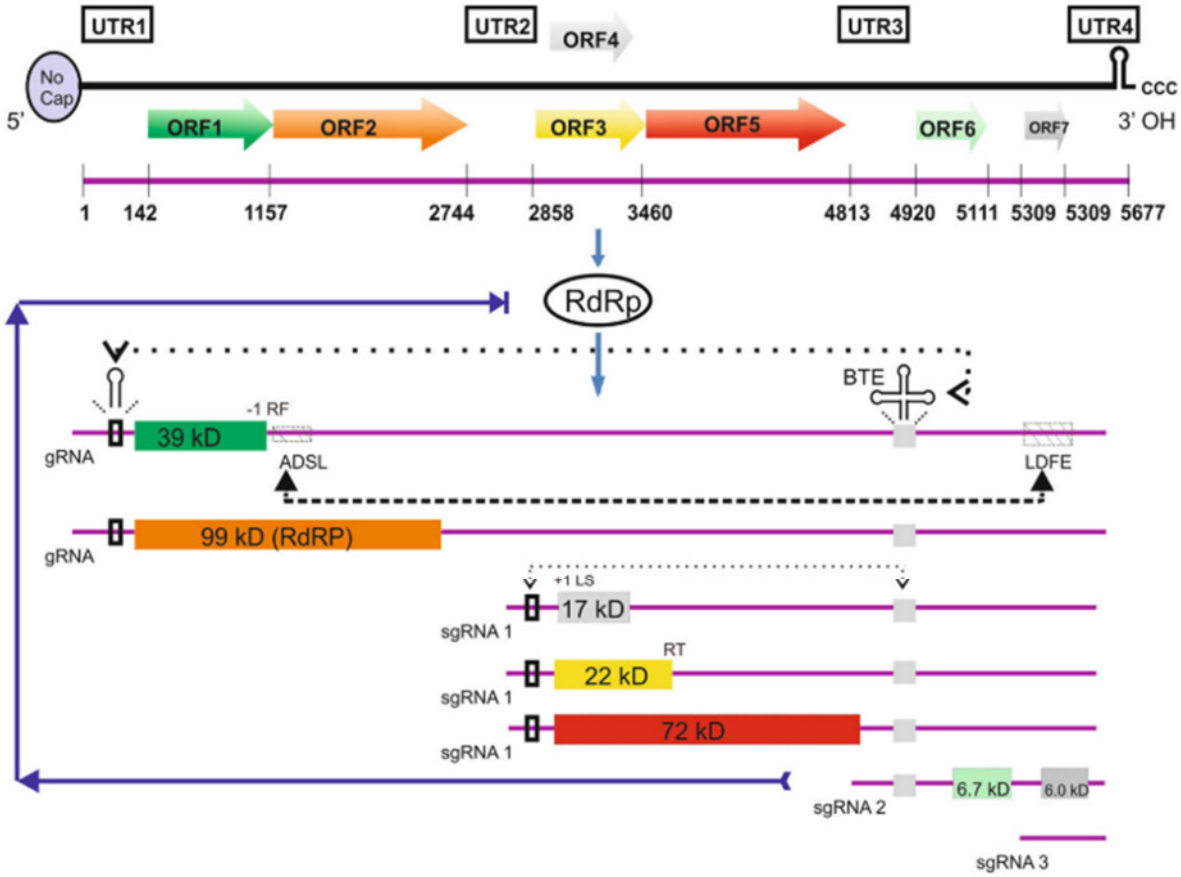

Figure 7: Schematic order of *Barley yellow dwarf virus* RNA (Ali et al. 2014).

The RNA shows six open reading frames (ORF), where ORF 1 and ORF 2 are directly translated from the genomic RNA and the other ones are translated from subgenomic RNAs (Ali et al. 2014). ORF 1 and ORF2 encode for proteins required for virus replication (RNA-dependent RNA polymerase). ORF3 includes the major coat protein (CP) and ORF4 encodes a 17 kDa protein, which is essential for systemic transport in plants (Chay et al. 1996). ORF5 encodes a 50 kDa protein which is expressed as a 72 kDa protein by read-through suppression of the ORF3 stop codon. ORF5 is required for aphid transmission and virion stability (Ali et al. 2014). The ORF6 flanking RNA sequence seems to be involved in virus replication and the protein itself supports virus replication (Mohan et al. 1995).

Isolates of BYDV were assigned to different genera of the *Luteoviridae*. BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-kerII and BYDV-kerIII belong to the genus *Luteovirus*. *Cereal Yellow Dwarf Virus*, CYDV-RPV and CYDV-RPS were categorized to the genus *Polerovirus* and the isolates BYDV-GPV, BYDV-SGV were not assigned to a genus so far (https://talk.ictvonline.org/taxonomy/; 17.04.2018). The isolates were named after their

predominant vectors (e.g. CYDV-RPV is transmitted by the aphid species *Rhopalosiphum padi*).

Luteoviruses are transmitted by aphids in a non-propagative circulative manner (Whitfield et al. 2015). Feeding 15 min in the phloem tissue of an infected plant is sufficient for virus acquirement. Viruliferous aphids transmit the virus through injection of saliva during feeding time. The maximum transmission efficiency is proposed to be between 12h and 24h, but aphids are also able to transmit the virus in 1 to 3 hours (Gray et al. 1991). At least 25 different aphid species were reported to be vectors for BYDV (Halbert and Voegtlin 1995). Because *R. padi* and *Sitobion avenae* are the most common aphids, the virus isolates BYDV-PAV and BYDV-MAV are most important in Europe (Habekuss et al. 2003; Plumb 1996).

Symptoms of BYDV infection include stunted growth, yellowing especially of leaf tips and reduction of number of ears per plant or thousand kernel weight (Choudhury et al. 2017; Kosova et al. 2008; Lüpken et al. 2014; Riedel et al. 2011) that lead to substantial yield losses of more than 70% (Suneson and Ramage 1957). Additionally, the root growth of infected plants is significantly inhibited (in barley 35%) within a few days after inoculation (Haber and Comeau 1992; Erion and Riedell 2012). Based on experiments with wheat, highest yield losses after BYDV infection are observed in plants, which were sown in mid-April to May (Thackray et al. 2009). Due to climate change, aphids will be more active during autumn resulting in a higher risk of BYDV infection (Swaminathan 2011). Habekuss et al. (2003) reported BYDV infection rates of 21% to 45% in winter barley fields in 2002. Treatment with insecticides is the most effective way to counteract virus infection indirectly by killing the aphids so far. This can reduce the aphid population from 24% on untreated fields to 1 % on treated fields resulting in a reduced virus infection (Habekuss et al. 2003). However, due to environmental reasons and consumer protection, insecticides are not the treatment of first choice (Aktar et al. 2009; Blacquière et al. 2012; Lichtenstein et al. 1962). Therefore, breeding for resistant cultivars is the most environmentally sound way to prevent yield losses.

Since 1961 tolerance to BYD is known in barley (reviewed in Burnett et al. 1996). Tolerance is defined as the ability of the host plant to reduce the physiological/agronomic effect of the infection by diseases irrespective of the amount of the pathogen (Pagán and García-

Arenal 2018). Today three major genes are known to confer tolerance to BYDV. The recessive gene *ryd1* was detected by Suneson (1955), but because of its low efficiency it is not used in breeding programmes (Kosova et al. 2008). Rasmusson and Schaller (1959) described the dominant gene *Ryd2* in Ethiopian barley lines localized on chromosome 3H (Collins et al. 1996). This gene confers tolerance to BYDV-PAV and BYDV-MAV but is ineffective to CYDV-RPV (Baltenberger et al. 1987). In 2004, a third dominant resistance gene (*Ryd3*) was detected in an Ethiopian landrace ('L94'; Niks et al. 2004) on chromosome 6H, conferring tolerance to BYDV-PAV and BYDV-MAV. Efforts for high resolution mapping were already carried out by Lüpken et al. (2014). Both dominant genes were used in breeding programmes (Kosova et al. 2008) and efforts to enhance the resistance by pyramiding were carried out by Riedel et al. (2011). This pyramiding leads to quantitative resistance instead of tolerance of the DH lines against BYDV-PAV-ASL-1. Besides the three major genes, different QTLs were identified on chromosomes 2H, 3H, 4H and 7H against BYDV-PAV (Scheurer et al. 2001b) and on chromosomes 1H, 4H and 7H against BYDV-PAV and BYDV-MAV (Toojinda et al. 2000).

1.3 Genetics of virus resistance and isolated resistance genes

In barley, the genes *rym4/5* and *rym11* for bymovirus resistance and no resistance gene for BYDV tolerance are described until now. For a targeted search for candidate genes, it is useful to know about the respective genetics of virus resistance and the currently isolated genes.

About half of the known virus resistance genes are inherited in a dominant manner (Hull 2014). Host genes for the recognition of specific pathogen effectors are called R-genes (Gururani et al. 2012). The R-gene mediated resistance is mostly race-specific and only effective against races expressing the appropriate effector gene (Avr-gene). These avirulence genes are produced by the pathogen either to change the physiological state of the host plant for better infection or to interfere with the activation of the defense system of the host plant. There is a direct gene-for-gene relationship between the R-genes and the corresponding Avr-genes (reviewed in Gururani et al. 2012). Most of these R-genes contain leucine-rich repeats (LRR), a nucleotide binding site (NBS) and an amino-terminal signaling domain; therefore, they are called NBS-LRR genes (Belkhadir et al. 2004). Some cloned R-

genes for plant viruses represent genes of the NB-LRR family (Cournoyer and Dinesh-Kumar 2011). For instance, the *Sw-5* locus, conferring broad and stable resistance to *Tomato spotted wilt virus* (TSWV) in tomato, or the *Soybean mosaic virus* (SMV) resistance gene RSV1 are CC-NBS-LRR genes (Brommonschenkel et al. 2000; Hayes 2004).

For recessive resistance two hypotheses were formulated by Fraser (1990). According to the first hypothesis, there is an active mechanism where the infested plant produces an inhibitor that stops the infection process of the virus or a factor that recognizes viral proteins and starts the resistance reaction. Second, the resistance is regarded as a result of a passive mechanism. That means that the loss of a special host factor or its mutation is responsible for the prevention of the completion of the virus infection (Diaz-Pendon et al. 2004) as it is shown for recessive resistance to potyviruses (Ben Khalifa et al. 2012, Cosson et al. 2012, Julio et al. 2015, Mazier et al. 2011).

1.3.1 The function of translation initiation factors

Potyviruses and Luteoviruses are +ssRNA viruses which are dependent on the host cell for replication (Lopez-Moya and Garcia 2008). As reviewed by Caranta and Dogimont (2008) and Sanfaçon (2015), numerous studies showed that recessive resistances against several plant viruses are due to the incompatibility of viral components to eukaryotic translation initiation factors (eIF). Translation initiation is depending on the formation of a closed-loop format (Ali et al. 2014). Cap-dependent translation (Figure 8A) is enhanced by the interaction of the polyA-tail of the host mRNA with the poly-A-binding protein (PABP) and binding of the translation initiation factor 4e (eIF4e) to the 5' cap of the mRNA. Subsequently, eIF4G binds to eIF4e and PABP, which leads to a ring formation of the mRNA. The 4F-complex is formed by the translation factors 4E and 4G, where 4G also interacts with 4A. This eIF4A-protein is a DEAD-box ATPase and unwinds the mRNA and facilitates ribosome scanning. These translation initiation factors are also key elements for virus gene translation and represent interesting targets for antiviral strategies (Sanfaçon 2015). The interaction of the eIF3 complex with eIF4G and the 40S ribosomal subunit guides the 43S pre-initiation complex (40S subunit and eIF2-GTP-$tRNA^{Met}$ ternary complex) to the mRNA. Hereafter, the 40S subunit, which includes the factors eIF2 and eIF5, scans the mRNA for the start-codon as shown in Figure 8 (Robaglia and Caranta 2006).

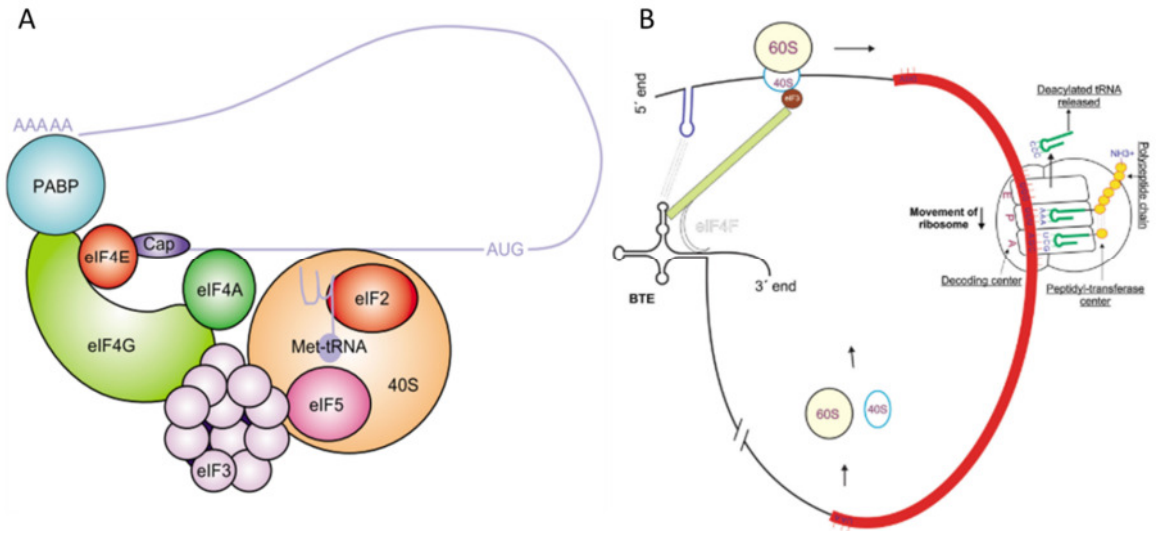

Figure 8: Schematic picture of translation initiation in eukaryotic plants.
A shows cap-dependent translation (Caranta and Dogimont 2008) and B shows cap-independent translation (Ali et al. 2014).

For cap-independent translation (Figure 8B), as required for translation initiation of BYDV genes, the 3' BTE-element binds to a sequence on the 5' end of the untranslated region of BYDV-RNA. This formation recruits eIF4F and eIF3 and starts the translation machinery (Ali et al. 2014).

When translation is initiated, eukaryotic elongation factors (eEFs) provide continuous protein synthesis. Elongation factor 1A forms a complex with GTP and tRNAs and transports these to the ribosome (Mateyak and Kinzy 2010). In plants, two isoforms of eIF4G and eIF4E, encoded by multi-gene families, exist. The specialization of viruses to use one of these isoforms leads to the fact that mutation of a single isoform can cause resistance without affecting the plant itself. Especially for potyviruses, co-evolution of the viral VPg protein and the translation initiation factor 4E is reported (Charron et al. 2008; Gallois et al. 2010). It was shown that single nucleotide exchanges prevent interactions between the viral VPg protein with eIFiso4e in *Arabidopsis thaliana* (Gallois et al. 2010), *Capsicum* spp. (Ayme et al. 2006) and *Lycopersicon hirsutum* (Moury et al. 2004). For barley, Stein et al. (2005) showed that the barley yellow mosaic resistance locus *rym4/5* encodes the translation initiation factor *HveiF4e.* A total of 66 different alleles of this factor have been described (Hofinger et al. 2011). Mutations within the viral VPg protein lead to susceptibility of the plant to respective viruses (Habekuss et al. 2008a; Habekuss et al. 2008b; Kuehne et al. 2003; Li and Shirako 2015).

Shopan et al. (2017) unraveled the resistance of *Brassica juncea* to *Turnip mosaic virus* (TuMV). They identified a new translation initiation factor responsible for resistance: *eIF2Bβ*. This factor acts as a guanine nucleotide exchange factor in the early initiation process.

1.3.2 The role of chaperones and folding enzymes

According to Verchot (2012), different ways of interaction of chaperones with potyviral proteins exist. One hypothesis is that the potyviral replicase is anchored to the endoplasmatic reticulum (ER) with the 6K-VPg-protein (Figure 9A). The HSC70-3 protein is associated to the translation initiation complex (as described above) and supports translation (Verchot 2012). Second, as reported in *Nicotiama benthamiana* for Potato virus A infection (Hafrén et al. 2010) *HSP70* and *CPIP*, a co-chaperone, suppress coat protein (CP) accumulation and blocks virion assembly (Figure 9B).

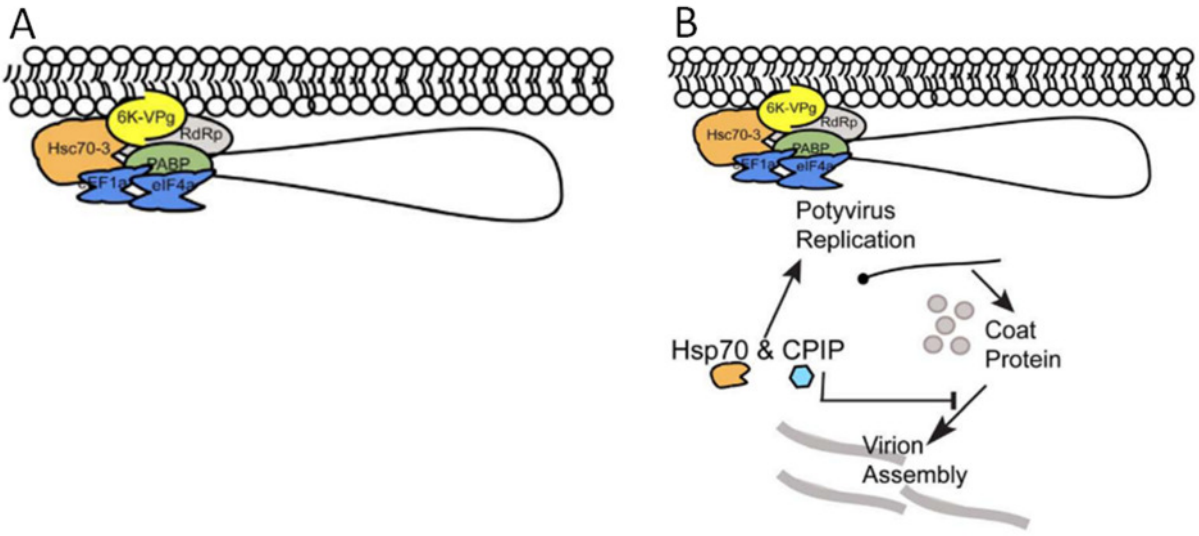

Figure 9: Schematic view of interaction of chaperones to potyviral proteins.
A: HSC70-3 is associated to the translation initiation complex. B: Impact of HSP70 and CPIP to potyviral replication (Verchot 2012).

CPIP delivers CP to *HSP70*, which facilitates ubiquitination and leads to degradation of this protein. This prevents termination of replication-associated translation of viral proteins (Hafrén et al. 2010).

In barley, the protein disulfide isomerase like 5-1 (*HvDPDIL5-1*) encoded by the BYMV resistance locus *rym11* was recently isolated (Yang et al. 2014b). This protein belongs to

the protein family of protein disulfide isomerases (PDI) and acts in cells as a chaperone for correct protein folding (Figure 10). It belongs to the ER quality control machinery (ERQC, Verchot et al. 2012) and is able to catalyze the oxidation of protein sulfhydryl groups and reduces and isomerises protein disulfide bonds (Laboissiere et al. 1995). This protein consists of a thioredoxin (TRX) domain and a C'-terminal tetrapeptide EDEL which is responsible for the control of the retention of the protein within the ER (Yang et al. 2014b).

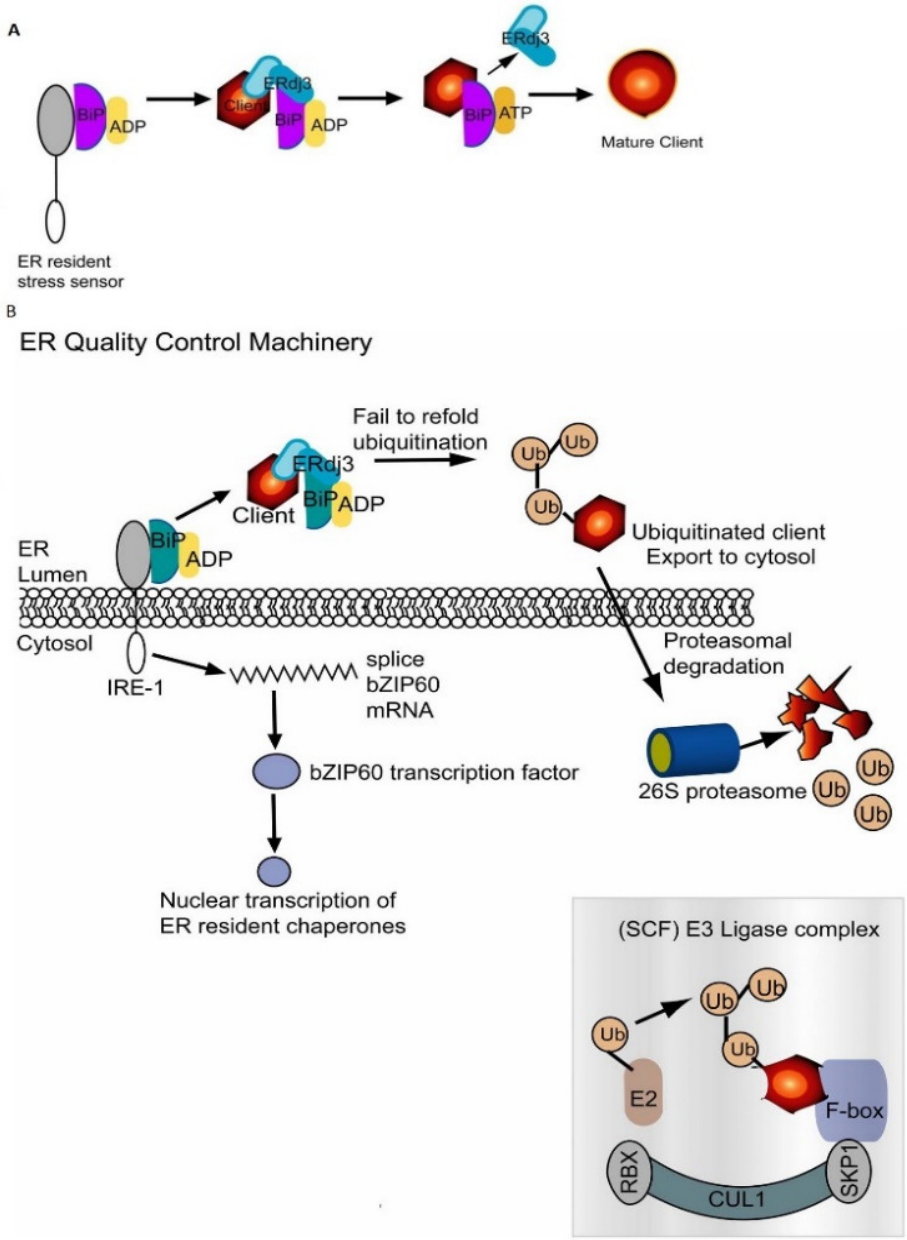

Figure 10: Schematic view of the ERQC machinery (modified after Verchot et al. 2012).
A shows the maturation of proteins in the ER using the HSP machinery. B shows the ER quality control machinery and how degradation of missfolded proteins works.

Figure 10 A shows the maturation of proteins using the Hsp70 homolog BiP. By recognition of misfolded proteins, a J-domain protein (Erdj3) recruits BiP and forms a complex with the misfolded protein. By conversion of ADP to ATP, the maturation of the protein follows. Figure 10B shows the ERQC machinery. After recognition of the misfolded protein by Erdj3 and BiP the misfolded protein is ubiquitinated in the ER and degraded by the 26S proteasome in the cytosol.

1.4 Map-based gene isolation

As map-based gene isolation is a commonly used method for gene identification, this method will be used in this work.

In the literature, two ways for gene isolation are described (Peters et al. 2003). The first is forward genetics (from phenotype to gene) and the second is reverse genetics (from gene to phenotype). As reviewed in Peters et al. (2003), reverse genetics are based on sequence information gained from genome or expressed sequence tag (EST) sequencing. The gene can directly be used to find the corresponding phenotype or function by e.g. transformation studies or targeting-induced local lesions in genomes (TILLING) approaches (McCallum et al. 2000).

For forward genetics, the phenotype for the trait of interest is known first and then it is searched for the causative gene. This approach is called map based cloning (MBC) and mainly follows the three steps of (i) genetic mapping and fine-mapping, (ii) subsequent physical mapping including identification of a candidate gene and (iii) proof of biological function of the candidate gene. This method was used for mapping different genes in barley (e.g. *mlo* by Büschges et al. 1997, *sdw3* by Vu et al. 2010, *rym4/5* by Pellio et al. 2005, *Ryd3* by Lüpken et al. 2014, *Rfm1* by Rizzolatti et al. 2017) followed by the isolation of the respective genes (e.g. Yang et al. 2014; Stein et al. 2005).

The main objective of MBC is the parallel reduction of genetic and physical distances between markers and the gene of interest. After crossing parental lines with different phenotypic characteristics regarding the gene of interest, a high resolution mapping population has to be build up. This population is analysed with flanking markers in a next step, followed by marker saturation of this interval by using different methods and resources to get diagnostic markers and to identify candidate genes (Stein and Graner 2005). To achieve this, anchoring the genetic map to the physical map is of particular

interest. The genome size of barley is 5.1 Gb and according to the chromosomal localization of the gene of interest e.g. distally or proximally 1 cM may correspond to a different physical length i.e. number of base pairs (Kuenzel et al. 2000).

The publication of the physical map of barley by Mayer et al. (2012) and of the barley reference sequence (Mascher et al. 2017) simplifies the identification of candidate genes. Anchoring flanking markers to the physical map leads to fast and precise localization of the chromosomal region of interest. Subsequently, all postulated genes located in the interval can easily be analyzed for possible candidate genes (Jander et al. 2002). This anchoring and gene analysis lays the foundation for isolation and validation of candidate genes. The last step of MBC is the verification of candidate genes. This can be achieved in different ways like complementation analysis or analysing mutants. For complementation, which was applied to *rym4/5* and *rym11,* the cDNA or gDNA of the susceptible allele was transferred to a resistant plant (Stein et al. 2005; Yang et al. 2014b) and segregation ratios for resistance of the T_1-families were analysed subsequently. In addition, mutation analysis was done by screening a TILLING-population (Yang et al. 2014). Searching for mutants and subsequent phenotyping and genotyping can support the thesis of the respective candidate gene. Also, knock-down of particular candidate genes using RNA interference (RNAi) is used for functional validation of these genes (Chen et al. 2013; Douchkov et al. 2016; Xiong et al. 2018).

The development of CRISPR/Cas is a powerful tool for precise modification or knock-out of genes (Barrangou et al. 2007; Sharma et al. 2017). This method induces directed double strand breaks, which may be repaired with slight modifications. This method has been established in barley by Budhagatapalli et al. (2015). Subsequently, the modified plant has to be evaluated phenotypically by a test for resistance or susceptibility.

1.5 Aim of this thesis

As the only isolated virus resistance genes in barley are the barley yellow mosaic virus resistance genes *rym4/5* and *rym11*, this work focuses on:

(i) Mapping the resistance gene *rym13* at high resolution, increase the marker density and identification of candidate genes as a prerequisite for the isolation of this BaMMV/BaYMV resistance gene.

(ii) Reduction of the interval harbouring *Ryd3* and identification of candidate genes for this BYDV tolerance gene.

For these purposes progenies of the cross 'Taihoku A' x 'Plaisant' and 'L94' x L94-QTL3 were available. 'Taihoku A' is a Taiwanese six rowed barley cultivar and the donor of *rym13*. 'Plaisant' is a BaMMV/BaYMV susceptible cultivar derived from France. In preliminary tests the resistance gene *rym13* was located on chromosome 4HL (Humbroich et al. 2010). Based on analyzing 154 DH-lines, it was assumed that the resistance to BaMMV and BaMMV-Teik is encoded by the same locus or because of the low resolution encoded by two independent genes, which are more closely linked than at 0.65 cM. No information on the genetics of resistance to BaYMV/BaYMV-2 in 'Taihoku A' was available. Previous mapping of *Ryd3* was conducted by Lüpken et al. (2014) with the mapping population of 'L94' x L94-QTL3. L94 is an Ethiopian accession carrying *Ryd3* (Niks et al. 2004), while L94-QTL3 carries the 6H fragment of the susceptible cultivar 'Vada' (van Berloo et al. 2001). Lüpken et al. (2014) localized the gene within an interval of 1.907 cM with 10 co-segregating markers. To dissolve this co-segregating region and to shorten the interval, the population size has to be increased and new markers have to be developed.

2 High resolution mapping of the BaMMV resistance gene *rym13* using genomic resources

2.1 Introduction

Barley yellow mosaic disease is caused by two different bymoviruses, i.e. *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV), which are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* (Adams et al. 1988, Kusaba and Toyama 1970, McGrann and Adams 2003, Toyama and Kusaba 1970). This virus disease occurs in several countries of the world (Habekuss et al. 2008b, Hariri et al. 2003, Hill 1981, Huth 1990, Kashiwazaki et al. 1990, Lee et al. 1996, Nomura et al. 1996, Steyer et al. 1995). In Germany, the disease was first detected in 1978 (Huth and Lesemann 1978) and two strains are described for BaMMV and BaYMV each (Habekuss et al. 2008b, Huth 1989, 1990). Typical symptoms of barley yellow mosaic disease are yellow patches in the field and yellow stripes on young leaves. Furthermore, plants are stunted. Plumb et al. (1986) reported yield losses up to 50% when susceptible plants are grown on infested fields. In addition, it was shown that as a result of an increased susceptibility to low temperature, total yield losses may occur (Huth 1988). Due to high yield losses and a constant spread of the infested area during the last thirty years (Kuehne 2009), there is an increasing demand to counteract virus infection by breeding resistant cultivars.

In several screening programs followed by genetic analyses, 18 different barley yellow mosaic virus resistance genes (Ordon and Perovic 2013) were detected corresponding to at least nine loci (Ordon et al. 2009; Kai et al. 2012). All of these resistance genes, except $Rym14^{Hb}$, $Rym16^{Hb}$ and *Rym17* are inherited in a recessive manner. The resistance genes $Rym14^{Hb}$ and $Rym16^{Hb}$ are introgressed from *Hordeum bulbosum* (Ruge et al. 2003; Ruge-Wehling et al. 2006) and *Rym17*, located on chromosome 3H, was first described in a six-rowed *H. vulgare* accession (Kai et al. 2012) from Pakistan.

Isolation of first resistance genes reveals most likely different mechanisms of resistance to barley yellow mosaic disease. Resistance encoded by *rym4/5*, isolated by Stein et al. (2005), is due to mutations in the eukaryotic translation initiation factor *eIF4E*. RNA viruses like potyviruses need to recruit the translation factory of their host for successful reproduction.

The bipartite bymovirus encodes only for 12 proteins which are mainly responsible for virus movement, vector transmission and pathogenicity (Lopez-Moya and Garcia 2008). The VPg protein is essential for the replication of the virus. There is a direct evolutionary relationship between this protein and the translation initiation factor *eiF4E* (Charron et al. 2008). This factor binds to the 5 prime terminal cap of the mRNA. Subsequently it recruits eIF4G and eIF4A to form the eIF4F complex. Translation starts by binding of the eIF4F complex to the small ribosomal subunit through interaction of eIF4G with eIF3. After this, the 40S subunit scans the mRNA for the start-codon (Robaglia and Caranta 2006). Meanwhile 66 alleles of this translation initiation factor are known (Hofinger et al. 2011, Perovic et al. 2014, Yang et al. 2016).

On the other hand, *rym11* encodes for the protein disulfide isomerase like 5-1 (*HvPDIL5*-1, Yang et al. 2014b) that most likely catalyzes the correct folding of proteins (Khan and Mutus 2014). Allele mining of these susceptibility factors was conducted by Yang et al. (2016) with a set of 365 wild and 2557 domesticated barley accessions which resulted in an identification of 30 haplotypes for *HvPDIL5-1*.

In Germany, nearly all barley cultivars released are resistant to BaMMV/BaYMV mainly due to the *rym4/5* locus (Bundessortenamt 2017). In the late 1980‘s BaYMV-2, which is able to overcome *rym4*, was detected (Huth 1989). This pathotype carries a variation of a single amino acid within the VPg protein (Kuehne et al. 2003; You and Shirako 2012). In the following years *rym5,* a different allele of *Hv-eiF4E,* was introduced into European cultivars. In 2004, in Germany two virus isolates occurred which were able to overcome *rym5*, i.e. BaMMV-Teik and BaMMV-Tasl (Habekuss et al. 2008b). Again a change of amino acids in the VPg protein seems to be the reason for the breakdown of *rym5*. This was also demonstrated for the virus strain BaMMV-Sil occurring in France 2002 (Kanyuka et al. 2004).

To achieve resistance to all these strains, marker based pyramiding (Werner et al. 2005; Werner et al. 2007) of independent resistance genes/alleles is an efficient strategy. To realize this with a minimum of linkage drag and as a prerequisite for gene isolation, high resolution mapping of the gene of interest has to be conducted. In recent publications, high resolution mapping was already carried out to provide the basis for isolating the resistance genes *rym4/rym5, rym7* and *rym11* (Lüpken et al. 2013; Pellio et al. 2005; Yang et al. 2013).

The present study is focused on high resolution mapping of the recessive resistance gene *rym13* located on chromosome 4HL (Werner et al. 2003; Humbroich et al. 2010) derived from the Taiwanese cultivar 'Taihoku A'.

2.2 Materials and Methods

2.2.1 Construction of a high resolution mapping population

For construction of a high resolution mapping population, a segregating population with a total of 5,191 F_2 plants of the cross 'Taihoku A' x 'Plaisant' was developed; 'Taihoku A' is resistant to BaMMV, BaYMV, BaYMV-2, BaMMV-Teik and BaMMV-SIL whereas 'Plaisant' is a susceptible French cultivar. F_2 plants were genetically analyzed with the co-dominant simple sequence repeats (SSRs) markers HVM67 and GBM1015 flanking *rym13* (Humbroich et al. 2010). Genomic DNA was extracted at the two-leaf stage using the method described by Dorokhov and Klocke (1997). Analysis of the markers was done according to previously published papers (Li et al. 2003; Liu et al. 1996; Rostoks et al. 2005; Thiel et al. 2003; Varshney et al. 2007) by fluorescence-detection on the genetic analysis system CEQ 8000 (Beckman-Coulter). Heterozygous recombinant F_2 plants were selfed in the greenhouse and twelve progenies per plant were re-analyzed with respective markers to identify homozygous recombinants. The homozygous recombinant plants obtained in F_2 and in F_3 were propagated as segmental recombinant inbred lines (RILs) for subsequent marker saturation of the target interval and for phenotyping in F_4. DNA of the obtained RILs was extracted according to Stein et al. (2001).

2.2.2 Validation of mapping the resistance gene

To validate the mapping of the developed markers and the resistance gene, an independent second mapping population of the cross of 'Hanna' x 'HOR11019' (IPK genebank, Gatersleben) was established as described before for the population 'Taihoku A' x 'Plaisant' consisting of 988 F_2-plants.

2.2.3 Marker saturation using genomic resources

For marker saturation of the respective target interval harbouring the resistance gene *rym13,* markers HVM67, GBM1015 and WMS06 were integrated in high density mapping populations developed by Sato et al. (2009), Thiel et al. (2003) and Perovic et al. (2013). Furthermore, data obtained by genotyping the parental lines with the Illumina 9k custom BeadChip (Comadran et al. 2012) were used for further marker development.

For the development of new markers derived from chip analyses, primers were designed using the web-based software Primer3 (http://frodo.wi.mit.edu/primer3/, Rozen and Skaletsky 2000) including an M13 tail (5´-CACGACGTTGTAAAACGAC-3´) added to the 5´end of the forward primer. The purified PCR products (MinElute® 96 UF PCR Purification Kit, Qiagen, according to manufacturer's protocol) amplified on the parental cultivars 'Taihoku A' and 'Plaisant' were subjected to cycle-sequencing from both ends on the ABI 377XL sequencer using BigDye v3.1 terminator sequencing chemistry (ABI Perkin Elmer, Weiterstadt, Germany). Subsequently, sequences were analyzed using the software Sequencher 5.0 (Gene Codes). Size polymorphisms like insertions/deletions (InDel) were directly analyzed on ethidium bromide stained 1.5% agarose gel or by detection on a capillary analyzer CEQ 8000 (Beckman-Coulter, Krefeld, Germany) using M13 tails as described above. Based on detected single nucleotide polymorphisms (SNPs) appropriate restriction enzymes were selected using the web-based program NEBcutter V2.0 (http://tools.neb.com/NEBcutter2/index.php, Vincze et al. 2003) to develop CAPS (cleaved amplified polymorphic sequences) markers. These PCR products were digested for three hours at respective temperature referred to manufacturer's protocol (Fermentas, NEB) by adding 0.07 U of the particular enzyme to 15 µl reaction volume. Next, the samples were analyzed on ethidium bromide stained 2% agarose gels.

In a next step, the genome zipper (GZ) developed by Mayer et al. (2009, 2011) was used for new marker development. All previously located markers were mapped by Blast search against rice (*Oryza sativa*), *Brachypodium distachyon* and *Sorghum bicolor* within this gene model. The sequences of the genes from the synthenic species located in the interval of interest were used for homology search using the IPK Barley Blast Server Assembly from Whole Genome Shotgun sequencing of barley, cultivar Morex (http://webblast.ipk-gatersleben.de/barley/viroblast.php, Deng et al. 2007). The sequence information from the

contigs located on chromosome 4HL with an e-value < 0.005 were used for further marker development as described above. With regard to intron/exon-structure of the genes, primers were developed to span whole genes covering 200-1000 bp, each.

After publication of the physical map of barley (Mayer et al. 2012; Mascher et al. 2013a), the sequences of the markers were mapped and a distinct chromosomal region was detected. Using information from the FTP download page (Mayer et al. 2012) and BARLEYMAP (http://floresta.eead.csic.es/barleymap; Cantalapiedra et al. 2015), potential candidate genes in between the resistance flanking markers were identified and primers were developed from sequence information using the above-mentioned tools. If a SNP was detected by sequencing and no restriction enzyme was found, a competitive allele-specific PCR and endpoint fluorescence genotyping (KASP^{TM}) assay was developed (Semagn et al. 2014; LGC, UK).

2.2.4 PCR-protocols for newly developed primers

For all newly developed primer pairs the PCR was performed on Gene Amp® PCR System 9700 (Applied Biosystems) using 50 ng/µl genomic DNA, 1 x PCR buffer (Buffer B without $MgCl_2$, Solis Biodyne, Tartu, Estonia), 1.5 mM $MgCl_2$ (Solis Biodyne), 0.6 mM dNTPs (Thermo Scientific), 0.06 µM M13-tailed forward primer, 0.6 µM reverse primer, 0.3 µM fluorescence-dye-labeled M13 universal primer and 0.3 U FIREPol® polymerase (Solis Biodyne). The amplification conditions were 5 min at 94°C followed by 12 cycles of 30 sec at 94°C, 30 sec annealing (in each cycle temperature decreases for 0.5 degree from 62 to 56°C), 30 sec at 72°C. These cycles were followed by 35 cycles at 94°C denaturation, 30 sec at 56°C annealing and 30 sec at 72°C elongation. The final elongation step was performed for 10 min at 72°C (Silvar et al. 2012). All used markers and are listed in table 2 (see supplemental data table S1 for marker sequences of candidate genes).

Concerning the KASP-primers, for PCR 20 ng DNA was used following the manufacturer's protocol. Amplification and subsequent genotyping was conducted with a Biorad CFX 96^{TM} real-time system. Samples were analysed using the software Bio-Rad CFX Manager 3.1.

Table 2: Polymorphic markers analyzed on the 'Taihoku A' x 'Plaisant' mapping population.

Marker	Marker type	Size of parental alleles [bp] (Taihoku A/Plaisant)	Endonuclease	Source
WMS06	SSR	155/167	-	Li et al. 2003
GBM1015	SSR	218/290	-	Thiel et al. 2003
HVM67	SSR	138/142	-	Liu et al. 1996
k08133	CAPS	400/350	HaeIII	Sato et al. 2009
k07201	CAPS	Multiple bands	SsiI	Sato et al. 2009
k00389	CAPS	Multiple bands	AluI	Sato et al. 2009
k08610	CAPS	100/150	SspI	Sato et al. 2009
k08313	CAPS	200/250	BsmAI	Sato et al. 2009
k00136	CAPS	Multiple bands	SacII	Sato et al. 2009
k03089	CAPS	330/230	MboII	Sato et al. 2009
k03289	CAPS	Multiple bands	TaqI	Sato et al. 2009
k00460	CAPS	340/310	TspRI	Sato et al. 2009
k08786	CAPS	75/80,130	MseI	Sato et al. 2009
S076_C337	CAPS	600,200,250/700,200	SsiI	9k iselect chip
SCRI_RS_108369	CAPS	Multiple bands	Bsh1236I	9k iselect chip
B094_C051	CAPS	Multiple bands	MspI	9k iselect chip
B296_C165	InDel	800/1000	-	9k iselect chip
B365_C876	InDel	900/1000	-	9k iselect chip
BOPA_766	CAPS	100/150	BseGI	9k iselect chip
2_1130	CAPS	Multiple bands	HaeIII	9k iselect chip
B250_C469	InDel	339/331	-	9k iselect chip
S164_C941	InDel	750/1000	-	9k iselect chip
S357_C104	CAPS	600,200/750	Sau96I	9k iselect chip
S116_C985	CAPS	800/700,150	AjiI	9k iselect chip
S829_C691	CAPS	Multiple bands	TspRI	9k iselect chip
Bradi77420	CAPS	200,300/400	MspI	Mayer et al. 2011
SCRI_RS_192456	InDel	354/374	-	Mayer et al. 2011
contig_273304	CAPS	1000/300,700	Cfr31I	Mayer et al. 2011
contig_43083	CAPS	Multiple bands	Cfr31I	Mayer et al. 2011
NiASHv2073c21	CAPS	1000/350,550	HincII	Mayer et al. 2011
Bradi78120	CAPS	100/150	MlyI	Mayer et al. 2011
CSN1-4	CAPS	1000/400	HincII	Candidate gene 1

Table 2: continued.

Marker	Marker type	Size of parental alleles [bp] (Taihoku A/Plaisant)	Endonuclease	Source
PCI_1-1	CAPS	750/550,300	PdmI	Candidate gene 2
C1g1	CAPS	Multiple bands	AciI	Candidate gene 3
C1g2	KASP	-	-	Candidate gene 4
C1g3_1	KASP	-	-	Candidate gene 5
C1g3_2	KASP	-	-	Candidate gene 6
C1g4	KASP	-	-	Candidate gene 7
E04_c1g73	KASP	-	-	Candidate gene 8
E04_c1g74	KASP	-	-	Candidate gene 9
K09_c1g89	KASP	-	-	Candidate gene 10
H20_c1g62	KASP	-	-	Candidate gene 11

2.2.5 Phenotypic tests

Resistance tests for BaMMV were carried out in growth chambers using mechanical inoculation (Habekuss et al. 2008a). After sowing, the plants were grown under long day conditions (16h day/8h night, 12°C). At the third leaf stage ten plants per RIL were inoculated mechanically with isolate BaMMV-ASL1 two times at an interval of 5 to 7 days as described by Habekuss et al. (2008a). Five weeks after the first inoculation, the resistance of each line was scored visually and samples for double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) were taken.

Field trials were conducted at two locations in Germany (Schladen: 52°00'58.9"N 10°30'37.5"E and Bornum: 52°15'57.9"N 10°45'16.1"E in Lower Saxony) in 2011/2012, 2012/2013, 2013/2014 and 2014/2015 to test for BaYMV-2 and BaMMV resistance. Besides 30 plants per RIL, susceptible (cultivar 'Uschi') and resistant standards (cultivar 'Tokyo', carrying the resistance gene *rym5,* and 'Carola', carrying the resistance gene *rym4*) were grown as standards. For resistance screening, a mixed sample of each line was taken and analysed by DAS-ELISA. Enzymatic assays were carried out as described by Clark and Adams (1977). Plants with an extinction value E_{405} > 0.1 were rated as susceptible (supplemental data table S2).

2.2.6 Genetic mapping

For genetic mapping, the resolution was calculated by the number of RILs, the number of meioses in F_2 and the number of recombination between single markers. As reviewed by Collard et al. (2005), the map distance equals the recombination frequency, if the map is smaller than 10 cM. Due to the fact, that the initial map spans an interval of 5.8 cM (Humbroich et al. 2010) we used this approximation to calculate the genetic map.

2.2.7 Genotyping-by-sequencing (GBS)

A set of 130 RILs of the cross 'Taihoku A' x 'Plaisant', 25 lines from the original DH-population (Humbroich et al. 2010) and 23 DH-lines of a cross from a F_{11} (of the cross 'Rubina' x 'Post') x HOR11019, which carries a resistance gene allelic to *rym13* with respect to BaMMV (personal communication A. Habekuß), were used for genotyping-by-sequencing (GBS) as described by Wendler et al. (2014). The DNA concentration of all genotypes was determined by a PicoGreen® assay (BioTek, Synergy HT). The normalized DNA (20 ng/µl) was used for pooling and subsequent GBS according to Wendler et al. (2014). Results were obtained by sequencing on Illumina® HiSeq™ 2000 (Illumina, Inc.). Obtained data were analyzed using the Galaxy platform (Blankenberg et al. 2001; Giardine et al. 2005; Goecks et al. 2010). After adapter and quality trimming (trim galore version 0.2.8.1; <20), read mapping of the GBS data was performed using BWA (Li and Durbin 2009) with standard settings to map the reads to the morex_assembly (Mayer et al. 2012). Next, using the program mpileup (Li et al. 2009), with genotype likelihood computation, SNP calling was performed followed by anchoring the data to the POPSEQ map (Mascher et al. 2013a). Biallelic SNPs were detected and subsequently filtered for differences between the resistant and susceptible parental line and a minimum coverage of five reads per SNP using the program SnpSift (Cingolani et al. 2012). Fisher's exact test was used to examine the relevance of each SNP for resistance. Resistance data for BaMMV and BaYMV were mapped independently.

2.2.8 Whole-Genome Sequencing (WGS)

In order to get more information about the BaMMV resistance harbouring interval, WGS was conducted with two RILs (348a which is resistant, and 480a which is susceptible to BaMMV). They were selected according to their genotypic accordance to the parental lines

'Taihoku A' and 'Plaisant' with respect to the target interval. Genomic DNA was fragmented using Covaris microTubes on a Covaris 220 Instrument and the sequencing library was constructed as reported by Himmelbach et al. (2014) and Mascher et al. (2013b). Sequencing was conducted on Illumina® HiSeq™ 2000 (Illumina, Inc.). Data were analyzed as described above for GBS-data. Analyzed data were filtered using the program SnpSift. For filtering, a minimum quality score of 999 and a minimal coverage of five reads per SNP and genotype were used.

After mapping the SNP markers to the physical map and subsequent assignment to bacterial artificial chromosomes (BACs), putative candidate genes were predicted using AUGUSTUS (version 3.0.3, Stanke et al. 2008) based on BLAST against the TAIR and UNIPROT database. Primers were developed using the previously described methods.

2.2.9 Diagnostic value of developed markers

To obtain information on the diagnostic value of respective markers, the closest linked markers were tested on a set of 93 diverse genotypes (supplemental table S3). This set includes susceptible as well as resistant cultivars with all known BaYMV/BaMMV resistance genes except genes *Rym17* and *rym18*.

2.3 Results

2.3.1 High resolution mapping population

A mapping population consisting of 5,191 F_2 plants was screened with the two co-dominant flanking SSR markers HVM67 and GBM1015, which results in a genetic resolution of 0.0096 % recombination. Out of these F_2 plants, ten homozygous recombinant plants and 552 heterozygous recombinant plants were identified (Figure 11a). 447 F_3 families were converted in 431 independent homozygous segmental recombinant inbred lines. Due to loss of plants, e.g. because of non-survival of F_2 or F_3 plants, the genetic resolution was reduced to 0.012 % recombination.

For further marker saturation, the genetic information of different high density mapping populations was used. Out of the high density mapping population 'H602' x 'Haruna Nijo' (Sato et al. 2009) 10 out of 18 potential markers were mapped in the high resolution mapping population (Figure 11b; green markers). Within the other high density maps,

markers HVM67 and GBM1015 were not polymorphic or there was no additional marker information for the interval of interest.

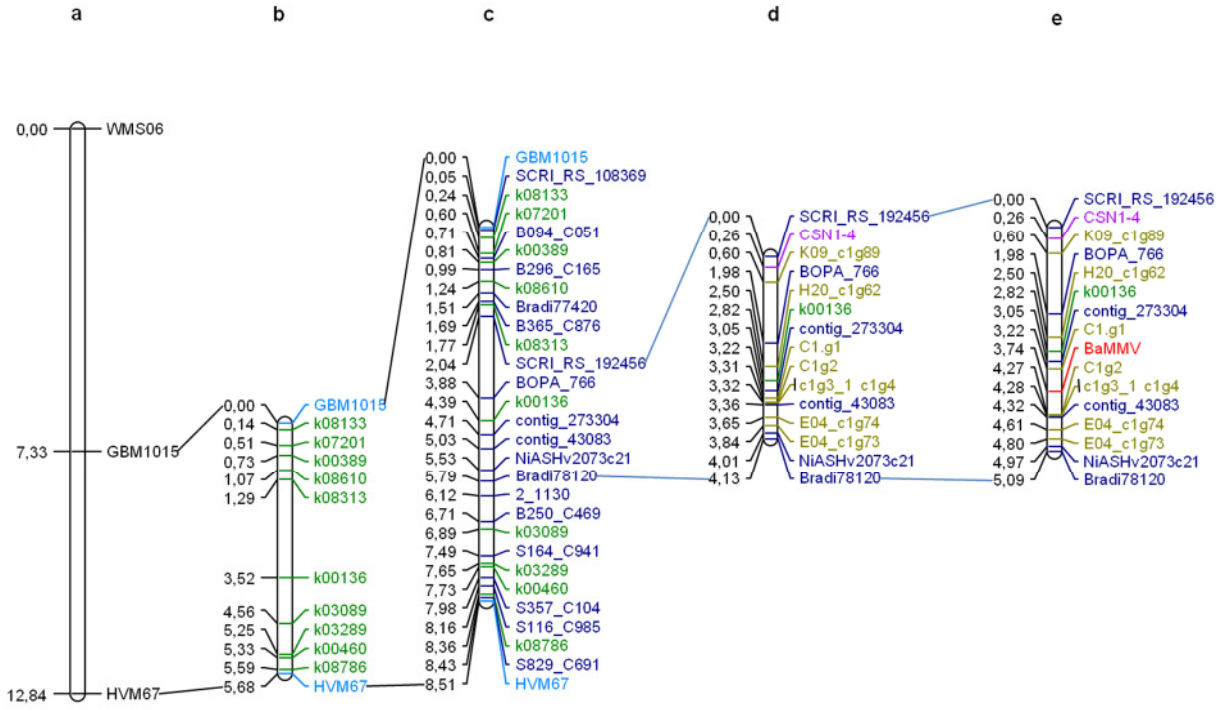

Figure 11: Partial maps of chromosome 4HL.
a: Map based on 5,191 F_2 plants. b: Mapping of markers originating from Sato et al. (2009) based on the analysis of 429 RILs. c: Map based on 431 RILs with mapped markers originating from 9k iselect chip and genome zipper. d: Partial map of chromosome 4HL after integrating new markers originating from GBS and from WGS experiments based on 173 informative RILs. e: Partial map of chromosome 4HL including resistance data based on 173 informative RILs. Light blue colour are initially flanking markers, green markers originate from Sato et al. (2009), dark blue markers were developed from iselect chip and genome zipper, yellow and purple markers represent candidate genes from GBS and WGS experiment, red colour highlights the resistance gene.

Using the information from the 9k iselect chip, 26 new primer pairs were developed, out of which 12 were polymorphic and mapped in the interval of interest (Figure 11c; dark blue markers). Mapping the available markers to the genome zipper leads to additional 25 potential markers. Three of them showed polymorphisms between the parental lines and were mapped in the interval of interest (Figure 11c, Bradi77420, NiASHv2073c21 and Bradi78120). In summary, using this approach 27 markers were localized in the target region.

2.3.2 Next generation sequencing

Using the approach of GBS leads to the identification of a total number of 11,948 SNPs between the parental lines 'Taihoku A' and 'Plaisant' of which 8,306 were mapped over all chromosomes (Figure 12a).

The highest correlation to the phenotypic data for BaMMV (p-value < 1e-12) was identified for 14 SNPs located on chromosome 4HL at 111.9 cM. One of these SNPs (morex_contig_50476), marked with NA, could not be anchored to the POPSEQ-map (Mascher et al. 2013a, Figure 12b and 12c). For BaYMV resistance the best hits were found on chromosome 3H and 7H (Figure 13 and supplemental data table S4). The hits on chromosome 3H (142-148 bp) seem to be located in the region of the *rym4/5* locus.

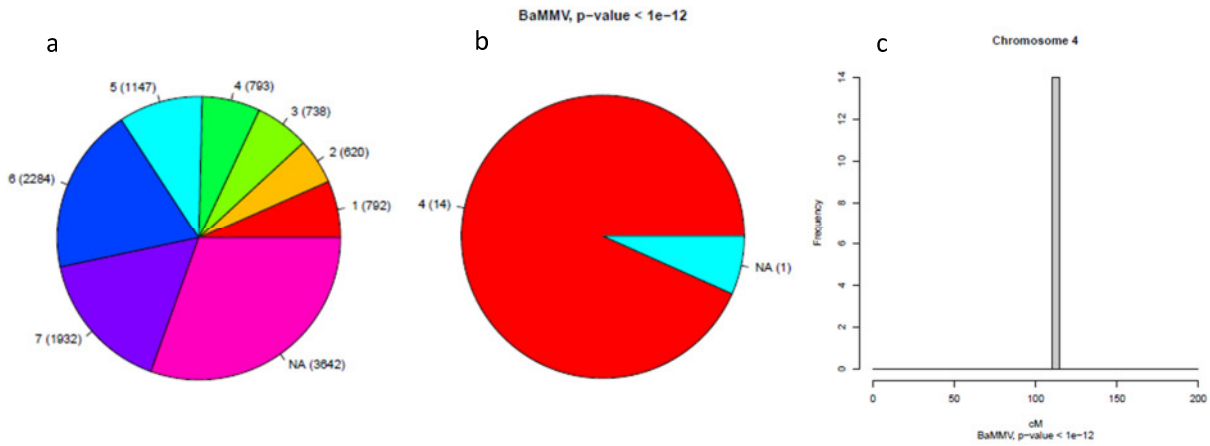

Figure 12: Graphical representations of GBS results.
a: Overview on the localization of all SNPs detected (NA= not anchored to any chromosome). b: SNPs linked to BaMMV-resistance with a p-value p< 1e-12. c: Chromosomal region of detected SNPs.

After the GBS experiment, the previously developed markers were anchored to the POPSeq-map and it turned out, that the region harbouring the BaMMV resistance spans an interval of 0.85 cM (from 111.12 to 111.97 cM). From eight potential candidate genes (Table 3) primers were developed, four of them showed polymorphism and one of them could be mapped in the interval of interest (Figure 11; CSN1-4, purple marker). The other three markers were mapped distal to the interval of interest.

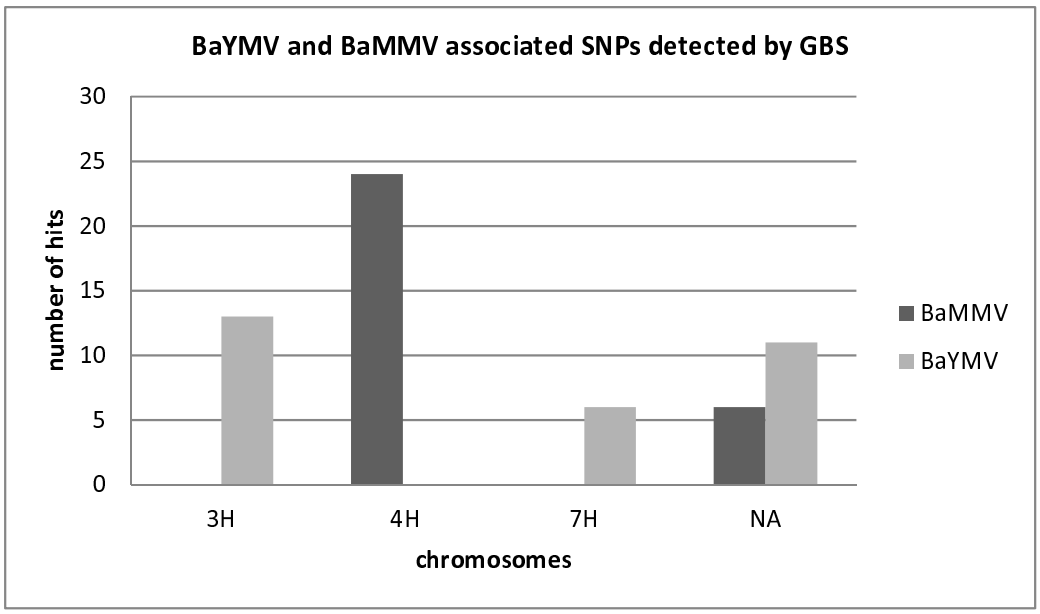

Figure 13: Distribution of BaYMV and BaMMV associated SNPs detected by GBS regarding their chromosomal localization.
(NA= not anchored to any chromosome)

Table 3: Putative candidate genes identified on the basis of GBS.

Name	InterPro number	GO term
NB-ARC	IPR002182	0043531 ADP binding
MYB-like	IPR017877	0003677 DNA binding
HSF	IPR000232	0006355 regulation of transcription, DNA-templated
		0043565 sequence-specific DNA binding; transcription factor activity, sequence-specific DNA binding
WD repeat	IPR001680	0005515 protein binding
PCI	IPR000717	0005515 protein binding
Per1	IPR007217	1990513 CLOCK-BMAL transcription complex
HSP70	IPR013126	0044183 protein binding involved in protein folding
CSN1	IPR033008	0000338 protein deneddylation
		0008180 COP9 signalosome

After subsequent whole-genome-sequencing (WGS) and data analysis, one sequenced BAC (HVVMRXALLMA0364I06; 133.098 bp) was detected spanning the interval between the marker contig_43083 and contig_275255. The BAC HVVMRXALLMA0364I06 consists of 14 genes listed in table 4. Five annotated genes of this BAC, which may be involved in virus

resistance, were used to develop new markers. The genes encode for three BTB/POZ domain containing proteins, one Gag/pol protein and one NB-ARC domain-containing disease resistance protein. The amplified PCR products were sequenced subsequently. For all genes, except the Gag/pol protein encoding gene, SNPs were detected. For one gene encoding the BTB/POZ domain containing protein (c1g1), the SNP was converted into a CAPS marker. The other detected SNPs were converted into KASP markers and mapped subsequently (Figure 11d). In addition, surrounding BACs were searched for candidate resistance genes and after allele-specific sequencing of eight genes (Table 4), seven polymorphic markers were converted to KASP markers and mapped subsequently (Figure 11d).

Table 4: Genes on BAC HVVMRXALLMA0364I06 and surrounding BACs (predicted using AUGUSTUS, version 3.0.3, Stanke et al. 2008, based on BLAST against TAIR and UNIPROT database).

Protein-Accession	Primer name	Blast-Hit-Accession	AHRD-Quality-Code	Human-Readable-Description
HVVMRXALLMA0364I06_C5.g11	-	tr|Q2QRF2|Q2QRF2_ORYSJ	*-*-[4.839]	Transposon protein, putative, CACTA, En/Spm sub-class, expressed
HVVMRXALLMA0364I06_C19.g14	-	tr|E2GK51|E2GK51_BRYDI	*-*-[2.24]	Gag/pol protein
HVVMRXALLMA0364I06_C2.g10	-	tr|Q69LW0|Q69LW0_ORYSJ	*-*-[4]	Myosin heavy chain-like protein
HVVMRXALLMA0364I06_C1.g6	-			Unknown protein
HVVMRXALLMA0364I06_C6.g12	-	tr|Q10HF0|Q10HF0_ORYSJ	***-[4.6]	Retrotransposon protein, putative, Ty1-copia subclass
HVVMRXALLMA0364I06_C1.g4	C1g4	tr|Q337Y9|Q337Y9_ORYSJ	***-[3.377]	BTB/POZ domain containing protein, expressed
HVVMRXALLMA0364I06_C1.g5	-	AT5G52280.1	*-*-[3.823]	Myosin heavy chain-related protein LENGTH=853
HVVMRXALLMA0364I06_C1.g2	C1g2	AT3G07040.1	***-[3.039]	NB-ARC domain-containing disease resistance protein LENGTH=926
HVVMRXALLMA0364I06_C1.g3	C1g3_1; C1g3_2	tr|Q337Z5|Q337Z5_ORYSJ	***-[3.501]	BTB/POZ domain containing protein
HVVMRXALLMA0364I06_C1.g1	C1.g1	tr|Q7XED5|Q7XED5_ORYSJ	***-[3.415]	BTB/POZ domain containing protein
HVVMRXALLMA0364I06_C14.g13	-	tr|Q10HF0|Q10HF0_ORYSJ	***-[4.6]	Retrotransposon protein, putative, Ty1-copia subclass

Table 4: continued.

Protein-Accession	Primer name	Blast-Hit-Accession	AHRD-Quality-Code	Human-Readable-Description
HVVMRXALLMA0364I06_C2.g7	-	tr|Q2QRD8|Q2QRD8_ORYSJ	***-[4.111]	Retrotransposon protein, putative, Ty3-gypsy subclass
HVVMRXALLMA0364I06_C2.g8	-	tr|Q2QNM7|Q2QNM7_ORYSJ	*-*-[4.762]	Retrotransposon protein, putative, Ty3-gypsy subclass
HVVMRXALLMA0364I06_C2.g9	-	tr|Q2R8I2|Q2R8I2_ORYSJ	***-[4.659]	Retrotransposon protein, putative, Ty3-gypsy subclass
HVVMRXALLMA0267H20_C1.g65	-	tr|Q337Z5|Q337Z5_ORYSJ	***-[3.56]	BTB/POZ domain containing protein
HVVMRXALLMA0267H20_C1.g56	-	tr|B9SBE2|B9SBE2_RICCO	***-[1.846]	WD-repeat protein, putative
HVVMRXALLMA0300N16_C2.g52	-	tr|Q10RN8|Q10RN8_ORYSJ	*-*-[4]	Heavy metal-associated domain containing protein, expressed
HVVMRXALLHA0157E04_C1.g73	E04_c1g73	tr|B6SWY8|B6SWY8_MAIZE	***-[2]	ASK20
HVVMRXALLMA0267H20_C1.g56	-	tr|B9SBE2|B9SBE2_RICCO	***-[1.846]	WD-repeat protein, putative
HVVMRXALLHA0157E04_C1.g74	E04_c1g74	tr|B8YPY0|B8YPY0_EICCR	*-*-[3.044]	Plasma membrane H+-ATPase
HVVMRXALLMA0354K09_C1.g89	K09_c1g89	tr|Q10SN5|Q10SN5_ORYSJ	***-[2.977]	Hydrolase, alpha/beta fold family protein, Expressed

2.3.3 High resolution mapping

For the mapping population 'Taihoku A' x 'Plaisant', F_4 plants were tested for their resistance behaviour in field and growth chamber experiments (supplemental data table S2). The segregation for BaYMV infection in field trials was 221 resistant RILs : 206 susceptible RILs (chi² 1r:1s=0.527), indicative for monogenic inheritance of BaYMV/BaYMV-2 resistance. At the same time segregation ratio for BaMMV infection was 38 resistant RILs : 388 susceptible RILs (chi² 1r:1s = 287.56), which indicates a deviating inheritance of BaMMV resistance. Considering the presence of a dominant modifier gene and a fixation of resistant and susceptible genotypes in F_4 because of previous selection, a segregation ratio of 82.5 resistant and 294.5 susceptible RILs would be expected (chi² = 0.0364). Figure 11e shows the combination of phenotypic data (obtained by mechanical BaMMV inoculation in the growth chamber) and genotypic data of this cross. It has to be noticed that there is no even distribution of the developed markers in all maps of Figure 11. There is a gap between the

markers K09_c1g89 and BOPA_766 (Figure 11e). Adding the resistance data to the genotypic data leads to an increasing size of the map because of a set of double recombinants.

2.3.4 Mapping of markers in an independent population

The phenotypic data for the BaMMV resistance reaction of segmental recombinant inbred lines of the validation population 'Hanna' x 'HOR11019' fits to a 1:1 segregation (Chi^2 = 2.13; 8 resistant and 15 susceptible RILs). As shown in Figure 14, the markers are evenly distributed and the size of the partial map is less than half of the size from the original mapping population. In this small population there are seven markers co-segregating with the resistance locus. Five of them were markers from the four candidate genes encoding for BTB/POZ domain containing protein, one Gag/pol protein and one NB-ARC domain-containing disease resistance protein.

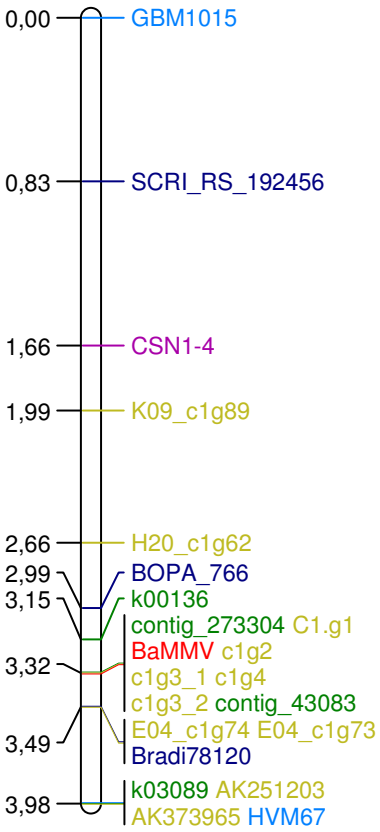

Figure 14: Partial map of chromosome 4HL of the mapping population 'Hanna' x 'HOR11019'. The map is based on analysis of 32 RILs originating from 301 F_2 plants. Different colours of the markers indicate their origin parallel to Figure 10.

2.3.5 Testing the diagnostic value of co-segregating markers

The nearest flanking markers were also tested in a set of 93 different barley genotypes. Differentiating results were obtained for the markers BOPA_766, contig_43083, c1g1 and c1g4. Only the genotypes 'Muju covered 2' and 'MBR530' show the same resistant allele as 'Taihoku A' (Figure 15 and supplemental data table S3), whereas the other genotypes carry the susceptible allele like 'Plaisant'.

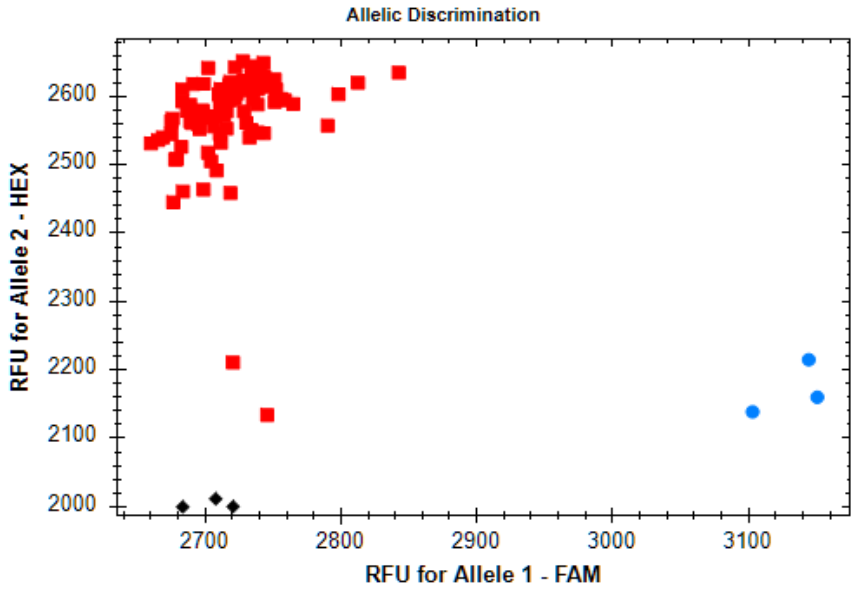

Figure 15: Allele discrimination plot of KASP marker c1g4.

Blue dots represent the susceptible allele derived from 'Plaisant' and orange dots represent the resistant allele from 'Taihoku A'. Black dots represent no-template controls.

2.4 Discussion of mosaic virus resistance

This chapter focuses on high resolution mapping of the resistance gene *rym13*. By analyzing 5,191 F_2 plants, a resolution of 0.0096 % recombination was obtained.

Phenotypic classification of more than 400 RILs was conducted and different segregation ratios for BaMMV (38 resistant and 388 susceptible) and BaYMV/BaYMV-2 (221 resistant and 206 susceptible) indicate an independent inheritance of these resistances in our mapping population. BaYMV/BaYMY-2 is obviously inherited in a monogenic manner (chi² 1r:1s=0.527), whereas one major gene modified by another dominant gene (chi² 1r:1s = 287.56, chi² 82.5 r: 294.5 s = 0.0364) is one explanation of BaMMV resistance.

The different results for phenotypic segregation of BaMMV and BaYMV resistance indicate that at least two independent loci are involved in resistance against these mosaic viruses. The independent inheritance of these two resistances is also reflected in the GBS data (Figure 12). After analysis of 131 RILs with different resistance behaviour, the most promising SNPs for resistance to BaMMV and BaYMV/BaYMV-2 were detected in different chromosomal regions (supplemental data table S4). SNPs closely linked to BaMMV resistance were mapped only on chromosome 4HL in a region of 111 cM (Figure 12). The published flanking markers of *rym13* (GBM1015 and HVM67) were located in the region of 110 cM by using the sequence information and the online tool barleymap (Cantalapiedra et al. 2015). This leads to the conclusion, that the resistance gene *rym13* is only effective against BaMMV whereas the BaYMV resistance of the cultivar 'Taihoku A' is encoded by another resistance gene. SNPs highly correlated to BaYMV resistance were identified on chromosome 3H in the region of the resistance locus *rym4/5* and on chromosome 7H, respectively. In summary, by using this approach it was clearly shown that BaMMV and BaYMV/BaYMV-2 resistances of ´Taihoku A´ are inherited independently.

For marker saturation, the initial flanking markers (GBM1015, HVM67) were mapped in three barley high density maps (Comadran et al. 2012, Sato et al. 2009 and Thiel et al. 2003). All together, 24 new markers could be mapped in the interval of resistance using this approach. This method was also used successfully for fine-mapping of the genes *rym11* (Lüpken et al. 2013), *rym7* (Yang et al. 2013), *Ryd3* (Lüpken et al. 2014) and two QTLs responsible for powdery mildew resistance (Silvar et al. 2012). The use of synthenic information originating from the genome zipper (Mayer et al. 2011) applied in our study also led to fast marker development and saturation of the interval of interest for powdery mildew resistance (Silvar et al. 2013). The information obtained from genomic resources is an efficient and simple tool for marker saturation of any region of interest, which is in accordance with Silvar et al. (2015), who reported of a high accuracy for marker development using genomic resources like the genome zipper (Mayer et al. 2011).

With regard to an equal distribution of markers in the interval of interest, a lack of markers between SCRI_RS_192456 and BOPA_766 was observed (Figure 11c). One reason may be a high recombination frequency in this region. As demonstrated by Kuenzel et al. (2000), regions with different recombination frequency may occur on individual chromosomes.

Regions with different recombination frequencies were also observed in the study of Lüpken et al. (2014), where the the gene *Ryd3* co-segregates with ten markers.

To close this gap and further increase marker saturation, genotyping-by-sequencing (GBS) and whole-genome-sequencing (WGS) experiments as powerful tools for fast marker development were conducted (Mascher et al. 2014). These experiments result in mapping of different candidate genes between the flanking markers. A huge set of SNPs (in this experiment 11,948 SNPs) was detected on the whole genome, and by using the barley reference genome (Mayer et al. 2012) they can easily be ordered by sequence comparison of the GBS data and the sequenced morex contigs. Wendler et al. (2014) and Mascher et al. (2014) demonstrated the possibility to enhance marker assisted breeding by using these next-generation-sequencing methods. Moreover, conducting GBS, WGS and subsequent data analysis revealed interesting candidate genes for the resistance gene *rym13*.

Against barley yellow mosaic disease only the resistance genes *rym4/5* and *rym11* were isolated up to now. *rym4/5* encodes the eukaryotic translation initiation factor 4e (Hv*eIF4e*). Mutations within these factors prevent binding of viral RNA (especially the VPg-protein) to the translation initiation complex and stop the usage of the plant translation machinery for viral reproduction (Robaglia and Caranta 2006; Stein et al. 2005). These susceptibility factors are wide spread within plants (for review see Yeam 2016). *rym11* encodes a protein disulfide isomerase (*HvPDI* 5-1; Yang et al. 2014b). Resistant genotypes show loss-of-function mutations of this protein which catalyses the correct folding of proteins (Wilkinson and Gilbert 2004).

As an interaction partner of *eiF3e*, the *COP9* signalosome encoding gene *CSN* was considered as a potential candidate gene for *rym13*. As reported for Arabidopsis, COP9 regulates the eukaryotic translation initiation factor 3e and has an impact on protein translation (Yahalom et al. 2001, 2008). The *CSN1* gene is located proximal to the resistance locus (Figure 11d), thus it could no longer be considered as the resistance gene after mapping. Screening the genes located in the region of interest, leads to the candidate gene, which is coding for a PCI domain containing protein. Because it was shown that *CSN* and *eiF3e* genes contain this PCI domain (Pick et al. 2009), this gene was considered as a candidate. After remapping of the developed marker, it was not located in the region of the resistance locus (Figure 11d; northern to the marker SCRI_RS_192456). Excluding all these

known resistance mechanisms for potyvirus resistance, gives hint that another new resistance mechanism seems to be responsible for *rym13* mediated resistance in barley. Gene annotation of the BAC spanning the interval between the closest flanking markers contig_273304 and contig_43083, led to genes encoding two different proteins: three genes encoding for BTB/POZ domain containing proteins (c1g1, c1g3 and c1g4) and one NB-ARC domain-containing disease resistance protein (c1g2). BTB had first been described in *Drosophila melanogaster* and it was shown that this zinc finger motif binds to transcriptional regulatory elements (Zollman et al. 1994). In mammals, it was described to have antiviral effects (Sadler et al. 2015). POZ was described by Bardwell and Treisman (1994) as a conserved protein-protein interaction motif. It was shown that NPR1 (Non-Expressor of PR1) contains a BTB/POZ domain and plays a major role in regulatory mechanisms concerning plant defence in *Arabidopsis thaliana* (Shi et al. 2010). Furthermore, it was demonstrated by Zhang et al. (2016) that the expression level of *NPR1* was significantly increased during potyvirus infection as shown for tobacco vein banding virus infected plants and for HC-Pro transgenic tobacco. This study indicates an interaction of potyviral HC-Pro to BTB/POZ domain containing proteins. The second protein family of possible candidate genes are NB-ARC domain containing proteins. These were suggested to interact directly to heat shock proteins, which are supposed to interact to transcription initiation factors like *eIF4G* in rice stripe virus infection (Kim et al. 2012).

The validation of these genes was conducted in two different ways. First, an independent mapping population ‘Hanna’ x ‘HOR11019’ was established to get more detailed information concerning *rym13* mediated BaMMV resistance. This was also conducted because of the distorted segregation ratio for BaMMV resistance in the initial mapping population. The segregation of resistant and susceptible genotypes fits a 1:1 segregation with chi^2=2.13 in this mapping population. Analyzing the first 32 developed RILs, it was possible to confirm the position of the resistance gene *rym13* between the initially flanking markers. Co-segregation of four candidate genes with the BaMMV resistance gene (c1g1, c1g2, c1g3 and c1g4) was detected. Hence, it would be necessary to increase the population size to dissolve the co-segregating markers to trace the candidate gene for resistance.

The nearest flanking markers were tested on a set of 93 barley genotypes to determine their diagnostic value. Genotypes were chosen covering all known BaMMV/BaYMV resistance

genes except *Rym17* and *rym18.* The markers BOPA_766, c1g1, c1g4 and contig_43083 revealed the *rym13* allele for the genotypes 'Taihoku A', 'MBR 530', 'Muju covered 2' and 'HOR11019'. For 'HOR11019' it was shown that its resistance gene is allelic to *rym13* (personal communication Dr. Antje Habekuß). 'MBR530', a Serbian landrace, also seems to be another new source of resistance carrying *rym13.* Resistance concerning BaMMV and BaYMV infection has been shown already in field tests (personal communication Dr. Antje Habekuß). Until now, 'Muju covered 2' is regarded as a source for *rym12*, which also is located in a similar region on chromosome 4HL (Graner et al. 1996). Ordon and Friedt (1993) tested a set of different genotypes for allelism, but the combination of *rym13/rym12* was not included. Our results give first hint, that *rym12* and *rym13* may represent the same gene but different haplotypes like this was shown for *rym1* and *rym11* (Yang et al. 2014a). According to Yang et al. (2014a), *rym13* is not allelic to *rym1* or *rym11*. The respective genotypes 'Mokusekko3', 'W757-112' and 'Russia 57' were also included in our test-set of genotypes and all of them carry the susceptible allele due to the above-mentioned markers.

Summed up, this study shows the development of closely linked markers that provide the possibility for effective marker based selection for *rym13* in barley breeding. Different candidate genes were identified and mapped in the interval of resistance. Subsequently, these candidate genes have to be verified via complementation analysis using CRISP/Cas technique (Sharma et al. 2017) or analysing TILLING populations (Gottwald et al. 2009). As none of the candidate genes corresponds to already known genes encoding resistance, *rym13* may probably code for a new mechanism of resistance. Combining different mechanisms of resistance is a good foundation for broad-spectrum resistance to virus infection in future varieties (Werner et al. 2005). Using the published sequence of barley (Mascher et al. 2017) will further increase the speed of marker development and gene isolation in the future.

3 Anchoring *Ryd3* to the physical map of barley

3.1 Introduction

Besides many other diseases affecting barley, barley yellow dwarf disease, first detected in California in 1951 (Oswald and Houston 1953), is of high economic importance (Lister and Ranieri 1996). Viruses causing barley yellow dwarf belong to the *Luteoviridae* and the genus *Luteovirus*, i.e *Barley yellow dwarf virus (BYDV)* (BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-kerII and BYDV-kerIII) and the genus *Polerovirus,* i.e. *Cereal yellow dwarf virus* (CYDV) with the strains CYDV-RPV and CYDV-RMV). The isolates BYDV-GPV, BYDV-SGV were not assigned to one genus so far (https://talk.ictvonline.org/taxonomy/; 17.04.2018). Names of the different strains are according to their main vector, e.g. CYDV-RPV is transmitted by the aphid species *Rhopalosiphum padi*. At least 25 different aphid species were reported to be vectors for these viruses (Halbert and Voegtlin 1995), whereas *R. padi* and *Sitobion avenae* are the most important vectors in Europe. Consequently, the virus isolates BYDV-PAV and BYDV-MAV are the most important here (Habekuss et al. 2003; Plumb 1996).

Typical symptoms for BYDV infection include stunted growth, yellowing especially of leaf tips and a reduction of yield components resulting in substantial yield losses of up to 80% to 90% (Choudhury et al. 2017; Kosova et al. 2008; Lüpken et al. 2014; Suneson and Ramage 1957). Regarding climate change, higher temperatures in the early vegetation period (Swaminathan 2011) increase the risk of yield losses by BYDV infection. As demonstrated by Thackray et al. (2009) for wheat, early sown stands have the highest risk for infection. For example, in 2002, infection rates of 21% to 45% in winter barley were observed (Habekuss et al. 2003). Treatments with insecticides are an effective way to counteract virus infection indirectly (Habekuss et al. 2003), but because of environmental reasons this is not the treatment of first choice. Breeding for resistant barley cultivars is considered the most environmentally friendly way to prevent yield losses by BYDV infection.

In 1955, the recessive gene *ryd1* was detected (Suneson 1955). Because of its low efficiency this gene was not used in barley breeding programmes (Kosova et al. 2008). Some years later, in 1959, the semi-dominant gene *Ryd2* was detected in Ethiopian barley landraces (Rasmusson and Schaller 1959). This gene was located on chromosome 3H (Collins et al. 1996) and confers tolerance to BYDV-PAV and BYDV-MAV but it is ineffective against CYDV-

RPV (Baltenberger et al. 1987; Niks et al. 2004). Subsequently, *Ryd3* was detected in the Ethiopian landrace L94 by Niks et al. (2004) and located on chromosome 6H. This gene confers tolerance to BYDV-PAV and BADV-MAV. These two genes were intensively used in breeding programmes (Kosova et al. 2008). Riedel et al. (2011) described the effect of pyramiding these genes on virus infection and especially on different yield parameters. DH lines with the combination of *Ryd2* and *Ryd3* revealed quantitative resistance to BYDV-PAV instead of tolerance. The only gene causing qualitative resistance to BYDV, namely *Ryd4*^{Hb}, was introgressed from *Hordeum bulbosum* (Scholz et al. 2009). Besides these major genes, some QTLs were identified on chromosome 2H, 3H, 4H and 7H against BYDV-PAV and on chromosome 1H, 4H and 7H against BYDV-PAV and BYDV-MAV (Scheurer et al. 2001a; Toojinda et al. 2000).

Lüpken et al. (2014) carried out first efforts for high-resolution mapping of *Ryd3*. Analyzing 3,210 F_2 plants, the gene was mapped within an interval of 1.907 cM with a large co-segregating region represented by 10 markers. As supposed by Lüpken et al. (2014), the mapping population was too small to unravel the co-segregating region. By increasing the population size, the aim of this study was (i) to separate the co-segregating markers, (ii) to develop new markers by next-generation-sequencing methods (iii) to identify potential candidate genes using the barley reference genome (Mascher et al. 2017).

3.2 Material and methods

3.2.1 High resolution mapping population

Seeds from crosses of the BYDV-tolerant line L94 and the near isogenic line L94-QTL3, which carries a fragment on chromosome 6H originating from the susceptible Dutch spring barley cultivar 'Vada' (Niks et al. 2004), were used to increase the population size of the mapping population described by Lüpken et al. (2014) to 7,427 F_2 plants. Seeds of the parental lines were kindly provided by R. Niks. Plants were analyzed with the co-dominant flanking markers GBS0655 and GBM1063 in order to identify heterozygous and homozygous recombinant plants. Twelve progenies of heterozygous recombinant plants were sown in F_3 and analyzed with the respective markers in order to identify homozygous recombinants. DNA was isolated following the protocol of Dorokhov and Klocke (1997). The DNA extraction protocol of Stein et al. (2001) was used for extracting high quality DNA for further analysis of the segmental recombinant inbred lines (RILs). All PCRs were carried out following the protocols described by Lüpken et al. (2014). The pyrosequencing marker U35_13709 was converted into a KASP^{TM} marker (Table 5, LGC Genomics GmbH) and amplified using manufacturer's protocol.

3.2.2 Phenotypic tests

Tests for BYDV tolerance of 250 newly developed segmental RILs were carried out in 2015, 2016 and 2017 in gauze house trials in Quedlinburg, Germany (51°46'20.9"N 11°08'47.0"E). The parental lines and each RIL was tested with at least 10 plants in two variants. All F_4 plants were grown to the one-leaf stage in the greenhouse (18-20°C, 16 h photoperiod with 10 klx, 60-80% humidity). The first variant was artificially inoculated with viruliferous *Rhopalosiphum padi* (isolate BYDV-PAV) and the second variant was the healthy control. The aphids were omitted after an inoculation period of five days and at the beginning of April, the plants were transferred to the gauze houses. Visual scoring of symptoms was conducted at heading (BBCH 59-61) with a scale from 1 (tolerant) to 9 (highly susceptible). In parallel, samples for enzyme linked immunosorbent assay (ELISA) were taken. At harvest time, plant height, number of the ears per plant, grain yield and thousand kernel weight was determined. Subsequently, the level of tolerance for each trait was calculated in percent for each RIL relative to the uninfected control plants ((infected/uninfected)*100).

3.2.3 Statistical analysis

For statistical analysis, the RILs were grouped into two groups based on the co-segregating marker GBMS0107. Group A comprises all RILs showing the same allele as the parental line L94 and group B consists of the RILs expressing the allele of the susceptible parent L94-QTL3. Each RIL represents one replication in the respective group and a one-way analysis of variance was perfomed for the phenotypic traits with SAS software v. 9.4. Subsequently, multiple comparison of means (α=0.05) was performed using the Tukey test.

3.2.4 Development of new markers

The existing markers were roughly anchored to the first draft of the physical map (Mayer et al. 2011) using the web resource barleymap (Cantalapiedra et al. 2015). Using this resource, four new primer pairs were developed (Table 5). Using the sequence information of markers located in the resistance harbouring interval, KASP™ markers were designed and KASP™ assays according to manufacturer's protocol (LGC, Teddington, UK) were conducted for mapping.

Table 5: Newly developed markers for *Ryd3* and their sequence information.

Primer name	Primer sequence (forward in case of InDel or CAPS marker; Allele_FAM in case of KASP marker)	Primer sequence (reverse in case of InDel or CAPS marker; Allele_HEX in case of KASP marker)	Primer_common (in case of KASP marker)
SCRI_RS_188305	AAATTCTCGCATTTCTAGTTTCTTCC	ACTAAATTCTCGCATTTCTAGTTTCTTCT	GAGCCGAAACCTTGTGTGATGTCTT
MLOC-18435	ATCTACTAAAGCATAAAGACATGAAAGT	ATCTACTAAAGCATAAAGACATGAAAGC	CTTTTGCAGTTCACCATTACTAGTACTGTT
U35_13709	CTAGGCGCTTCTCATATCCC	ATCTCTAGGCGCTTCTCATATCCT	AATGGCTGTCTCTGCCATTGGGTTT
SCRI_RS_233266	TGCTGTGGACGTTCTCATGA	ACATGAACAAGACAGGCCCT	-

3.2.5 Genotyping-By-Sequencing

A set of 20 BYDV susceptible RILs, 19 BYDV tolerant RILs and the parental genotypes Vada (susceptible) and L94 (tolerant) were chosen for GBS. The normalized DNA (20 ng/µl) was used for pooling and subsequent GBS according to Wendler et al. (2014). Results were obtained by sequencing on an Illumina® MiSeq™ (Illumina, San Diego, USA). Obtained data

were analyzed using the Galaxy platform implemented at the JKI (Blankenberg et al. 2001; Giardine et al. 2005; Goecks et al. 2010). After adapter and quality trimming (trim galore version 0.2.8.1; quality <30, read length >50), read mapping of the GBS data was performed using BWA (Li and Durbin 2009) with standard settings to map the reads to the pseudomolecules of barley (Mascher et al. 2017). Using the program mpileup (Li et al. 2009), with genotype likelihood computation, SNP calling was performed. Imputation of missing data was performed with the program Beagle (Browning and Browning 2016). Biallelic SNPs were detected and subsequently filtered for differences between the tolerant and susceptible parental lines and a minimum coverage of five reads per SNP using the program SnpSift (Cingolani et al. 2012).

3.2.6 Anchoring of markers to the barley reference sequence

All markers were anchored to the physical map of barley (Mascher et al. 2017). The published sequences were blasted using the IPK barley BLAST Server against the sortedChromosomes database. The best hits on chromosome 6H were chosen for further analysis.

3.3 Results

3.3.1 High-resolution mapping and anchoring to the physical map

To increase the resolution of the mapping population, 4,217 F_2 plants were analysed extending the mapping population to in summary 7,424 F_2 plants. Within the new set of plants screened, 343 heterozygous recombinant individuals and 44 homozygous recombinant plants were detected. Out of these 232 newly developed segmental RILs were subsequently screened for BYDV tolerance (Table 6).

All variables, which are associated with tolerance (plant height, grain yield, thousand-kernel-weight, ears per plant and symptom expression), showed high correlation to the *Ryd3* genotype for the marker GBMS0107 ($p<0.001$).

Table 6: Phenotypic data obtained in gauze-house experiments after artificial BYDV-inoculation. Data represent the level of BYDV tolerance in percent for each RIL relative to the uninfected control variant ((infected/uninfected)*100). Different letters indicate significant differences (Tukey test, apha=0.05).

	L94	L94-QTL3	Group A	Group B
Plant height	93.85 a	38.99 c	97.87 a	60.03 b
Grain yield	133.43 a	4.35 b	144.19 a	17.01 b
Thousand-kernel-weight	93.15 a	55.16 b	93,34 a	67.03 b
Ears per plant	150.11 a	11.36 b	161.31 a	32.93 b
Symptom expression	2.0 a	7.75 b	1.79 a	6.68 b
N	5	4	107	125

For each trait analysed, the differences between groups were significant. RILs belonging to group A revealed similar results as the tolerant genotype L94, while group B is similar to the non-tolerant genotype L94-QTL3.

The segregation observed (107 tolerant vs. 125 non-tolerant) fits to the expected 1:1 segregation (Chi^2 = 1.3966). Compared to the uninoculated control plants, *Ryd3* infection leads to 90 % decrease of grain yield, 82 % decrease in numbers of ears per plant, a decreased height of the barley plants of 39 % and a reduction in thousand-kernel-weight of 28 %.

For further high resolution mapping of *Ryd3*, the formerly developed markers by Lüpken et al. (2014) were analysed on the extended population and additionally the four newly developed markers SCRI_RS_188305, SCRI_RS_233266, MLOC-18435 and U_13709 were mapped (Figure 15b). By analyzing the new RILs, some of the initially co-segregating markers were separated by recombinations. The marker Bmag009 was mapped northern of *Ryd3* with 13 recombinations, and the markers U35_16315-345-563, GBMS0135, HVM0014 and Bmac0018 were separated from the resistance locus by one recombination event. Two recombinations were estimated between GMBS0178, U35_02578, DArT_bp-3722, SCRI_RS_233266, MLOC_18435 and *Ryd3*. The only marker still co-segregating with *Ryd3* is GBMS0107 (compare Fig. 16A and 16B).

3.3.2 Genotyping-by-sequencing and assignment to the reference sequence

Genotyping-by-sequencing was conducted to get more marker information for the initial co-segregating interval. A number of 3,759 SNPs differing between the parental lines were generated by a sequencing experiment. Anchoring the SNPs to the reference sequence of barley revealed an unequivocal mapping of the BYDV-tolerance locus on chromosome 6H. The interval harboring the tolerance gene was narrowed down to 120.76 mio bp and harbors a relatively large number of genes (463HC and 857 LC genes), so that candidate gene analysis was not conducted at this time. To shorten the interval, the known markers were mapped in the published reference genome, too. Table 7 and Figure 16c show the mapping results in terms of base pairs. Table 7 represents the best hits within the 6H chromosomal region for each marker with start and end position in base pairs.

Table 7: Anchoring of markers to the published sequence data of barley (Mascher et al. 2017).

Primername	Start	End	Best E-value	Size
GMS006	113625818	113625969	0.083	151
GBS655	144016079	144016554	0.007	475
HVM0022	224501135	224501301	0.083	166
HVM0014_1	208945433	208945453	0.024	20
HVM0014_2	177161918	177161939	0.29	21
Bmag009	313089525	313089696	6.0 E-04	171
U02578_540-712_bp132	324408367	324408889	0.0	522
DArT_bp_3722_bp355	331594757	331595131	0.0	374
HVM0074	350438914	350439101	0.024	187
SCRI_RS_188305	366400251	366401016	0.0	765
MLOC_18435	340303832	340304697	0.0	865
SCRI_RS_233266	338780109	338780919	0.0	810
U35_16315	313920248	313920466	0.002	218
U35_13709	359588660	359588850	0.29	190
WBE201	372102450	372102470	1.0	20
GBM1063	388366287	388366496	0.002	209
Bmac0018	293925223	293925242	0.083	19
HVM0031	152363182	152363199	1.0	17
GBS822	117458695	117460403	0.007	1708
U35_13709	359588650	359588750	3.0 E-43	100
GBS0164	383275578	383276083	2.0 E-04	505
WBE103	383274923	383276083	6.0 E-04	1160
GBMS0107	242161761	242161778	1.0	17
GBMS0135	317369852	317369871	0.083	19
GBMS0178	324202439	324202458	0.083	19

All markers could be anchored to an interval spanning 210.6 mio bp, whereas the co-segregating markers from Lüpken et al. (2014) were located within an interval of 154.4 mio bp.

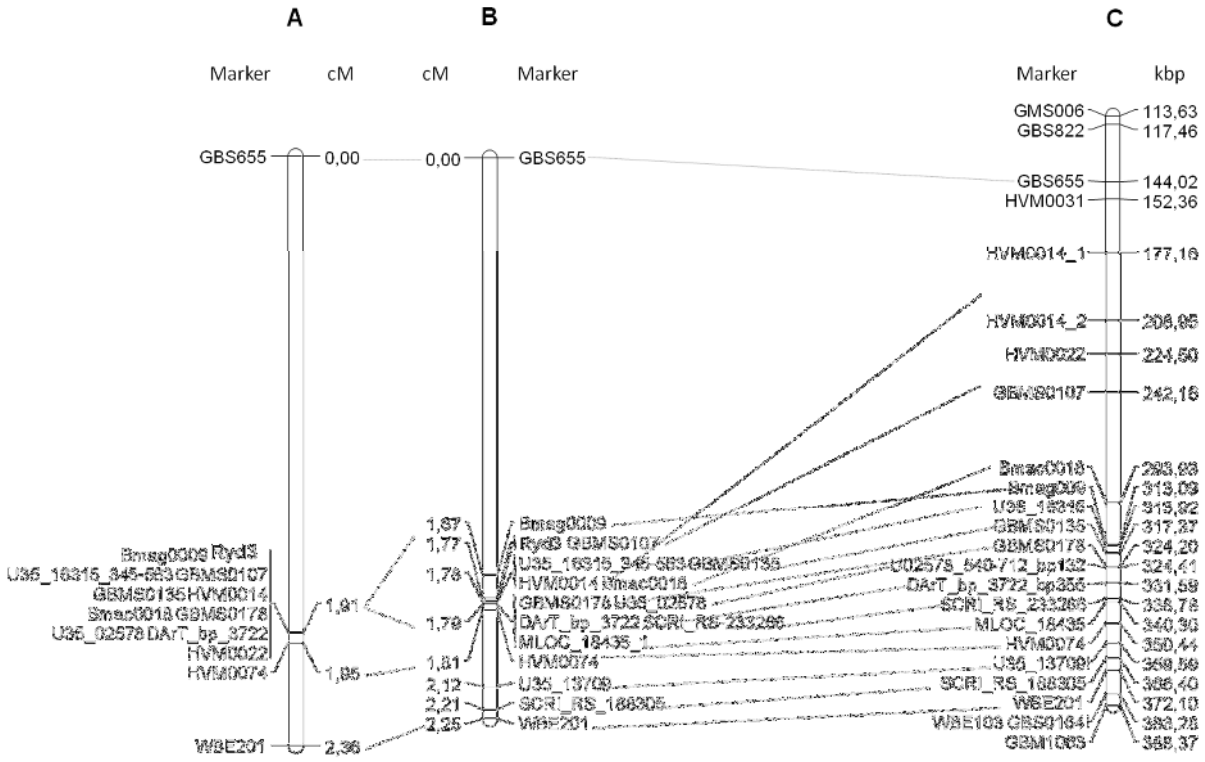

Figure 16: High resolution mapping of the Barley yellow dwarf tolerance gene *Ryd3* and anchoring to the physical map.
A: Previously published map by Lüpken et al. (2014). B: High resolution map obtained by analysing 329 RILs (6310 F_2-plants). C: Anchoring mapped markers to the reference genome of barley (Mascher et al. 2017).

Most of the markers show linear order by comparing the mapping results with the positions on the reference genome. Only the markers Bmag009 and HVM0014 were located on different positions.

Figure 17 shows the anchored marker data in direct comparison to the mapping data of the segmental RILs. The marker order is co-linear with exception of the markers Bmag0009 and HVM0014.

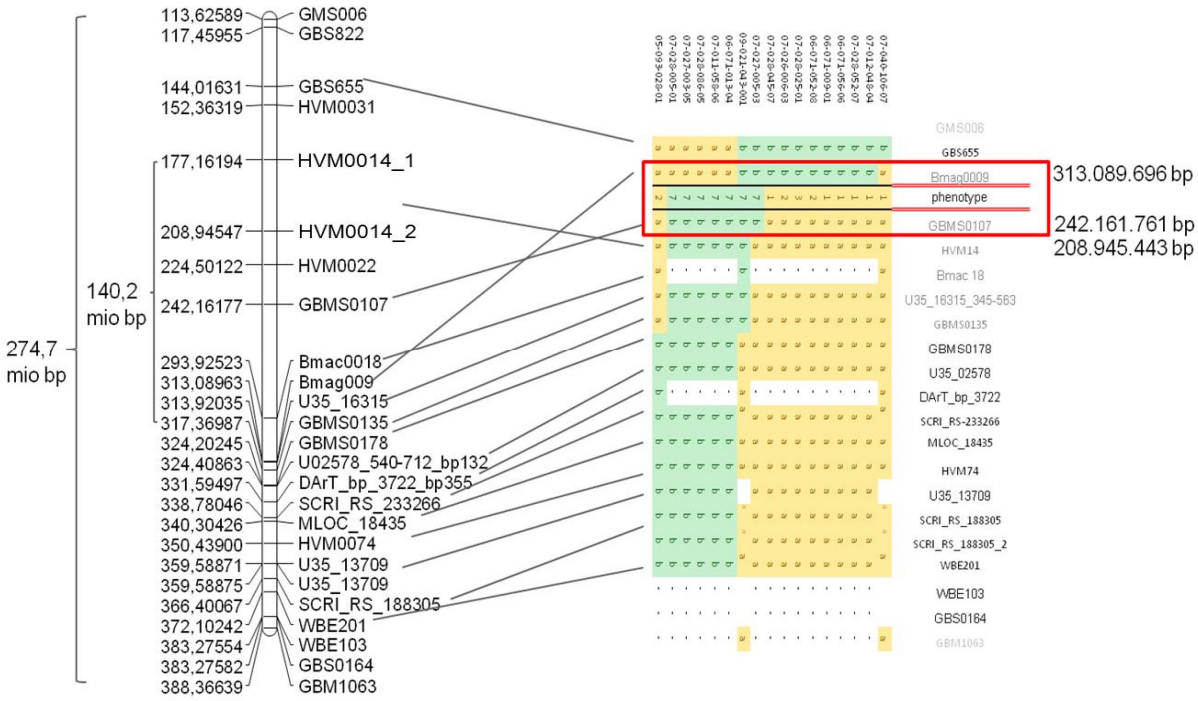

Figure 16: Comparison of markers anchored to the sequence data and the genotypic data from the mapping population L94 x L94-QTL3.

Regarding the genetic mapping, one recombination event was observed between the markers GBMS0107 and HVM14. Thus, the interval harboring *Ryd3*, flanked by the markers Bmag009 and GBMS0107, was shortened to 70.93 mio bp. Due to the inversion, the interval for candidate gene search was enlarged to the marker next to GBMS0107, i.e. HVM014 to 104.1 mio bp. Further analysis of this interval reveals the presence of 387 HC-genes and 768 LC-genes. Regarding the LC genes, 592 are unknown or undescribed genes and 108 are supposed to be retrotransposons or transposable elements. Fifty of the HC-genes are unknown or undescribed genes, whereas the other ones were used for further candidate gene analysis. At position 285,905,500 bp two genes encoding chaperone proteins (DNAJ-related, HORVU6Hr1G047760 and HORVU6Hr1G047770) are located, which are known to be involved in virus resistance (Du et al. 2013, Hofius et al. 2007, Liu and Whitham 2013, Soellick et al. 2000). We conducted allele specific sequencing of the genes HORVU6Hr1G047760 and HORVU6Hr1G047770 on parental lines and five tolerant and susceptible RILs each. The sequencing data did not reveal SNPs in these genes (supplemental data S5 and S6).

3.4 Discussion on BYDV tolerance

The mapping population of Lüpken et al. (2014) was extended to a total number of 7,424 F_2 plants resulting in a resolution of 0.0067 % recombination. By phenotyping newly developed segmental RILs by artificial BYDV inoculation, *Ryd3* was located in the same marker interval as by Lüpken et al. (2014) and the monogenetic inheritance already postulated by Niks et al (2004) was proven. Impacts on agronomic traits of a BYDV infection were in the same range as already reported for the former mapping population (Lüpken et al. 2014). This study shows that BYDV infection can lead to yield losses of up to 90%. Jarošová et al. (2016) reported yield losses of 5 to 80 % depending on the cultivar, which are in the same range. The slightly higher yield losses in this study may be due to the artificial inoculation.

Using sequence information originating from the online tool barleymap (Cantalapiedra et al. 2015) and the first draft of the barley sequence (Mayer et al. 2011, 2012) new markers were developed for the interval. Because the number of co-segregating markers was very high and no marker interval was present at the beginning of the mapping process, it was difficult to develop new markers at the appropriate position. Contrary to the demonstration of the possibilities using these resources by Silvar et al. (2015) and Färber et al. (in preparation), our newly developed markers did not map in the co-segregating region (Figure 16 b and c). As shown by Kuenzel et al. (2000) each chromosome consists of regions with different recombination frequency, the resistance gene *Ryd3* seems to be located in an area of low recombination complicating the mapping process. This is also supported by the fact, that a high resolution mapping population of 3,200 F_2 plants was not sufficient to dissolve the co-segregating region (Lüpken et al. 2014). Screening about 5,000 F_2 plants leads to sufficient separation of markers for candidate gene analysis in former publications to isolate different resistance genes in barley (Büschges et al. 1997, Lüpken et al. 2013, Stein et al. 2005, Yang et al. 2014, Färber et al. in preparation). As already shown for *vrs1*, increasing the mapping population to 9,800 gametes leads to the successful isolation of the gene responsible for the six-rowed phenotype of barley (Komatsuda et al. 2007). By genotyping and phenotyping all newly developed RILs, we were able to dissolve the co-segregating region. Genotyping-by-sequencing as a next generation sequencing technique in combination with a reference genome is a very powerful tool for gene isolation as shown in recent publications (e.g. Jost et al. 2016, Mascher et al. 2014). Regarding the *Ryd3* locus, applying this technique leads to

further delimitation of the interval of interest. We were able to anchor the SNPs linked to *Ryd3* to the reference genome (Mascher et al. 2017) and narrow the chromosomal region of chromosome 6H harbouring *Ryd3* down to 120.76 mio bp.

After anchoring all mapped markers to the reference sequence, they show co-linearity in the high resolution map compared to the sequenced data, except the markers flanking *Ryd3*, i.e. Bmag009 and HVM014, which are inverted. By identifying one recombination event between the markers GBMS0107 and HVM014, the co-segregating interval was shortened to 70.93 mio bp. Due to the inversion, the interval for candidate gene search was enlarged to the marker next to GBMS0107, i.e. HVM014. This interval comprises 104.14 mio bp and harbors 387 HC-genes and 768 LC-genes. Regarding only the characterized genes, a large number of genes (405) with a wide range of biological functions (starting from ATP-transport proteins to kinases and photosystem dependent proteins) are located in this interval.

Yang et al. (2014) described a chaperone as susceptibility factor to BaMMV/BaYMV. Within the interval of interest for *Ryd3,* chaperones, e.g. heat shock protein (HSP) 40, also named DNAJ protein because of a conserved DNAJ-domain (Zhong et al. 2018), are located. Genes encoding this protein are co-segregating with *Ryd3*. Several publications reported about increased tolerance to biotic stress (Lee et al. 2018; Wang et al. 2014), induced hypersensitive response (Liu and Whitham 2013) or increased resistance to fungal pathogens (Wang et al. 2014; Zhong et al. 2018) by DNAJ like proteins. Regarding viral diseases, this protein is also of interest. Liu and Whitham (2013) showed that susceptibility to *Soybean mosaic virus* (SMV) was increased by silencing this gene. During virus infection in tobacco, type I DNAJ proteins functions as a co-chaperone (Du et al. 2013). For *Potato Virus Y* (PVY) infection DNAJ seems to be essential as it is associated to the capsid protein and represents a susceptibility factor that is probably recruiting HSP70 for viral assembly or cellular spread of the virus (Hofius et al. 2007). Soellick et al. (2000) showed that the movement protein of *Tomato spotted wilt virus* (TSWV) also recruits DNAJ like proteins for intercellular transport of the virus. These results indicate that the DNA-J like protein encoding genes represent candidates for *Ryd3.* As two of these genes can be found in the interval of interest (HORVU6Hr1G047760 and HORVU6Hr1G047770), allele specific sequencing was conducted. However, no SNPs differentiating between tolerant and non-

tolerant lines within the genes HORVU6Hr1G047760 and HORVU6Hr1G047770 were detected, excluding the possibility that SNPs of these genes are involved in the *Ryd3* encoded BYDV tolerance. Further experiments concerning differences in gene expression should be conducted to exclude a differential expression pattern involved in *Ryd3* encoded BYDV tolerance.

Translation initiation factors were also identified in this interval. Former publications show that these genes are quite frequently involved in virus resistance of plants (Le Gall et al. 2011; Robaglia and Caranta 2006; Stein et al. 2005). Banerjee et al. (2012) reported that the translation initiation factor eiF4F binds to the 3‘ UTR of BYDV, which directly affects the protein synthesis efficiency. Some recent results also revealed interaction of BYDV with different translation initiation factors for cap-independent translation (Powell et al. 2018). This mechanism is used to translate mRNAs lacking the 5’ 7-methylguanosine cap, as it is the case for BYDV. The 3’ BTE-element binds to a sequence on the 5’ end of the untranslated region of BYDV-RNA. This formation recruits eIF4F and eIF3 and starts the translation mechanism (Ali et al. 2014). In the interval of *Ryd3*, a translation initiation factor IF-1 was detected (HORVU6Hr1G049440). Also a gene encoding a GTP binding elongation factor Tu family protein can be found in the interval (HORVU6Hr1G0501). Using information from the InterPro database (http://www.ebi.ac.uk/interpro/), this protein is also named EF1A. The elongation factor 1A *(eEF1A)* also binds to BYDV as shown by Gustilo and Goss (2014).

Additionally, the eukaryotic translation initiation factor 4B1 (*eIF4B1*) is located in the region of the *Ryd3* locus (HORVU6Hr1G045130). Powell et al. (2018) reported about binding of ATP-bound *eiF4B* to the BTE-element to recruit the 40S ribosomal subunit for translation initiation.

Because of the huge physical size of the interval, also additional candidate genes were identified. For example bZIP transformation factors (HORVU6Hr1G051110), a eukaryotic translation initiation factor SUI1 family protein (HORVU6Hr1G042320), maturase k (HORVU6Hr1G046830) or mediator subunit 8 (HORVU6Hr1G043270) can be found in the interval. Yoon and Donahue (1992) postulated, that the suppressor of initiator codon mutations (*Sui*) in yeast may interact with *eiF1* or *eiF1-A*. Mediator 8 (Med8) was demonstrated to act as a transcription regulator for plant immunity. Li et al. (2018) showed that Med8 of *Arabidopsis thaliana* regulates plant immunity to *Botrytis cinerea* through

interaction with the transcription factor FAMA. Efforts to unravel the responses of mungbean (*Vigna radiata*) to *Mungbean yellow mosaic virus* (MYMV) infection were carried out using 2D-protein expression analysis by Cayalvizhi et al. (2015). They assumed maturase k to be strongly involved in resistance mechanism. Therefore, it is necessary to have a deeper look at this interval.

In summary, this chapter shows the possibilities of next generation sequencing techniques in combination with the barley reference genome to identify possible candidate genes. For further identification and verification, a TILLING (targeting-induced local lesions in genomes) population was established applying 40 mM EMS (ethyl methansulfonate) to 10,000 kernels of the parental line Vada-qtl3 as described by Gottwald et al. (2009). The M1 seeds were harvested and are available for further investigations concerning *Ryd3*-tolerance. Using the TILLING technique, several genes were identified and verified (Gauffier et al. 2016; Gottwald et al. 2009; Lababidi et al. 2009, Yang et al. 2014).
Using the information described above and the TILLING population may lead to the identification of a candidate gene for *Ryd3*.

4 Final joint discussion

Regarding virus resistance in barley, the only genes isolated until now are *rym4/5* and *rym11* (Stein et al. 2005; Yang et al. 2014b). Hence, this thesis targets the identification of candidate genes for the BaMMV/BaYMV resistance gene *rym13* and the BYDV tolerance gene *Ryd3*.

The development of co-segregating markers and detailed knowledge on the mechanism of action of these genes is a prerequisite for their targeted use in barley breeding and the pyramiding of genes with a different mode of action maybe resulting in longer lasting resistance.

4.1 Segregation of resistance/tolerance vs. susceptibility

Phenotyping with regard to *rym13* was conducted in field trials for BaMMV and BaYMV-2 resistance/susceptibility by natural infection and in growth chamber experiments for BaMMV resistance by mechanical inoculation.

In the growth chamber, 377 segmental RILs were inoculated mechanically with BaMMV and tested for resistance by DAS-ELISA. A total of 84 RILs turned out to be resistant and 293 susceptible. Therefore, the hypothesis of a 1r:1s segregation can obviously be rejected (chi^2= 115.86). By considering a dominant modifying factor and a fixation of the resistance/susceptibility in F_4 by marker based selection, a segregation ratio from 82.5 resistant and 294.5 susceptible RILs would be expected. The observed segregation perfectly fits this hypothesis with a chi^2 value of 0.0364.

Modifier genes were already reported in different other species. In a review of modifier genes and protective alleles in humans and mice Nadeau (2003) reported about tubby mutant mice showing variation in susceptibility to hearing loss. Subsequently, a modifier of tubby hearing (moth1) was identified by positional cloning. Protective alleles are a class of modifiers that can suppress disease in otherwise susceptible individuals. This would result in more resistant than susceptible RILs and thus these protective alleles can be excluded. In plants, the hypersensitive response following *Cucumber mosaic virus* (CMV) infection in *Arabidopsis thaliana* seems to be influenced by a modifier gene as an odd segregation ratio was observed, which can be explained by a recessive modifier gene that only modulates the hypersensitive response in heterozygous plants (Takahashi et al. 1994a). Swiezynski et al.

(1981) reported that the resistance to *Potato virus M* (PVM) was inherited by one major dominant gene which is modified by at least one other gene in *Solanum gourlayi*. In wheat, the combination of different leaf rust resistance genes resulted in a higher resistance than for the single genes alone (German and Kolmer 1992).

Field tests for the resistance reaction were conducted at two different locations in Lower Saxony (Germany). BaYMV-2 resistance results in Bornum showed 226 resistant and 205 susceptible RILs, whereas in Schladen 230 resistant and 218 susceptible RILs were observed. Although these results are quite similar, 20 RILs (4.6%) were observed showing a different reaction at the two locations. This may be due to uneven distribution of virus particles in the fields. As there is no difference in the number of differentiating genotypes regarding both locations, the occurence of different virus strains may not be the reason for these events. Regarding RILs with identical resistance reaction, the segregation for BaYMV-2 infection on both fields was 221 resistant: 208 susceptible RILs. This segregation fits a 1r:1s segregation (Chi^2= 0.527).

With respect to BaMMV, a larger deviation between the two locations was observed. The latest monitoring for BaYMV/BaMMV in Germany was conducted in 2009/2010 (Schäfer et al. 2010) and reveals the continuous spread of BaYMV-2 in this country. Even the occurrence of new strains has to be considered because of the co-evolution of viruses and host plants (Charron et al. 2008; Moury et al. 2014; Wu et al. 2015) and the fact that both locations have been used for virus testing since decades. As shown in the literature, the change of one amino acid in the central region of the VPg protein (aa 1307 from lysine to asparagine or histidine) is sufficient to break the *rym4*-mediated resistance (Kuehne et al. 2003). Considering again only clear phenotypes, a segregation of 38 resistant and 388 susceptible RILs is recorded. The chi^2 value for a 1r:1s segregation is chi^2=287.56 in this case so that the hypothesis of monogenic resistance has to be rejected. Regarding the hypothesis of a dominant modifier gene as assumed in the case of the greenhouse experiments, a chi^2-value of 41.83 was calculated resulting also in a rejection of this hypothesis. Because of this, the resistance data from artificial inoculation in the greenhouse were used for mapping the BaMMV resistance gene *rym13*. All resistance data are displayed in supplemental data for chapter 2 (Supplemental table S2).

Considering the entire phenotypic data and the different segregation ratios, an independent inheritance of BaMMV and BaYMV-2 resistance in the cultivar 'Taihoku A' can be assumed.
For high resolution mapping of *Ryd3,* the phenotypes of 250 newly developed RILs were estimated in gauze house experiments. A clear 1:1 segregation of BYDV-tolerant and non-tolerant RILs (chi^2 = 1.296) was observed. This finding confirms the results of Niks et al. (2014) and Lüpken et al. (2014).

4.2 High resolution mapping of the resistance/tolerance genes

Humbroich et al. (2010) mapped the gene *rym13* to the telomeric region of chromosome 4HL. For further fine mapping the map-based cloning approach was used and a high resolution mapping population containing 5,192 F_2 plants was established. This mapping population is able to dissolve genetic distances to 0.0096 % recombination.
To conduct marker saturation of the *rym13* harbouring interval, the markers GBM1015 and HVM67 were mapped to three already published high density maps (Comadran et al. 2012, Sato et al. 2009 and Thiel et al. 2003). As shown in figure 11, 24 new markers were mapped in the interval using this approach. The use of publicly available high density maps is a useful method as already shown for fine-mapping of the resistance genes *rym11* (Lüpken et al. 2013), *rym7* (Yang et al. 2013), *Ryd3* (Lüpken et al. 2014) as well as two QTLs responsible for powdery mildew resistance (Silvar et al. 2012). Using syntenic information to other grass species, provided by the genome zipper (Mayer et al. 2011) facilitated precise marker development in the interval of interest. As also demonstrated by Silvar et al. (2015, 2013) and Poursarebani et al. (2013), the use of the genome zipper is a powerful tool due to the syntenic relationship of the grass species (Gaut 2002). Regarding the distribution of the mapped markers, a gap in the *rym13* map between the markers SCRI_RS_192456 and BOPA_766 was observed (Figure 11c). Kuenzel et al. (2000) showed that regions with differing recombination frequencies occur on each single chromosome. This gap may indicate such a block of low recombination.
To close this gap and for further marker development, genotyping-by-sequencing (GBS) and whole-genome-sequencing (WGS) was conducted. These techniques are powerful tools for marker development. Analyzing 131 RILs with distinct resistance reactions, 11,948 SNPs

were detected covering the whole genome in our GBS-experiment. After remapping to the physical map of barley they were ordered according to their position. By searching for the best hits concerning the resistance reactions for BaYMV and BaMMV, the hypothesis of an independent inheritance and localization of the resistance genes was confirmed. Most reliable SNPs for BaYMV-2 resistance were located on chromosomes 3H and 7H. The SNPs mapped on 3H were found in the chromosomal region of the *rym4/5* locus. This locus encodes for the translation initiation factor 4E (Stein et al. 2005). For better understanding of the BaYMV-2 resistance in the population derived from the cross with 'Taihoku A', it is necessary to have a deeper look at the other SNPs derived by GBS.

For BaMMV resistance, all relevant SNPs were found on chromosome 4H in a distinct region around 111 cM. Contrary to the data for BaYMV-2 resistance, the BaMMV resistance gene was detected in a defined region, due to the selection in the F_2 generation for the particular interval between flanking markers located in this region. Using sequence information from barley (Mayer et al. 2012) in combination with the online tool barleymap (Cantalapiedra et al. 2015), the flanking markers GBM1015 and HVM67 were mapped in a region of 110-112 cM. SNPs detected after GBS were located in this distinct area, which confirms that GBS is a very useful and reliable tool for further marker development. The work of Wendler et al. (2014) and Mascher et al. (2014) also showed the power of next-generation-sequencing methods.

For marker development within the initial interval of *Ryd3* mediated BYDV tolerance, the genetic resources of the online tool barleymap (Cantalapiedra et al. 2015) and anchoring the markers to the first draft of the barley sequence (Mayer et al. 2012) were not efficient because of the high number of co-segregating markers and due to the fact that *Ryd3* is located in an area of low recombination. This is in contrast to the successful marker development for *rym13* or the powdery mildew resistance QTL on chromosome 7 (Silvar et al. 2015) where several markers could be developed within the interval of interest. Analyzing the GBS data and subsequent anchoring of all markers to the reference sequence of barley (Mascher et al. 2017) reveals a fast and precise determination of the target interval but a high number of candidate genes. Due to the localization of *Ryd3* in a region with suppressed recombination (14-60 Mb per cM, Kuenzel et al. 2000), it will be challenging to further detect the respective candidate gene.

4.3 Candidate genes for virus resistance or tolerance

4.3.1 Known resistance genes against viral diseases in plants

In barley, the resistance mechanisms for *rym4/5* and *rym11* have already been isolated and functionally characterized. The *rym4/5* locus encodes the eukaryotic translation initiation factor 4E (Stein et al. 2005), and the *rym11* gene encodes a protein disulfide isomerase like gene (*HvPDIL5-1*; Yang et al. 2014b). Mutations within the translation initiation factor prevent binding of the viral VPg-protein to the translation initiation complex and translation will not be initiated (Kuehne et al. 2003). Variation in the sequence of the disulfide isomerase-like protein is supposed to be essential for virus establishment and/or replication in barley (Yang et al. 2014). No eukaryotic translation initiation factors and protein disulphide isomerase like genes can be found in the region of *rym13*, which indicates a new resistance mechanism of this resistance gene. As a regulator of the eukaryotic translation initiation factor 3e, COP9 signalosome (CSN) was reported in Arabidopsis (Yahalom et al. 2001, 2008). A gene coding for the CSN1 subunit of this protein can be found in the region of *rym13*. After mapping to the RIL-population, the *CSN1* gene was located proximal to the resistance locus and for this reason, it is not considered as a candidate gene anymore. Another interesting gene in the *rym13* harbouring interval is the gene encoding for a PCI domain containing protein. Pick et al. (2009) showed that *CSN* and *eIF4e* genes carry this domain. After mapping, *PCI* was located proximal to *rym13*. Excluding these genes as candidates leads to the conclusion that another undescribed mechanism must be responsible for the recessive virus resistance encoded by *rym13*.

Regarding *Ryd3*, cap-independent translation is required because the mRNA of BYDV lacks the 5'-7-methylguanosine cap. The viral 3'BTE-element binds to parts of the 5' end untranslated region of the RNA. This loop formation recruits eiF4F and eiF3, which results in translational start (Ali et al. 2014). Powell et al. (2018) demonstrated the binding of ATP-bound eiF4B to the BTE-element of BYDV to recruit the 40S ribosomal subunit for translation initiation. This mechanism is of great interest as the translation initiation factor 4B1 (*eiF4B1*) is located in this region (HORVU6Hr1G045130). To investigate if this gene is a potential candidate, allele specific sequencing may give a first hint. As several translation initiation (*IF-1*, HORVU6Hr1G049440; *eIF4B1,* HORVU6Hr1G045130; SUI1 family protein,

HORVU6Hr1G042320) or elongation factors (e.g. elongation factor Tu family protein, HORVU6Hr1G0501) can be found in the interval of *Ryd3*, a resistance to BYDV and translation initiation may be connected. This is supported by the studies of Gustilo and Goss (2014) and Banerjee and Goss (2014), who reported the binding of eiF4F to the 3' UTR of BYDV and binding of eF1A to BYDV.

4.3.2 Newly identified candidate genes

By analysing BACs harbouring *rym13* flanking markers, we were able to find one BAC (HVVMRXALLMA0364I06), which comprises the two segments contig_273304 and contig_43083. This BAC consists of 14 genes, whereof genes encoding for two different proteins were chosen for allele specific sequencing. The genes encode for three BTB/POZ domain containing proteins (c1g1, c1g3 and c1g4) and one for a NB-ARC domain containing protein (c1g2). The genes coding for these proteins can be considered as candidate genes because of their biological function and will therefore be analysed in detail in additional studies.

4.3.2.1 The BTB/POZ domain containing protein

The BTB (Broad-Complex, Tramtrack and Bric a brac) domain was first described in Drosophila (Zollman et al. 1994). The crystal structure of the BTB domain from human promyelocytic leukemia zinc finger (PLZF) was determined by Ahmad et al. (1998). This conserved protein-protein interaction domain shows a zinc finger motif, which binds on transcriptional regulatory elements. This protein self-associates into a dimer and consists of a cluster of α-helices and short β-sheets at the top and bottom of the molecule (Figure 18). The protein also shows a potential ligand binding region.

In mammals the deletion of the BTB domain from the Myc interacting zinc finger protein 1 (M1Z1) leads to hyperinflammatory response and limits the expression of inflammatory gene products (Sadler et al.). The POZ domain also is reported to be a protein-protein interaction motif (Bardwell and Treisman 1994). In plants this domain was found in NPR1 (Non-repressor of PR1) and plays a role in regulatory mechanisms of defence to *Pseudomonas syringae* in *Arabidopsis thaliana* (Shi et al. 2010). Constitutive expression leads to 100 fold less bacterial growth on leaf assays. *NPR1* encodes a transcription co-activator and interacts with transcription factors to increase the expression of plant defence

genes. It is reported, that the potyviral HC-Pro binds to ds-RNA as a widely used strategy to suppress gene silencing as a defence reaction in plants (Lakatos et al. 2006; Mérai et al. 2006). Yang et al. (2016) showed an activation of expression of *NPR1* in *Tobacco vein banding mosaic virus* (TVBMV) infected plants. In addition, HC-Pro transgenic tobacco plants showed this increase. These findings suggest a connection of potyviral HC-Pro and the BTB/POZ domain within NPR1.

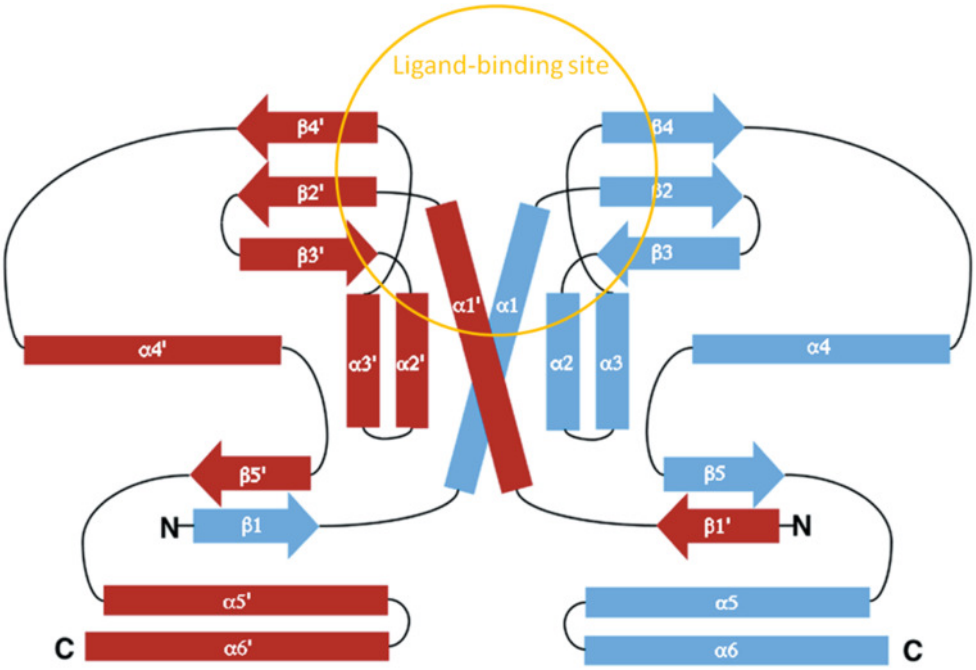

Figure 17: Structure of BTB – domain dimer of PLZF (modified after Ahmad et al. 1998).

4.3.2.2 The NB-ARC domain containing protein

The second protein family chosen as candidate for *rym13* are NB-ARC domain containing proteins. These proteins show a signal motif, which is shared by regulators of cell death and plant resistance genes (R-genes). The protein consists of a nucleotide binding (NB) and two ARC subdomains (van Ooijen et al. 2008). ARC is the abbreviation for APAF-1 (apoptotic protease-activating factor-1), R proteins and CED-4 (*Caenorhabditis elegans* death 4-protein), because in these proteins the domain was detected. The NB-ARC domain is a functional ATPase domain, which supports protein-protein interactions (van der Biezen and

Jones 1998). Figure 19 shows the computed structure of the NB-ARC domain of the resistance protein I-2.

In rice stripe virus infection, NB-ARC proteins were suggested to interact directly with heat shock proteins which are supposed to interact to transcription initiation factors like eIF4G (Kim et al. 2012).

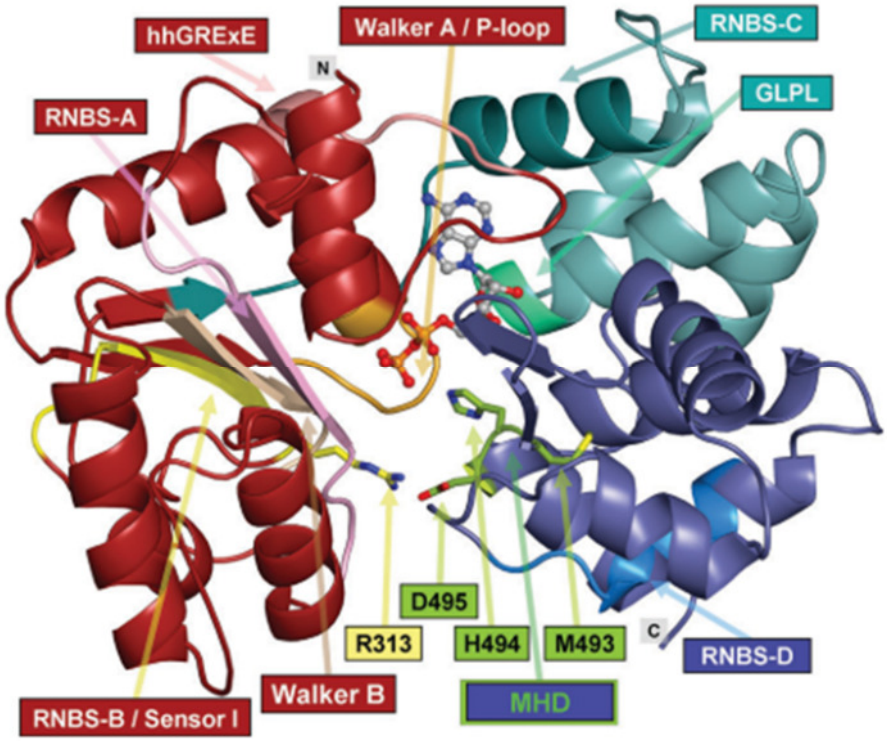

Figure 18: Computed structure oft he NB-ARC domain of I-2 (van Ooijen et al. 2008).

4.3.2.3 DNAJ-like protein

The interval of interest concerning *Ryd3* harbours a number of chaperone-like proteins. As Yang et al. (2014) described chaperones as susceptibility factors to plant viruses, especially BaMMV/BaYMV, this gene family has to be considered as candidate for BYDV as well (Verchot 2012). The chaperone HSP40 shows a conserved DNAJ-domain (Zhong et al. 2018). Two DNAJ-like proteins were detected in the *Ryd3* harbouring interval. This gene family can increase tolerance to abiotic stress as well as resistance to fungal pathogens (Lee et al. 2018, Liu and Whitham 2013, Wang et al. 2014, Zhong et al. 2018). Liu and Whitham (2013) demonstrated increased susceptibility of soybean plants to *Soybean mosaic virus* after

silencing HSP40. During virus infection in tobacco, type I DNAJ proteins function as co-chaperones (Du et al. 2013). The DNAJ protein seems to be essential for *Potato virus Y* (PVY) infection as it is associated to the capsid protein. It represents a susceptibility factor, which probably recruits HSP70 for viral assembly or cellular spread of the virus (Hofius et al. 2007). Intercellular transport of the virus is facilitated by the recruitment of DNAJ like proteins by movement proteins of *Tomato spotted wilt virus* (TSWV; Soellick et al. 2000). As this indicates the possibility for the DNAJ-like protein to represent a candidate for the *Ryd3* tolerance gene, primers were developed for allele specific sequencing. However, sequencing of susceptible versus tolerant RILs and parental lines did not reveal SNPs within the particular genes HORVU6Hr1G04776 and HORVU6Hr1G047770 (supplemental data S5 and S6). This result rules out these genes as candidates for *Ryd3*.

4.4 Outlook

Sequencing of the model plant *Arabidopsis thaliana* in 2000 (The Arabidopsis Genome Initiative 2000) marked the starting point to sequencing of genomes of many different plants like rice (Goff et al. 2002), poplar (Tuskan et al. 2006), sorghum (Paterson et al. 2009) and many more (Published plant genomes). Figure 19 shows the increasing number of recently sequenced genomes. In the end of 2018, nearly 360 genomes have been sequenced and are publically available. This is very important since the knowledge of reference sequences is generally very helpful for gene isolation.

By publishing the first draft sequence of barley by Mayer et al. (2012), the possibilities for gene isolation in this plant were tremendously improved. The parallel decreasing costs for sequencing lead to an extended use of new sequencing technologies like GBS (Poland and Rife 2012). By mapping new sequencing data to the existing physical map (POPSEQ-map of barley, Mascher et al. 2013), different genes in barley could be mapped and isolated (*lax-a*, Jost et al. 2016; *mnd*, Mascher et al. 2014). In this thesis, next-generation-sequencing methods were used in combination with available sequence information to identify candidate genes.

In the beginning of 2017, the high quality reference genome of the barley cultivar ‘Morex’ was published (Mascher et al. 2017). By mapping flanking markers and the candidate genes

for the resistance gene *rym13* to a distinct region, as already indicated by Milner et al. (2018) it is possible to determine the sequence in between and to annotate genes. Next step is allele specific sequencing of the genes on resistant and susceptible RILs of the populations 'Taihoku A' x 'Plaisant' and 'Hanna' x 'HOR11019'. Unraveling insertions or deletions leading to frameshifts or SNPs that confer changes in the amino acid sequence of the candidates would be the best case. As already shown for the *rym4/5* locus, a single SNP can confer resistance to yellow mosaic disease, and on the other hand, a single SNP can also help the virus to break down the resistance as also shown for the very same locus (Kuehne et al. 2003).

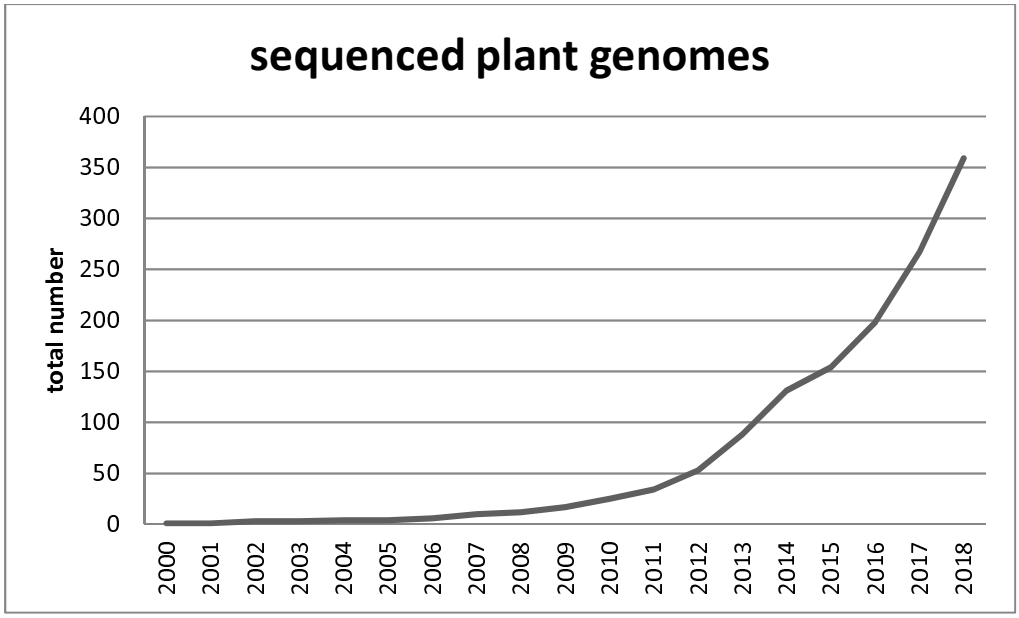

Figure 19: Graphical overview over the total number of sequenced plant genomes over the last years until the end of 2018 (http://www.plabipd.de).

Subsequently, the candidate genes have to be functionally validated. To achieve this, different ways can be followed. Analyzing TILLING populations is one of them. With this approach, the effects of point-mutations or deletions etc. in candidate genes can be analysed. To build up TILLING populations, seeds are treated with a mutagen, like ethyl metanesulphonate (EMS), to produce point mutations or chromosomal rearrangements by double strand breaks to induce random mutations (Taheri et al. 2017). For barley, different TILLING populations are available. Gottwald et al. (2009) developed a population comprising

10,279 M2 plants by applying EMS to the spring barley cultivar 'Barke'. This method was successfully used to identify different alleles of genes and to validate candidate genes in different publications, e.g. Gottwald et al. 2009 (HvHOX1), Yang et al. 2014b (HvPDI5-1), Lababidi et al. 2009 (Dhn), Mendiondo et al. 2016 (PROTEOLYSIS6), Stolarek et al. 2015a (HvPARP3), Stolarek et al. 2015b (HvKu80). Regarding *Ryd3*, we have established a mutated population for enhanced candidate gene identification. Since the tolerant line Vada-qtl3 was chosen for mutagenesis, selection of susceptible descendants in the next generation would be of interest. M1 seeds were harvested and are available for further analysis.

Ways to confirm distinct genes as candidates include complementation analysis or using genome editing techniques like ZFN, TALENs or CRISPR/Cas9 (see below). The genes *rym4/5* and *rym11* were confirmed by complementation. To induce susceptibility, full-length cDNA and gDNA constructs were transformed via *Agrobacterium tumefaciens* (Hensel et al. 2009) into resistant barley genotypes (Stein et al. 2005, Yang et al. 2014).

The named genome editing techniques use sequence specific nucleases which act as molecular scissors by inducing double strand breaks to the DNA. These breaks can be repaired by the cell in two ways: first, with non-homologous end joining, which is mostly imprecise and leads to deletions and mostly to a disrupted gene sequence. Repairing can also be achieved by homologous recombination, whereby gene targeting can be performed (Sprink et al. 2015). Zinc finger nucleases (ZFN) combine the specific DNA binding domain of a zinc finger with an endonuclease (FokI). Here 18 or 24 bases of a specific target sequence are recognized. This system has some disadvantages such as off-target effects, time-consuming screening and the procedure is sometimes toxic for the host cell (Sharma et al. 2017). Transcription activator-like effector nucleases (TALENs) combine the catalytic domain of an endonuclease (mostly FokI) with the DNA binding domain from transcription activator like effectors from *Xanthomonas spec* (Boch et al. 2009).

This method also has disadvantages like the high costs of designing and developing DNA-binding proteins (Sharma et al. 2017). Today the most widely used technique for precise genome editing is the clustered regularly interspaced short palindromic repeats technique in combination with a Cas9 nuclease (CRISPR/Cas). Different types of CRISPR/Cas system exist, but genome engineering is based on type II, which uses one single protein for RNA-mediated DNA recognition and cleavage. Using this method allows gene knockout, replacement and

multiplex editing in plants as shown in figure 20. Sharma et al. (2017) compared ZFN, TALENs and CRISPR/Cas9 techniques regarding the effect on non-target sites and determined CRISPr/Cas9 to have the lowest effects. Regarding the ease of multiplexing and the preparation capacity for large-scale libraries, CRISPr/Cas9 was identified as the one best suited. To study the function of a gene, modifications can be achieved in a precise way with the knowledge of the sequence of the gene. Potential functional SNPs can be discovered with this method, too.

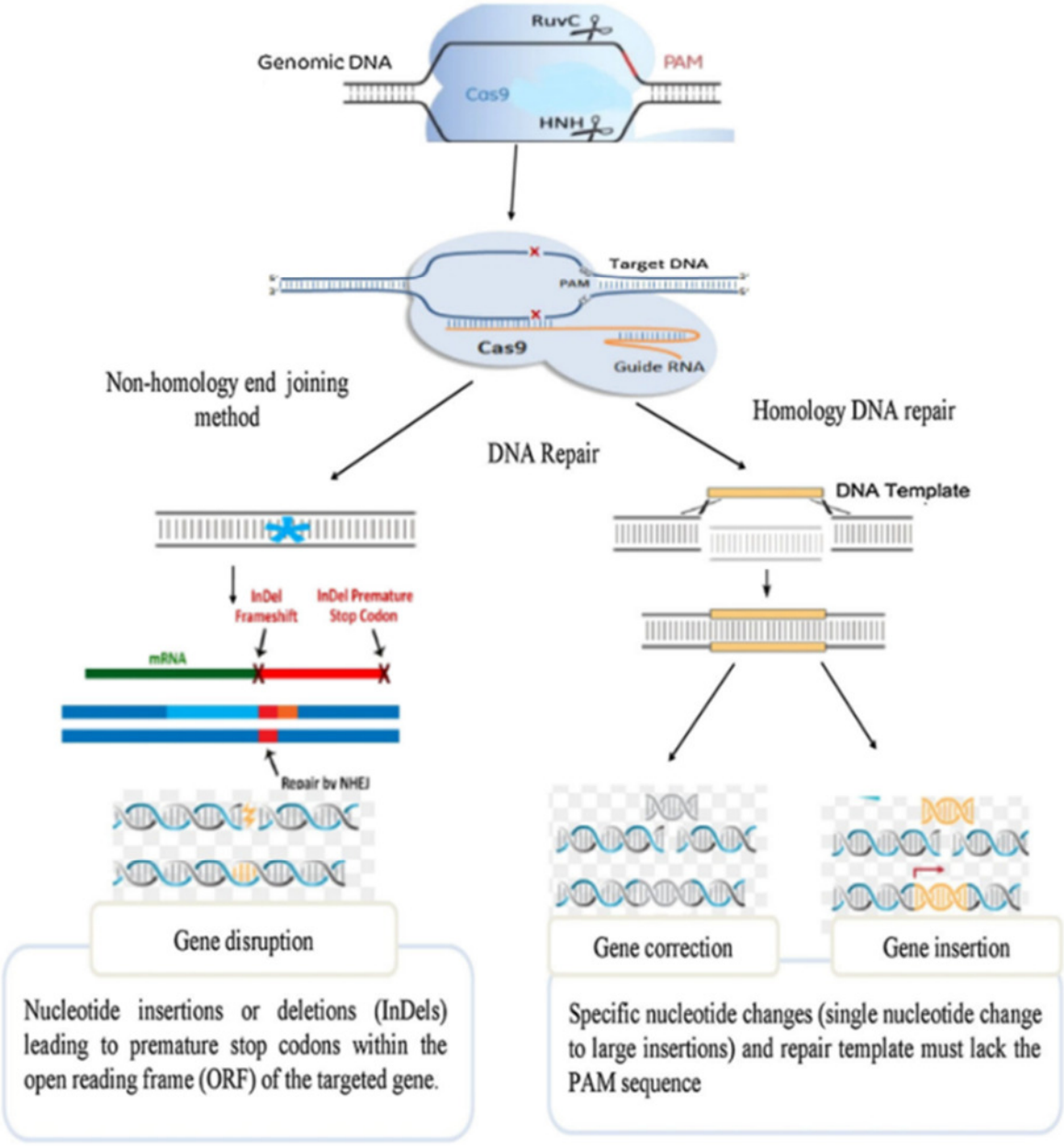

Figure 20: Mechanism of CRIPSR/Cas and possible applications (Sharma et al. 2017).

In summary, some potential candidates for the BaMMV resistance gene *rym13* as well as *Ryd3* conferring BYDV tolerance were detected and efforts for subsequent gene isolation were conducted in this thesis. This work lays the foundation for the isolation of the corresponding genes conferring resistance or tolerance to economically important viruses of barley and other cereals.

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7 Supplemental Data

Supplemental data 1: Sequence information for the new developed markers for candidate genes.

marker	type	enzyme	source	primer sequence (forward in case of InDel or CAPS marker; Allele_FAM in case of KASP marker)	primer sequence (reverse in case of InDel or CAPS marker; Allele_HEX in case of KASP marker)	primer_common (in case of KASP marker)
S076_C337	CAPS	SsiI	9kiselect chip	TTTTCCCAAAAGCAGCAACT	GGTCTGTGCCTTTCTCTTGC	
SCRI_RS_108369	CAPS	Bsh1236I	9kiselect chip	TTCACCCCTCTTCCTTCCTT	CAACTAACAAATCGCCAGCA	
B094_C051	CAPS	MspI	9kiselect chip	TGATGATAATGGGGGCATTT	GCCCTTCTTCTCCTTGTCCT	
B296_C165	InDel	-	9kiselect chip	CGGTAACAAGTCCTCCTCCA	TAAGAAGCAGCCAACGACCT	
B365_C876	InDel	-	9kiselect chip	ATATCACCCGACACCCTGAA	ATCACCCAGCTTGCGTTATC	
BOPA_766	CAPS	BseGI	9kiselect chip	CAGAACTTCCCTTGCTGGAA	CGTGCACAGCCCTTTATTTT	
2_1130	CAPS	HaeIII	9kiselect chip	ATAAAAACAGGGCAGCGAGA	GCCTTGTCCACCTCCTTGTA	
B250_C469	InDel	-	9kiselect chip	AGATCTGCCCGATGAGCTT	ATTGGAGATGCCCTTACTGC	
S164_C941	InDel	-	9kiselect chip	GTCGTCGTTGTTGACCAGTG	CGAACACCTCTGCCATGTAG	
S357_C104	CAPS	Sau96I	9kiselect chip	AGGATCACATCTTGGGCATT	ACATTGAAACAAGGGGTGGA	
S116_C985	CAPS	AjiI	9kiselect chip	TCAACTTCTGCAGGGTCCTT	ATTTTGGTTTGAGGGCTGGA	
S829_C691	CAPS	TspRI	9kiselect chip	CCTGAAATTTTTGGACGCATA	CTCAAGATGGCCTCCACATT	
Bradi77420	CAPS	MspI	Mayer et al. (2011)	GATTGAGCAATGCAAAGCAA	AAGATGCAAAGTAGTTCAGGGTTT	
SCRI_RS_192456	InDel	-	Mayer et al. (2011)	GCCCATGATGCTGCCTAT	CCGCGAGGATAATAAATTGG	
Contig_273304	CAPS	Cfr31I	Mayer et al. (2011)	AGGCGGCCAAATACATAGTG	ATCGTCTCCACGGATCAAAG	
Contig_43083	CAPS	Cfr31I	Mayer et al. (2011)	CAGGTGTGGGTTAGGCAGAT	CTCCAGCTCCTGTCTTGTCC	

Supplemental data 1: continued.

marker	Type	enzyme	source	primer sequence (forward in case of InDel or CAPS marker; Allele_FAM in case of KASP marker)	primer sequence (reverse in case of InDel or CAPS marker; Allele_HEX in case of KASP marker)	primer_common (in case of KASP marker)
NiASHv2073c21	CAPS	HincII	Mayer et al. (2011)	ACAGGAGCGCCAGAAACTAA	TAACAGCACCCACATTGCAT	
Bradi78120	CAPS	MlyI	Mayer et al. (2011)	GCGAGGTGGAGATCAAGTTC	TCCAAAAGGCCATGCTAGAG	
CSN1-4	CAPS	HincII	Candidate gene 1	CGAAAGAAGCAAGTGCACAA	CTATGGTCATGGCCACCTTT	
PCI_1-1	CAPS	PdmI	Candidate gene 2	AACCAGCACCTTGATCCAGA	ACTGAAGAGAACCGTGCTGA	
C1g1	CAPS	AciI	Candidate gene 3	AACGGCCTTCGATCAGATAGT	GACGACGCTCATATTCTTGG	
C1g2	KASP	-	Candidate gene 4	CGCTGATGTACAGGCTCTCAAGT	CGCTGATGTACAGGCTCTCAAGA	AAGAGAATCCATTTTCACCTCCCAAGAT
C1g3_1	KASP	-	Candidate gene 5	GAGAGACTGAAGCTGACCTGTG	GAGAGACTGAAGCTGACCTGTC	TCGTGGAAACACGTTTGCACAGCAT
C1g3_2	KASP	-	Candidate gene 6	TGAAGTGGCGGATAGGTACG	GCTTGAAGTGGCGGATAGGTACA	ACAGGTCAGCTTCAGTCTCTCCAT
C1g4	KASP	-	Candidate gene 7	GCATCTCAGCTCAAGGATCGCTT	CATCTCAGCTCAAGGATCGCTC	GGTTTCCCAGTCCGGTCCAGTA
E04_c1g73	KASP	-	Candidate gene 8	CAGGACGAAGGTGCCCGTGT	AGGACGAAGGTGCCCGTGC	TATTATTAACAGCACCCACATTGCATGAAA
E04_c1g74	KASP	-	Candidate gene 9	TAAGTTTATTAAACATTAATACATGTAGGAAT	AAGTTTATTAAACATTAATACATGTAGGAAC	TGGATGATCCCGAACAGGTTCCTTT
K09_c1g89	KASP	-	Candidate gene 10	CCTTGAAACATCCAAAGAAAAGGTAAG	CCTTGAAACATCCAAAGAAAAGGTAAT	CGAATTGGGGATCATCCAATGGAGAA

Supplemental data 2: Phenotypic data of all analyzed RILs for both viruses and locations compared to the greenhouse.

		BaMMV		BaYMV	
RILS	greenhouse	Bornum	Schladen	Bornum	Schladen
2	susceptible	resistant	resistant	resistant	resistant
6	susceptible	resistant	susceptible	resistant	resistant
8	susceptible	resistant	susceptible	resistant	resistant
9	susceptible	resistant	resistant	resistant	resistant
11	susceptible	susceptible	susceptible	resistant	resistant
15	resistant	resistant	susceptible	susceptible	susceptible
22	resistant	resistant	resistant	resistant	resistant
23	susceptible	susceptible	susceptible	resistant	susceptible
37	susceptible	resistant	susceptible	resistant	resistant
40	susceptible	resistant	susceptible	resistant	resistant
42	susceptible	resistant	susceptible	susceptible	susceptible
48	susceptible	resistant	susceptible	susceptible	susceptible
56	resistant	resistant	resistant	resistant	resistant
58	-	resistant	susceptible	resistant	resistant
60	susceptible	susceptible	susceptible	resistant	resistant
73	resistant	resistant	susceptible	resistant	resistant
77	-	resistant	resistant	resistant	resistant
84	susceptible	susceptible	susceptible	resistant	resistant
85	-	resistant	susceptible	resistant	resistant
94	susceptible	resistant	susceptible	resistant	resistant
95	susceptible	resistant	susceptible	resistant	resistant
98	resistant	resistant	susceptible	resistant	resistant
136	resistant	resistant	resistant	susceptible	susceptible
144	susceptible	resistant	susceptible	resistant	resistant
163	susceptible	resistant	susceptible	resistant	resistant
168	susceptible	resistant	susceptible	susceptible	resistant
170	resistant	susceptible	susceptible	resistant	resistant
172	susceptible	susceptible	susceptible	susceptible	susceptible
184	-	susceptible	-	resistant	-
185	susceptible	susceptible	susceptible	susceptible	susceptible
200	susceptible	resistant	susceptible	resistant	susceptible
204	susceptible	susceptible	susceptible	susceptible	susceptible
205	susceptible	resistant	susceptible	susceptible	susceptible
207	susceptible	resistant	susceptible	susceptible	susceptible
210	susceptible	susceptible	susceptible	susceptible	susceptible
212	susceptible	resistant	susceptible	susceptible	susceptible
214	-	resistant	susceptible	susceptible	susceptible
228	susceptible	susceptible	susceptible	resistant	resistant
230	-	susceptible	susceptible	resistant	resistant
238	susceptible	susceptible	susceptible	susceptible	susceptible
240	susceptible	resistant	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

		BaMMV		BaYMV	
RIL	greenhouse	Bornum	Schladen	Bornum	Schladen
258	susceptible	resistant	susceptible	resistant	resistant
262	susceptible	susceptible	susceptible	resistant	resistant
291	susceptible	resistant	susceptible	susceptible	susceptible
294	susceptible	susceptible	susceptible	susceptible	susceptible
310	susceptible	susceptible	susceptible	susceptible	susceptible
316	susceptible	susceptible	susceptible	resistant	resistant
339	susceptible	susceptible	susceptible	resistant	resistant
343	susceptible	resistant	susceptible	resistant	resistant
363	-	-	susceptible	-	susceptible
370	susceptible	resistant	susceptible	susceptible	susceptible
388	susceptible	susceptible	susceptible	resistant	resistant
393	resistant	resistant	resistant	resistant	resistant
395	susceptible	resistant	susceptible	susceptible	susceptible
408	susceptible	resistant	susceptible	susceptible	susceptible
421	-	susceptible	susceptible	susceptible	susceptible
430	resistant	-	susceptible	-	resistant
439	susceptible	-	susceptible	-	resistant
460	susceptible	susceptible	susceptible	resistant	resistant
467	susceptible	resistant	susceptible	susceptible	susceptible
475	susceptible	-	susceptible	-	susceptible
485	susceptible	-	susceptible	-	resistant
491	susceptible	resistant	susceptible	susceptible	susceptible
503	susceptible	-	susceptible	-	resistant
505	susceptible	susceptible	susceptible	susceptible	susceptible
507	susceptible	resistant	susceptible	susceptible	susceptible
519	resistant	resistant	susceptible	susceptible	susceptible
522	susceptible	resistant	susceptible	susceptible	susceptible
527	susceptible	susceptible	susceptible	susceptible	susceptible
541	-	susceptible	susceptible	resistant	resistant
544	susceptible	susceptible	susceptible	susceptible	susceptible
546	susceptible	resistant	susceptible	resistant	resistant
549	susceptible	susceptible	susceptible	susceptible	susceptible
562	susceptible	susceptible	susceptible	susceptible	susceptible
563	susceptible	susceptible	susceptible	susceptible	susceptible
564	susceptible	-	susceptible	-	resistant
565	susceptible	resistant	resistant	resistant	resistant
569	susceptible	resistant	susceptible	resistant	resistant
570	susceptible	resistant	susceptible	resistant	resistant
575	resistant	susceptible	susceptible	resistant	resistant
578	susceptible	resistant	susceptible	resistant	resistant
581	-	susceptible	susceptible	susceptible	susceptible
582	susceptible	susceptible	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
584	resistant	resistant	susceptible	susceptible	susceptible
585	resistant	resistant	susceptible	susceptible	susceptible
587	susceptible	susceptible	susceptible	susceptible	susceptible
588	-	susceptible	susceptible	susceptible	susceptible
014-a	susceptible	resistant	susceptible	resistant	resistant
016-a	-	resistant	susceptible	susceptible	susceptible
017-a	resistant	resistant	susceptible	resistant	resistant
018-a	susceptible	susceptible	susceptible	susceptible	resistant
025-a	susceptible	resistant	susceptible	resistant	resistant
026-a	susceptible	resistant	susceptible	resistant	resistant
027-a	-	resistant	-	susceptible	-
029-a	susceptible	resistant	resistant	resistant	resistant
030-a	susceptible	resistant	susceptible	resistant	resistant
031-a	susceptible	resistant	susceptible	resistant	resistant
032-a	susceptible	resistant	susceptible	-	-
034-a	susceptible	susceptible	susceptible	susceptible	susceptible
038-a	susceptible	resistant	susceptible	resistant	susceptible
039-a	susceptible	resistant	susceptible	susceptible	susceptible
042-b	-	resistant	susceptible	resistant	susceptible
043-a	susceptible	resistant	susceptible	susceptible	susceptible
047-a	-	resistant	susceptible	susceptible	susceptible
050-b	susceptible	resistant	susceptible	resistant	resistant
051-a	susceptible	resistant	susceptible	susceptible	susceptible
053-b	susceptible	resistant	susceptible	resistant	resistant
061-a	susceptible	resistant	susceptible	susceptible	susceptible
065-a	resistant	resistant	resistant	resistant	resistant
066-a	resistant	resistant	resistant	resistant	resistant
067-a	susceptible	resistant	susceptible	resistant	resistant
070-a	-	resistant	susceptible	resistant	susceptible
071-a	-	resistant	susceptible	resistant	susceptible
072-a	susceptible	resistant	susceptible	resistant	resistant
074-b	resistant	resistant	susceptible	resistant	resistant
076-a	resistant	resistant	resistant	susceptible	susceptible
078-a	-	susceptible	susceptible	resistant	resistant
080-b	susceptible	resistant	susceptible	resistant	resistant
087-b	-	resistant	susceptible	resistant	resistant
088-a	susceptible	resistant	susceptible	resistant	resistant
089-b	-	resistant	susceptible	resistant	resistant
093-a	susceptible	susceptible	susceptible	resistant	resistant
096-a	susceptible	susceptible	susceptible	resistant	susceptible
097-a	susceptible	resistant	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
100-a	susceptible	susceptible	susceptible	resistant	resistant
102-a	resistant	resistant	resistant	resistant	resistant
103-a	susceptible	susceptible	susceptible	resistant	resistant
105-a	susceptible	susceptible	susceptible	resistant	resistant
111-b	-	resistant	resistant	resistant	resistant
112-a	susceptible	susceptible	susceptible	susceptible	susceptible
117-b	susceptible	susceptible	susceptible	susceptible	susceptible
118-a	susceptible	susceptible	susceptible	resistant	resistant
122-b	susceptible	susceptible	susceptible	resistant	susceptible
124-a	susceptible	susceptible	susceptible	resistant	resistant
125b	susceptible	susceptible	susceptible	resistant	resistant
169-a	-	resistant	susceptible	susceptible	susceptible
176-a	susceptible	susceptible	susceptible	resistant	resistant
177-a	susceptible	resistant	susceptible	resistant	resistant
180a	-	resistant	susceptible	resistant	resistant
181-a	-	resistant	susceptible	resistant	susceptible
183-a	susceptible	susceptible	resistant	susceptible	susceptible
186-a	resistant	susceptible	susceptible	susceptible	resistant
187-c	-	-	susceptible	-	susceptible
191-a	susceptible	resistant	susceptible	susceptible	susceptible
193-a	susceptible	resistant	susceptible	susceptible	susceptible
196-a	susceptible	susceptible	susceptible	susceptible	susceptible
197-a	susceptible	susceptible	susceptible	susceptible	susceptible
199-c	susceptible	resistant	susceptible	susceptible	susceptible
201-b	susceptible	susceptible	susceptible	susceptible	susceptible
202-a	susceptible	susceptible	susceptible	susceptible	susceptible
203-a	susceptible	resistant	susceptible	susceptible	susceptible
206-b	susceptible	susceptible	susceptible	susceptible	susceptible
208-a	susceptible	resistant	susceptible	susceptible	susceptible
209-c	susceptible	resistant	susceptible	susceptible	susceptible
211b	resistant	resistant	susceptible	susceptible	susceptible
213-b	susceptible	resistant	susceptible	susceptible	susceptible
215-a	susceptible	resistant	susceptible	susceptible	susceptible
216-c	-	resistant	susceptible	susceptible	susceptible
217-a	susceptible	resistant	susceptible	susceptible	susceptible
218-b	-	resistant	susceptible	resistant	resistant
220-a	susceptible	resistant	susceptible	resistant	resistant
222-b	susceptible	susceptible	susceptible	resistant	resistant
223-b	susceptible	resistant	susceptible	resistant	resistant
224b	susceptible	susceptible	susceptible	resistant	resistant
225-a	susceptible	resistant	susceptible	resistant	resistant

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
226-b	resistant	resistant	susceptible	resistant	resistant
227-b	susceptible	susceptible	susceptible	resistant	resistant
229b	susceptible	resistant	susceptible	resistant	resistant
231c	resistant	resistant	susceptible	susceptible	susceptible
232-b	susceptible	susceptible	susceptible	resistant	resistant
233-d	susceptible	resistant	susceptible	resistant	resistant
234-a	susceptible	resistant	susceptible	susceptible	susceptible
236-c	susceptible	resistant	susceptible	susceptible	susceptible
237-a	susceptible	resistant	susceptible	susceptible	susceptible
241-a	susceptible	resistant	susceptible	susceptible	susceptible
242-b	resistant	resistant	resistant	resistant	resistant
243-a	-	resistant	susceptible	resistant	resistant
245-a	susceptible	resistant	susceptible	resistant	resistant
246-b	resistant	resistant	resistant	resistant	resistant
247-a	-	resistant	resistant	resistant	resistant
248-a	susceptible	resistant	susceptible	resistant	resistant
249-a	susceptible	resistant	susceptible	resistant	resistant
251-a	-	resistant	susceptible	resistant	resistant
253-a	resistant	resistant	susceptible	resistant	resistant
254-a	susceptible	resistant	susceptible	resistant	resistant
255-a	-	resistant	susceptible	resistant	resistant
256-a	-	susceptible	susceptible	resistant	resistant
257-b	susceptible	resistant	susceptible	resistant	resistant
259-a	resistant	susceptible	susceptible	resistant	resistant
260-a	resistant	resistant	resistant	resistant	resistant
261-a	susceptible	susceptible	susceptible	resistant	susceptible
263-a	susceptible	resistant	susceptible	susceptible	susceptible
264-a	resistant	resistant	susceptible	resistant	resistant
265-b	susceptible	resistant	susceptible	susceptible	susceptible
266-a	susceptible	susceptible	susceptible	resistant	resistant
267-a	susceptible	resistant	susceptible	susceptible	susceptible
268-a	susceptible	susceptible	susceptible	susceptible	susceptible
269-c	susceptible	resistant	susceptible	susceptible	susceptible
270-a	susceptible	resistant	susceptible	susceptible	susceptible
271-a	susceptible	resistant	susceptible	susceptible	susceptible
272-b	susceptible	resistant	susceptible	susceptible	susceptible
273-a	susceptible	resistant	susceptible	susceptible	susceptible
274-b	susceptible	resistant	susceptible	susceptible	susceptible
275-a	susceptible	resistant	susceptible	susceptible	susceptible
276-a	susceptible	resistant	susceptible	susceptible	susceptible
277-a	susceptible	resistant	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
278-a	susceptible	resistant	susceptible	susceptible	susceptible
279-c	susceptible	resistant	susceptible	susceptible	susceptible
280-a	susceptible	resistant	susceptible	susceptible	susceptible
281-a	-	resistant	susceptible	susceptible	susceptible
282-a	susceptible	susceptible	susceptible	susceptible	susceptible
283-a	susceptible	susceptible	susceptible	susceptible	susceptible
284-a	susceptible	susceptible	susceptible	susceptible	susceptible
285-c	susceptible	susceptible	susceptible	susceptible	susceptible
286-b	resistant	susceptible	susceptible	susceptible	resistant
289-a	susceptible	susceptible	susceptible	resistant	resistant
290-a	susceptible	susceptible	susceptible	resistant	resistant
292-a	-	-	susceptible	-	susceptible
293-a	susceptible	susceptible	susceptible	susceptible	susceptible
295-a	susceptible	resistant	resistant	resistant	susceptible
296-a	susceptible	resistant	resistant	resistant	resistant
297b	susceptible	susceptible	susceptible	resistant	resistant
298-b	susceptible	susceptible	susceptible	resistant	resistant
299-a	susceptible	resistant	susceptible	resistant	resistant
300-a	susceptible	resistant	susceptible	resistant	resistant
301-a	susceptible	resistant	susceptible	susceptible	resistant
302-b	susceptible	susceptible	susceptible	resistant	resistant
303-a	susceptible	resistant	susceptible	resistant	resistant
304-a	susceptible	susceptible	susceptible	resistant	resistant
305-a	resistant	susceptible	susceptible	resistant	resistant
306-a	susceptible	susceptible	susceptible	resistant	resistant
307-a	-	susceptible	susceptible	resistant	resistant
308-a	susceptible	resistant	susceptible	resistant	resistant
309-a	-	resistant	resistant	resistant	resistant
311-a	susceptible	susceptible	susceptible	resistant	resistant
312-a	-	resistant	susceptible	resistant	resistant
313-a	susceptible	resistant	susceptible	resistant	resistant
314-a	susceptible	resistant	susceptible	resistant	resistant
315-a	susceptible	susceptible	susceptible	resistant	resistant
318-b	susceptible	susceptible	susceptible	resistant	resistant
320-a	susceptible	susceptible	susceptible	resistant	resistant
321-a	susceptible	susceptible	susceptible	resistant	resistant
322-a	susceptible	susceptible	susceptible	resistant	resistant
323-a	resistant	resistant	susceptible	resistant	resistant
324-a	susceptible	resistant	susceptible	resistant	resistant
326-b	resistant	resistant	susceptible	resistant	resistant
327-a	resistant	resistant	susceptible	resistant	resistant

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
328b	susceptible	susceptible	susceptible	resistant	resistant
329-a	resistant	susceptible	resistant	resistant	resistant
330-a	resistant	resistant	resistant	resistant	resistant
331-a	susceptible	susceptible	susceptible	susceptible	resistant
332-b	resistant	susceptible	resistant	resistant	resistant
333-a	-	resistant	susceptible	resistant	resistant
334-a	susceptible	resistant	susceptible	resistant	susceptible
335-c	susceptible	susceptible	susceptible	resistant	resistant
336-a	resistant	resistant	resistant	resistant	resistant
337-a	-	resistant	susceptible	resistant	susceptible
338-a	susceptible	resistant	susceptible	resistant	resistant
340-a	resistant	resistant	resistant	resistant	resistant
341-a	susceptible	resistant	susceptible	resistant	resistant
342-a	susceptible	resistant	susceptible	resistant	resistant
344-a	resistant	resistant	resistant	resistant	resistant
345-b	susceptible	susceptible	resistant	resistant	resistant
346-a	susceptible	resistant	susceptible	resistant	resistant
348-a	resistant	resistant	susceptible	resistant	resistant
354-a	resistant	susceptible	susceptible	resistant	resistant
355-a	susceptible	resistant	susceptible	resistant	resistant
356-b	-	resistant	susceptible	susceptible	resistant
357-a	resistant	susceptible	susceptible	resistant	resistant
358-a	resistant	resistant	susceptible	resistant	resistant
359-a	susceptible	resistant	susceptible	resistant	resistant
361-b	resistant	resistant	susceptible	susceptible	susceptible
362-a	susceptible	resistant	susceptible	susceptible	susceptible
366-b	-	resistant	resistant	resistant	resistant
367-c	susceptible	resistant	resistant	susceptible	susceptible
369-a	resistant	susceptible	resistant	resistant	resistant
371-a	resistant	resistant	resistant	resistant	resistant
372-a	susceptible	resistant	susceptible	resistant	resistant
373-a	susceptible	resistant	susceptible	resistant	resistant
374-a	-	resistant	resistant	resistant	resistant
375-a	resistant	resistant	resistant	resistant	resistant
376-a	susceptible	resistant	susceptible	resistant	resistant
377-a	resistant	resistant	resistant	resistant	resistant
378-b	susceptible	resistant	susceptible	resistant	resistant
379-a	susceptible	resistant	susceptible	resistant	resistant
380-a	resistant	resistant	susceptible	resistant	resistant
381-a	resistant	resistant	susceptible	resistant	resistant
382-a	susceptible	resistant	susceptible	resistant	resistant

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
387-a	susceptible	resistant	susceptible	resistant	resistant
389-a	susceptible	susceptible	susceptible	resistant	susceptible
390-a	resistant	resistant	resistant	susceptible	susceptible
391-a	susceptible	susceptible	susceptible	susceptible	susceptible
392-a	resistant	susceptible	susceptible	resistant	resistant
394-a	resistant	resistant	susceptible	susceptible	susceptible
396-a	resistant	resistant	susceptible	susceptible	susceptible
397-a	susceptible	resistant	susceptible	susceptible	susceptible
398-a	resistant	resistant	susceptible	resistant	resistant
399-a	susceptible	susceptible	susceptible	susceptible	susceptible
400-a	susceptible	susceptible	susceptible	resistant	resistant
401-a	susceptible	susceptible	susceptible	susceptible	susceptible
402-a	-	resistant	susceptible	susceptible	susceptible
403-a	resistant	susceptible	resistant	resistant	resistant
404-a	susceptible	susceptible	susceptible	susceptible	susceptible
405-a	susceptible	resistant	susceptible	susceptible	susceptible
406-a	susceptible	susceptible	susceptible	susceptible	susceptible
407-a	-	susceptible	susceptible	susceptible	susceptible
409-a	susceptible	resistant	susceptible	susceptible	susceptible
410-a	susceptible	susceptible	susceptible	resistant	resistant
411-a	-	susceptible	susceptible	susceptible	susceptible
412-a	susceptible	susceptible	susceptible	susceptible	susceptible
413-a	resistant	susceptible	susceptible	resistant	resistant
414-b	susceptible	resistant	susceptible	susceptible	susceptible
415-b	susceptible	-	susceptible	-	resistant
416-a	susceptible	susceptible	susceptible	susceptible	susceptible
417-a	susceptible	resistant	susceptible	resistant	resistant
418-a	susceptible	susceptible	susceptible	susceptible	susceptible
419-b	susceptible	resistant	susceptible	susceptible	susceptible
420-a	susceptible	susceptible	susceptible	susceptible	susceptible
422c	susceptible	susceptible	susceptible	resistant	resistant
423c	susceptible	-	susceptible	-	susceptible
424c	-	-	susceptible	-	resistant
425c	susceptible	resistant	susceptible	resistant	resistant
427c	susceptible	resistant	susceptible	resistant	resistant
429b	susceptible	susceptible	susceptible	resistant	resistant
431-a	susceptible	resistant	susceptible	resistant	resistant
432-a	-	susceptible	susceptible	resistant	resistant
433-a	-	resistant	susceptible	resistant	resistant
434-a	susceptible	susceptible	susceptible	susceptible	susceptible
435a	susceptible	susceptible	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
436-a	resistant	resistant	susceptible	susceptible	susceptible
437-a	susceptible	resistant	resistant	susceptible	resistant
438-a	resistant	resistant	susceptible	resistant	resistant
440-b	susceptible	susceptible	susceptible	resistant	resistant
442-a	resistant	susceptible	susceptible	resistant	resistant
443-a	susceptible	susceptible	susceptible	resistant	resistant
444-b	susceptible	resistant	susceptible	susceptible	susceptible
445-a	resistant	resistant	resistant	susceptible	susceptible
446-a	-	resistant	susceptible	susceptible	susceptible
447-b	,	resistant	susceptible	susceptible	susceptible
448-a	resistant	susceptible	susceptible	susceptible	susceptible
449-a	susceptible	susceptible	susceptible	susceptible	susceptible
450-b	susceptible	susceptible	susceptible	resistant	resistant
451-a	susceptible	resistant	susceptible	susceptible	susceptible
452-a	susceptible	resistant	susceptible	susceptible	susceptible
454-a	susceptible	susceptible	susceptible	resistant	resistant
455-a	susceptible	resistant	susceptible	susceptible	susceptible
456-a	susceptible	susceptible	susceptible	resistant	resistant
457-c	susceptible	susceptible	susceptible	susceptible	susceptible
458-a	susceptible	susceptible	susceptible	susceptible	susceptible
459-b	susceptible	resistant	susceptible	susceptible	susceptible
461-a	resistant	resistant	susceptible	susceptible	susceptible
462c	susceptible	-	susceptible	-	resistant
463-a	-	resistant	susceptible	susceptible	susceptible
464-a	resistant	resistant	susceptible	susceptible	susceptible
465-b	susceptible	resistant	susceptible	susceptible	susceptible
468-b	-	resistant	resistant	-	susceptible
469-a	susceptible	susceptible	susceptible	resistant	resistant
470-a	susceptible	susceptible	susceptible	resistant	resistant
471-a	susceptible	resistant	susceptible	resistant	resistant
473-b	susceptible	resistant	susceptible	susceptible	susceptible
474-a	-	resistant	resistant	susceptible	susceptible
476-a	-	resistant	susceptible	susceptible	susceptible
477-b	susceptible	resistant	susceptible	susceptible	susceptible
478-a	-	resistant	resistant	susceptible	susceptible
479-a	-	resistant	resistant	susceptible	susceptible
480-a	susceptible	-	susceptible	-	susceptible
481-b	susceptible	susceptible	susceptible	susceptible	susceptible
482-a	resistant	resistant	susceptible	susceptible	susceptible
483-a	resistant	resistant	susceptible	susceptible	susceptible
484-b	resistant	resistant	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
486-a	susceptible	resistant	susceptible	resistant	resistant
489-a	resistant	resistant	susceptible	susceptible	susceptible
490-a	susceptible	resistant	susceptible	susceptible	susceptible
492-b	susceptible	resistant	susceptible	susceptible	susceptible
493-a	susceptible	susceptible	susceptible	-	susceptible
494-a	susceptible	resistant	susceptible	susceptible	susceptible
495-a	resistant	resistant	susceptible	susceptible	resistant
496-a	susceptible	resistant	susceptible	susceptible	susceptible
497-a	susceptible	resistant	susceptible	susceptible	susceptible
498-a	susceptible	resistant	susceptible	resistant	susceptible
499-a	susceptible	resistant	susceptible	susceptible	susceptible
500-a	susceptible	susceptible	susceptible	resistant	resistant
501-a	susceptible	susceptible	susceptible	susceptible	susceptible
502-a	susceptible	resistant	susceptible	resistant	resistant
504-a	resistant	susceptible	susceptible	resistant	resistant
506-a	resistant	resistant	susceptible	susceptible	susceptible
508-b	-	resistant	susceptible	resistant	resistant
509-a	susceptible	resistant	-	susceptible	-
510-b	-	resistant	susceptible	susceptible	susceptible
511a	resistant	susceptible	susceptible	susceptible	susceptible
514a	susceptible	resistant	susceptible	susceptible	susceptible
515a	susceptible	resistant	susceptible	susceptible	susceptible
516a	resistant	resistant	susceptible	susceptible	susceptible
517-a	susceptible	resistant	susceptible	susceptible	susceptible
518-a	susceptible	resistant	susceptible	resistant	resistant
520-a	susceptible	susceptible	susceptible	resistant	resistant
521-a	susceptible	susceptible	susceptible	susceptible	susceptible
523-a	susceptible	-	susceptible	-	susceptible
524-a	-	resistant	susceptible	susceptible	susceptible
525-a	-	resistant	susceptible	resistant	resistant
526-a	susceptible	resistant	susceptible	susceptible	susceptible
529-a	resistant	resistant	susceptible	susceptible	susceptible
530-a	susceptible	susceptible	susceptible	resistant	resistant
531-a	resistant	resistant	susceptible	susceptible	susceptible
532-a	-	susceptible	susceptible	susceptible	susceptible
533-a	susceptible	susceptible	susceptible	susceptible	susceptible
534-b	-	susceptible	susceptible	resistant	resistant
535-b	susceptible	resistant	susceptible	susceptible	susceptible
536-a	susceptible	susceptible	susceptible	susceptible	susceptible
537-a	susceptible	susceptible	susceptible	susceptible	susceptible
538-a	resistant	susceptible	susceptible	resistant	resistant

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
539-a	susceptible	resistant	susceptible	susceptible	susceptible
540-a	susceptible	resistant	susceptible	resistant	resistant
542-a	-	resistant	susceptible	resistant	resistant
543-b	susceptible	susceptible	susceptible	resistant	resistant
545-a	susceptible	susceptible	susceptible	resistant	resistant
547-a	resistant	resistant	susceptible	resistant	resistant
548-b	resistant	-	susceptible	-	resistant
550-a	susceptible	susceptible	susceptible	susceptible	susceptible
552-a	susceptible	resistant	susceptible	susceptible	susceptible
553-a	-	-	susceptible	-	susceptible
554a	susceptible	susceptible	susceptible	susceptible	susceptible
555a	susceptible	resistant	susceptible	susceptible	resistant
556a	susceptible	-	susceptible	-	susceptible
557a	susceptible	susceptible	susceptible	susceptible	susceptible
558a	susceptible	susceptible	susceptible	susceptible	susceptible
559a	susceptible	susceptible	susceptible	susceptible	susceptible
560a	susceptible	susceptible	susceptible	susceptible	susceptible
561a	susceptible	susceptible	susceptible	susceptible	susceptible
566a	susceptible	resistant	susceptible	susceptible	susceptible
567a	susceptible	resistant	susceptible	resistant	resistant
568a	-	resistant	resistant	susceptible	susceptible
571a	susceptible	susceptible	susceptible	resistant	resistant
572a	susceptible	susceptible	susceptible	susceptible	susceptible
573a	resistant	resistant	susceptible	susceptible	susceptible
574a	susceptible	susceptible	susceptible	susceptible	susceptible
576a	susceptible	resistant	susceptible	resistant	resistant
577b	resistant	resistant	susceptible	susceptible	resistant
579a	susceptible	susceptible	susceptible	susceptible	susceptible
586a	resistant	resistant	susceptible	resistant	resistant
589a	resistant	resistant	susceptible	susceptible	susceptible
590a	-	susceptible	susceptible	susceptible	susceptible
591a	-	resistant	susceptible	resistant	resistant
594a	-	susceptible	susceptible	susceptible	susceptible
595a	-	susceptible	susceptible	susceptible	susceptible
599a	-	susceptible	susceptible	resistant	resistant
601a	-	susceptible	susceptible	resistant	resistant
602a	-	susceptible	susceptible	susceptible	susceptible
603b	-	susceptible	susceptible	resistant	resistant
604a	-	susceptible	susceptible	resistant	resistant
605a	-	susceptible	susceptible	resistant	resistant
607a	-	susceptible	susceptible	susceptible	susceptible

Supplemental data S 2: continued.

RIL	BaMMV greenhouse	BaYMV Bornum	Schladen	BaMMV Bornum	BaYMV Schladen
# resistant	83	268	45	226	230
# susceptible	293	166	404	205	218

Supplemental data 3: Set of different genotypes to test the diagnostic value of markers. Genotypes with "s" show the susceptibility allele like 'Plaisant, whereas "r" means resistant allele like 'Taihoku A'.

Cultivar	Year of registration	Resistance	BOPA766	contig43083	C1g1	C1g4
10247	-	*rym 8*	s	s	s	s
Advance	2002	*rym4*	s	s	s	s
Affair	2002	*rym4*	s	s	s	s
Anastasia	2002	*rym5*	s	s	s	s
Anisette	2009	susceptible	s	-	s	s
Antalya	2004	susceptible	s	s	s	s
Babylone	1994	*rym4*	s	s	-	s
Barcelona	2001	susceptible	s	s	s	s
Bayava	2002	*rym4*	s	s	s	s
Birgit	1976	*rym4*	s	s	s	s
Bistro	2003	susceptible	s	s	s	s
Brunhild	1986	*rym4*	s	s	s	s
Bulgarien 347	1933	*rym 9*	s	s	s	s
Calador	2003	*rym4*	s	s	s	s
Campanille	2005	*rym4*	s	-	s	s
Canberra	2009	*rym4*	s	s	s	s
Carat	2002	*rym4*	s	s	s	s
Carola	1998	*rym 4*	s	s	-	s
Chikurin Ibaraki 1	-	*rym 15*	s	s	s	s
Cinderella	2004	*rym4*	s	s	s	s
Cosima	2001	*rym4*	s	s	s	s
Duet	1995	*rym4*	s	s	s	s
EA52	-	*rym3*	s	s	s	s
Edda	2001	susceptible	s	-	s	s
Elbany	2002	*rym4*	s	-	s	s
Existenz	2002	*rym4*	s	s	s	s
Express	1990	*rym 4*	s	s	s	s
Franka	1980	*rym4*	s	s	s	s

Supplemental data S 3: continued.

Cultivar	Year of registration	Resistance	BOPA766	contig43083	C1g1	C1g4
Fridericus	2006	*rym4*	s	s	s	s
Gaulois	1989	*rym4*	s	s	s	s
Gerval	2002	-	s	s	s	s
Gilberta	2001	susceptible	s	s	s	s
Goldmine	2000		s	s	s	s
Hanna	1992	susceptible	s	s	s	s
HOR1018	-	susceptible	s	s	-	-
HOR11019	-	*rym13*	r	r	r	r
HOR3365	-	*rym 7*	s	s	s	s
HOR4224	-	On chromosome 5HL not named	s	s	s	s
Hiberna	1993	*rym 10*	s	s	s	s
Highlight	2007	*rym4*	s	s	s	s
Igri	1976	susceptible	s	s	s	s
Jana	1990	*rym4*	s	s	s	s
Jessica	2003	*rym4*	s	s	s	s
Kamoto	2001	*rym 5*	s	-	s	s
Kathleen	2009	*rym 15*	s	s	s	s
Krimhild	1991	*rym4*	s	s	s	s
Kyoto	2002	*rym5*	s	-	s	s
Labea	1992	*rym4*	s	s	s	s
Landi	1995	*rym4*	s	s	s	s
Leibniz	2007	*rym4*	s	s	s	s
Lomerit	2001	*rym4*	s	-	s	s
Lunaris	2002	*rym4*	s	s	s	s
Madou	2001	*rym4*	s	s	s	s
Mädru	1959	*rym4*	s	s	s	s
MBR530	-	resistant	r	r	r	r
MBR532	-	resistant	s	s	s	s
Merlot	2002	*rym4*	s	s	s	s
Metaxa	2008	*rym4*	s	s	s	s
Miho Golden	-	*rym 6*	s	s	s	s
Mihori Hadaka	-	*Rym2*	s	s	s	s
Mokusekko3	-	*rym1, rym5*	s	s	s	s

Supplemental data S 3: continued.

Cultivar	Year of registration	Resistance	BOPA766	contig43083	C1g1	C1g4
Mombasa	2002		s	s	s	s
Muju covered2	-	*rym 12*	r	r	r	r
Naomie	2003	*rym4*	s	s	s	s
Naturel	-	*rym 4*	s	-	s	s
Nelly	1998	-	s	s	s	s
Nerz	2008	*rym4*	s	s	s	s
Noveta	1991	*rym4*	s	s	s	s
Passion	2002	*rym4*	s	s	s	s
Plaisant	1979	susceptible	s	s	s	s
Ragusa	1929	*rym 4*	s	s	s	s
Reni	2001	susceptible	s	s	s	s
Russia 57	-	*rym 11*	s	s	s	s
Sandra	2010	*rym4*	s	s	s	s
Saturn	2010	susceptible	s	-	s	s
Semper	2009	*rym4*	s	s	s	s
Sonate	1983	*rym4*	s	-	s	s
Souleyka	2009	*rym4*	s	s	s	s
Stendal	2010	*rym4*	s	s	s	s
Stephanie	2002	*rym4*	s	s	s	s
Structura	2002	*rym5*	s	s	s	s
Sympax	1993	*rym4*	s	s	s	-
Taihoku A	-	*rym13*	r	r	r	r
Theresa	1994	*rym4*	s	s	s	s
Tokyo	1996	*rym 5*	s	s	s	s
Traminer	2002	*rym4*	s	-	s	s
Venezia	2002	susceptible	s	s	s	s
Venus	1990	*rym4*	s	s	s	s
Verticale	2002	*rym4*	s	s	s	s
Viresa	1988	*rym4*	s	s	s	s
Vogelsanger Gold	1965	-	s	s	s	s
W757-112	-	*rym 11*	s	-	s	s
W757-612	-		s	s	s	s
Wendy	2008	*rym4*	s	s	s	s
Wintmalt	2007	*rym4*	s	s	s	s
Yokohama	2008	*rym4*	s	s	s	s

Supplemental data 4: BaYMV and BaMMV associated SNPs detected by genotyping by sequencing. The best 30 hits for each resistance are displayed. First 30 are linked to BaMMV resistance and the next 30 were assigned to BaYMV resistance.

morex_contig	pos	ref	alt	chr	cM	p-value_M	p-value_Y
morex_contig_275255	3364	T	C	4	111,966913	3,96E-18	0,23857231
morex_contig_244255	1469	T	C	4	111,966913	5,64E-17	0,23139555
morex_contig_244255	1447	C	T	4	111,966913	1,14E-16	0,27469764
morex_contig_244255	1463	A	G	4	111,966913	1,14E-16	0,27469764
morex_contig_80415	170	T	A	4	112,358357	1,73E-16	0,52445108
morex_contig_1562868	5564	C	A	4	113,385269	1,39E-15	0,30612497
morex_contig_80415	134	A	G	4	112,358357	1,69E-15	0,60889845
morex_contig_66148	729	A	G	4	111,966913	2,44E-15	0,29376754
morex_contig_275255	3268	G	A	4	111,966913	2,70E-15	0,16130009
morex_contig_1562868	5609	C	T	4	113,385269	4,12E-15	0,37225878
morex_contig_43083	5360	G	A	4	111,331445	8,99E-15	0,39448147
morex_contig_80415	111	C	T	4	112,358357	2,38E-14	0,73858955
morex_contig_50476	5571	T	C	NA	NA	3,33E-14	0,44786459
morex_contig_275255	3260	G	A	4	111,966913	9,00E-14	0,45255358
morex_contig_1630001	623	G	C	4	111,225213	2,27E-13	0,33660012
morex_contig_59373	1290	C	T	4	111,966913	1,60E-12	0,05538648
morex_contig_275255	3251	T	C	4	111,966913	1,92E-12	0,53312683
morex_contig_59373	1214	G	A	4	111,966913	3,12E-12	0,08433828
morex_contig_79527	1833	A	G	4	111,966913	4,80E-12	0,67573366
morex_contig_137912	1694	C	A	4	111,225213	5,68E-12	0,63965258
morex_contig_50476	7153	G	T	NA	NA	1,24E-11	0,6704656
morex_contig_65723	2843	A	T	4	112,358357	2,02E-11	0,0474422
morex_contig_50476	7142	C	A	NA	NA	2,11E-11	0,69189618
morex_contig_50476	5638	G	A	NA	NA	2,65E-11	0,35403567
morex_contig_1552692	72	T	G	NA	NA	3,11E-11	0,30170292
morex_contig_50476	5640	G	A	NA	NA	4,53E-11	0,37878066
morex_contig_1643348	510	A	G	4	111,966913	7,75E-11	0,52007585
morex_contig_1640421	132	C	A	4	112,358357	2,20E-10	0,1355181
morex_contig_59373	3480	T	C	4	111,966913	5,80E-10	0,60834577
morex_contig_1580492	2282	A	C	4	111,966913	6,20E-10	0,65045649
morex_contig_42966	310	G	C	NA	NA	0,13227598	7,56E-19
morex_contig_2503572	163	G	A	NA	NA	0,20320588	1,05E-18
morex_contig_1572506	1196	A	G	NA	NA	0,11305071	2,82E-18

Supplemental data S 4: continued.

morex_contig	pos	ref	alt	chr	cM	p-value_M	p-value_Y
morex_contig_1611026	1660	T	C	3	147,999838	0,11146905	1,35E-17
morex_contig_1611026	1694	A	G	3	147,999838	0,11146905	1,35E-17
morex_contig_1611026	1703	A	G	3	147,999838	0,11146905	1,35E-17
morex_contig_1572506	1244	G	C	NA	NA	0,11840305	3,14E-17
morex_contig_50110	2051	T	G	3	147,999838	0,14797319	4,13E-17
morex_contig_50110	2066	A	C	3	147,999838	0,13998662	7,22E-17
morex_contig_1572506	1121	C	A	NA	NA	0,1634502	8,62E-17
morex_contig_39181	3360	C	T	3	142,917847	0,00907284	1,11E-16
morex_contig_75133	1335	A	G	NA	NA	0,17005643	2,68E-16
morex_contig_75133	1359	T	C	NA	NA	0,17005643	2,68E-16
morex_contig_83725	618	T	C	3	147,999838	0,22712258	5,86E-16
morex_contig_8984	3262	C	T	3	142,917847	0,01726308	1,82E-15
morex_contig_69755	515	G	C	7	126,628895	0,03602755	1,98E-15
morex_contig_43068	6467	T	A	7	110,835694	0,00919439	2,71E-15
morex_contig_43068	6497	A	T	7	110,835694	0,00919439	2,71E-15
morex_contig_47200	2145	T	A	NA	NA	0,02145048	4,01E-15
morex_contig_170791	3101	C	A	3	143,130312	0,00400816	4,24E-15
morex_contig_1575277	1736	T	C	NA	NA	0,15898289	5,44E-15
morex_contig_52938	1637	C	G	7	116,076487	0,01193134	5,93E-15
morex_contig_59824	4750	T	C	3	143,909348	0,03834508	6,54E-15
morex_contig_2555775	521	T	C	3	154,886686	0,13461434	6,77E-15
morex_contig_46326	3352	A	C	7	116,076487	0,02462199	7,71E-15
morex_contig_47200	2138	G	A	NA	NA	0,01867954	8,33E-15
morex_contig_1568777	1669	T	G	3	139,164306	0,0227491	9,52E-15
morex_contig_1568777	1651	C	A	3	139,164306	0,02859251	1,34E-14
morex_contig_1646615	756	A	G	NA	NA	0,02859251	1,34E-14
morex_contig_1566191	7353	A	G	7	120,287875	0,00721598	1,67E-14

Supplemental data 5: Alignment HORVU6Hr1G047760 with sequencing data from susceptible and tolerant RILs and the lines L94 and Vada.
Introns are marked by asterisks.

```
>HORVU6Hr1G047760   #1                NNNNNNNNNN NNNNNNNNNN CTTTTCAACT ATCTGGCACC
<susceptible RILs_4.>#1>                                     T TTCTGGCACC
<tolerant RILs_47760>#1>                                        TGGCACC
                                      ..........................................
consensus           #1                NNNNNNNNNN NNNNNNNNNN CTTTTCAACT WTCTGGCACC
                                      ++++++++++ ++++++++++            *

>HORVU6Hr1G047760   #41               :TTGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
<susceptible RILs_4.#12               CTTGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
<tolerant RILs_47760#8                :TTGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
>Vada_47760         >#1>               CTGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
>L94_47760          >#1>                 TGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
                                      ..........................................
consensus           #41               :TTGAAAATC CCTATTTTTT GTACTCTAAT TCATTTGCCT
                                      **

>HORVU6Hr1G047760   #81               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG
<susceptible RILs_4.#52               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG
<tolerant RILs_47760#48               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG
>Vada_47760         #40               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG
>L94_47760          #39               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG
                                      ..........................................
consensus           #81               ATCTAAACTT GCTTCCGTAC CATAGGATAC TTGCCATGCG


>HORVU6Hr1G047760   #121              CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA
>CDS_47760          >#1>                                  ATGTTGTTGG GCACCAAACA
<susceptible RILs_4.#92               CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA
<tolerant RILs_47760#88               CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA
>Vada_47760         #80               CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA
>L94_47760          #79               CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA
                                      ..........................................
consensus           #121              CCCGTTGCTC TGCCAGCAAA ATGTTGTTGG GCACCAAACA


>HORVU6Hr1G047760   #161              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
>CDS_47760          #21               CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
<susceptible RILs_4.#132              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
<tolerant RILs_47760#128              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
>Vada_47760         #120              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
>L94_47760          #119              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA
                                      ..........................................
consensus           #161              CTTTTCACTT TCTACAACTG AACGATGTTC CAATTGTTCA


>HORVU6Hr1G047760   #201              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
>CDS_47760          #61               GGAGCTGGAA AGGT:::::: :::::::::: ::::::::::
<susceptible RILs_4.#172              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
<tolerant RILs_47760#168              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
>Vada_47760         #160              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
>L94_47760          #159              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
                                      ..........................................
consensus           #201              GGAGCTGGAA AGGTATGGTG TCTAATCTGA TTTTTATTTT
                                                ****** ********** **********
```

```
>HORVU6Hr1G047760   #241          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
>CDS_47760          #101          :::::::::: :::::::::: :::::::::: ::::::::::
<susceptible RILs_4.#212          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
<tolerant RILs_47760#208          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
>Vada_47760         #200          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
>L94_47760          #199          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
                                  ........................................
consensus           #241          CACAATACTC GATTTGATGA TGTCTGGAAG ATTCAACTGA
                                  ********** ********** ********** **********

>HORVU6Hr1G047760   #281          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
>CDS_47760          #141          :::::::::: :::::::::: :::::::::: ::::::::::
<susceptible RILs_4.#252          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
<tolerant RILs_47760#248          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
>Vada_47760         #240          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
>L94_47760          #239          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
                                  ........................................
consensus           #281          AAAGCAACCA AATGGTTTAT CATACTTGAG ATTAAAACAT
                                  ********** ********** ********** **********

>HORVU6Hr1G047760   #321          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
>CDS_47760          #181          :::::::::: :::::::::: :::::::::: ::::::::::
<susceptible RILs_4.#292          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
<tolerant RILs_47760#288          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
>Vada_47760         #280          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
>L94_47760          #279          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
                                  ........................................
consensus           #321          TGTGCTTGGT ACCCATACTA TCTGAATTCA CACCTGTCGG
                                  ********** ********** ********** **********

>HORVU6Hr1G047760   #361          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
>CDS_47760          #221          :::::::::: ::GATGTGCC CAACATGCTT GTGCACGGGA
<susceptible RILs_4.#332          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
<tolerant RILs_47760#328          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
>Vada_47760         #320          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
>L94_47760          #319          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
                                  ........................................
consensus           #361          TGTTTTCCAG GTGATGTGCC CAACATGCTT GTGCACGGGA
                                  ********** **

>HORVU6Hr1G047760   #401          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
>CDS_47760          #261          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
<susceptible RILs_4.#372          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
<tolerant RILs_47760#368          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
>Vada_47760         #360          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
>L94_47760          #359          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT
                                  ........................................
consensus           #401          ATGGCAATGG CAAGTGAGCA TGACCCACGT ATAGATCCCT


>HORVU6Hr1G047760   #441          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
>CDS_47760          #301          TTGACTAA
<susceptible RILs_4.#412          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
<tolerant RILs_47760#408          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
>Vada_47760         #400          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
>L94_47760          #399          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
                                  ........................................
consensus           #441          TTGACTAATG TTGGGCAATT TGACTTGTGC CTGGTGGTTA
```

Supplemental data 6: Alignment HORVU6Hr1G047770 with sequencing data from susceptible and tolerant RILs and the lines L94 and Vada.
Introns are marked by asterisks.

```
<Vada_47770_1       >#1>                                ACCGC GAAACAACAG CCCGTCGCCC
<L94_47770_1        >#1>                               CACCGC GAAACAACAG CCCGTCGCCC
<tolerant RILs      >#1>                        GT ATTCCACCGC GAAACAACAG CCCGTCGCCC
<susceptible RILs   #1                  TCGTACTCGT ATTCCACCGC GAAACAACAG CCCGTCGCCC
                                        ........................................
consensus           #1                  TCGTACTCGT ATTCCACCGC GAAACAACAG CCCGTCGCCC


<HORVU6Hr1GO47770   >#1>                                                  TTCTCCATC
<Vada_47770_1       #26                 CGAGCCGCCC GGCATTTATC CAAACACCGC CTTCTCCATC
<L94_47770_1        #27                 CGAGCCGCCC GGCATTTATC CAAACACCGC CTTCTCCATC
<tolerant RILs      #33                 CGAGCCGCCC GGCATTTATC CAAACACCGC CTTCTCCATC
<susceptible RILs   #41                 CGAGCCGCCC GGCATTTATC CAAACACCGC CTTCTCCATC
                                        ........................................
consensus           #41                 CGAGCCGCCC GGCATTTATC CAAACACCGC CTTCTCCATC


<HORVU6Hr1GO47770   #10                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT
>CDS_47770          >#1>                                                    ATGTCTT
<Vada_47770_1       #66                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT
<L94_47770_1        #67                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT
<tolerant RILs      #73                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT
<susceptible RILs   #81                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT
                                        ........................................
consensus           #81                 CGCCCCCGCC ACACCGCGAG TCGCGGACCG GCCATGTCTT


<HORVU6Hr1GO47770   #50                 GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
>CDS_47770          #8                  GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
<Vada_47770_1       #106                GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
<L94_47770_1        #107                GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
<tolerant RILs      #113                GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
<susceptible RILs   #121                GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC
                                        ........................................
consensus           #121                GCCTCGCCGC GTCCTCGACG ACGCCGCCAG CGTCAAGGCC


<HORVU6Hr1G047770   #90                 TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
>CDS_47770          #48                 TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
<Vada_47770_1       #146                TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
<L94_47770_1        #147                TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
<tolerant RILs      #153                TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
<susceptible RILs   #161                TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC
                                        ........................................
consensus           #161                TGCGGCCCAG CGTCTCTTTG TTGCGGTAGC CGCTCCTTCC


<HORVU6Hr1G047770   #130                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
>CDS_47770          #88                 CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
<Vada_47770_1       #186                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
<L94_47770_1        #187                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
<tolerant RILs      #193                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
<susceptible RILs   #201                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
                                        ........................................
consensus           #201                CGTTGGCGGT CCATTGAGCC TCATCGCGCT CTACGGAGGA
```

```
<HORVU6Hr1G047770   #170          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
>CDS_47770          #128          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
<Vada_47770_1       #226          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
<L94_47770_1        #227          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
<tolerant RILs      #233          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
<susceptible RILs   #241          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC
                                  ........................................
consensus           #241          GTGAAGGCTC CAGGGTTCTA ACGATGGTAC GGAGGTGCTC


<HORVU6Hr1G047770   #210          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
>CDS_47770          #168          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
<Vada_47770_1       #266          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
<L94_47770_1        #267          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
<tolerant RILs      #273          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
<susceptible RILs   #281          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA
                                  ........................................
consensus           #281          TTCCGCCGGC GACTCCAGGG CGGCCGGAGA CGGCAGCCTA


<HORVU6Hr1G047770   #250          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
>CDS_47770          #208          TCCAG::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #306          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
<L94_47770_1        #307          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
<tolerant RILs      #313          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
<susceptible RILs   #321          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
                                  ........................................
consensus           #321          TCCAGGTTGC CTTTTACACC AGCCTCCGCA TCAAATCTAA
                                       ***** ********** ********** **********


<HORVU6Hr1G047770   #290          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
>CDS_47770          #248          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #346          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
<L94_47770_1        #347          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
<tolerant RILs      #353          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
<susceptible RILs   #361          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
                                  ........................................
consensus           #361          TTTCCCCTTA TTTGTGCTTA CGAAATTGAT GGAATGACAA
                                  ********** ********** ********** **********


<HORVU6Hr1G047770   #330          GGTTGTTAAG TTACTACTTG GTGCTTTGTA TGATAAATTC
>CDS_47770          #288          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #386          GGTTGTTAAG TTACTACTTG GTGCTTTGTA TGATAAATTC
<L94_47770_1        #387          GGTTGTTAAG TTACTACTTG GTGCTTTGTA TGATAAATTC
<tolerant RILs       #393          GGTTGTTAAG TTACTACTTG GTGCTTTGTA
TGATAAATTC
<susceptible RILs   #401          GGTTGTTAAG TTACTACTTG GTGCTTTGTA TGATAAATTC
                                  ........................................
consensus           #401          GGTTGTTAAG TTACTACTTG GTGCTTTGTA TGATAAATTC
                                  ********** ********** ********** **********


<HORVU6Hr1G047770   #370          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
>CDS_47770          #328          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #426          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
<L94_47770_1        #427          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
<tolerant RILs      #433          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
<susceptible RILs   #441          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
                                  ........................................
consensus           #441          ATGGTTGTGC CGGAGTAAAC TTGAGATTTT ACAACTTCCT
                                  ********** ********** ********** **********


<HORVU6Hr1G047770   #410          GGTCTAGTAT ACGAGTTTTC AACATTTATC TTCGACTTAT
>CDS_47770          #368          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #466          GGTCTAGTAT ACGAGTTTTC AACATTTATC TTCGACTTAT
<L94_47770_1        #467          GGTCTAGTAT ACGAGTTTTC AATATTTATC TTCGACTTAT
<tolerant RILs      #473          GGTCTAGTAT ACGAGTTTTC AATATTTATC TTCGACTTAT
<susceptible RILs   #481          GGTCTAGTAT ACGAGTTTTC AACATTTATC TTCGACTTAT
                                  ........................................
consensus           #481          GGTCTAGTAT ACGAGTTTTC AACATTTATC TTCGACTTAT
                                  ********** ********** ********** **********
```

```
<HORVU6Hr1G047770   #450    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
>CDS_47770          #408    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #506    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
<L94_47770_1        #507    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
<tolerant RILs      #513    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
<susceptible RILs   #521    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
                            ..........................................
consensus           #521    GAGTGGGTAC ATATCATATA CTAGTGTGCT AAAGCGCAAA
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #490    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
>CDS_47770          #448    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #546    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
<L94_47770_1        #547    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
<tolerant RILs      #553    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
<susceptible RILs   #561    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
                            ..........................................
consensus           #561    ATTCTGCATC AAACTGGTAG ATATACTTAC TCCGTTTTCC
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #530    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
>CDS_47770          #488    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #586    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
<L94_47770_1        #587    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
<tolerant RILs      #593    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
<susceptible RILs   #601    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
                            ..........................................
consensus           #601    TAAATACATG CCCTTTTAGA GATTTCACTA CAAACTACGT
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #570    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
>CDS_47770          #528    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #626    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
<L94_47770_1        #627    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
<tolerant RILs      #633    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
<susceptible RILs   #641    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
                            ..........................................
consensus           #641    ACGGATTATA GATTGTAGAT TCACTCGTCT TACTCCGTAT
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #610    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
>CDS_47770          #568    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #666    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
<L94_47770_1        #667    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
<tolerant RILs      #673    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
<susceptible RILs   #681    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
                            ..........................................
consensus           #681    GTGTAGTCTG TAGTGGAATA TCTGACAGAC TTATATTTAG
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #650    GAACGGAGGG AGTATTTATC TACAGCGAAT GCCTTATGTA
>CDS_47770          #608    :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #706    GAACGGAGGG AGTATTTATC TACAGCGAAT GCCTTATGTA
<L94_47770_1        #707    GAACGGAGGG AGTATTTATC TACAACGAAT GCCTTATGTA
<tolerant RILs      #713    GAACGGAGGG AGTATTTATC TACAACGAAT GCCTTATGTA
<susceptible RILs   #721    GAACGGAGGG AGTATTTATC TACAGCGAAT GCCTTATGTA
                            ..........................................
consensus           #721    GAACGGAGGG AGTATTTATC TACAGCGAAT GCCTTATGTA
                            ********** ********** ********** **********

<HORVU6Hr1G047770   #690    CATCTTTTGG AATGCTTACT AGACTAACAT CTAGTACAAA
>CDS_47770          #648    :::::::::: :::::::::: :::::::::: ::::::::::
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<L94_47770_1        #747    CATCTTTTGG AATGCTTACT AGACTAACAT CTAGTACAAA
<tolerant RILs      #753    CATCTTTTGG AATGCTTACT AGACTAACAT CTAGTACAAA
<susceptible RILs   #761    CATCTTTTGG AATGCTTACT AGACTAACAT CTAGTACAAA
                            ..........................................
consensus           #761    CATCTTTTGG AATGCTTACT AGACTAACAT CTAGTACAAA
                            ********** ********** ********** **********
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<HORVU6Hr1G047770   #730          CATTCTCTAT AAACTCAGTT TACAAGAAGC CCGCTAGATC
>CDS_47770          #688          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_1       #786          CATTCTCTAT AAACTCAGTT TACAA
<L94_47770_1        #787          CATTCTCTAT AAGCTCAGTT TACAAGAAG
<tolerant RILs      #793          CATTCTCTAT AAGCTCAGTT TACAAGAAGC CCGCT
<susceptible RILs   #801          CATTCTCTAT AAACTCAGTT TACAAGAAGC CC
                                  ........................................
consensus           #801          CATTCTCTAT AAACTCAGTT TACAAGAAGC CCGCTAGATC
                                  ********** ********** ********** **********

<HORVU6Hr1G047770   #770          CACGGCAATT CGCTCATGCT TCTCCTCTCT AGTTTTTGCA
>CDS_47770          #728          :::::::::: :::::::::: :::::::::: ::TTTTTGCA
>Vada_47770_2       >#1>                       TGCT TCTCCTCTCT AGTTTTTGCA
>L94_47770_2        >#1>                       TGCT TCTCCTCTCT AGTTTTTGCA
>tolerant RILs      >#1>                          T TCTCCTCTCT AGTTTTTGCA
>susceptible RILs   >#1>                GCTCATGCT TCTCCTCTCT AGTTTTTGCA
                                  ........................................
consensus           #841          CACGGCAATT CGCTCATGCT TCTCCTCTCT AGTTTTTGCA
                                  ********** ********** ********** **

<HORVU6Hr1G047770   #810          TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
>CDS_47770          #768          TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
>Vada_47770_2       #25           TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
>L94_47770_2        #25           TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
>tolerant RILs      #22           TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
>susceptible RILs   #30           TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT
                                  ........................................
consensus           #881          TAATTGAAGG CCCAGAGACA ATACAAGACT TCGTTCAGAT

<HORVU6Hr1G047770   #850          GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
>CDS_47770          #808          GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
>Vada_47770_2       #65           GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
>L94_47770_2        #65           GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
>tolerant RILs      #62           GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
>susceptible RILs   #70           GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC
                                  ........................................
consensus           #921          GCAATCACAG GAGATTCAAG ACAACATCAA GAGTCGGCGC

<HORVU6Hr1G047770   #890          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
>CDS_47770          #848          AGCAAAATAT TCCTTTTAAT GGAAGAGGT: ::::::::::
>Vada_47770_2       #105          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
>L94_47770_2        #105          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
>tolerant RILs      #102          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
>susceptible RILs   #110          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
                                  ........................................
consensus           #961          AGCAAAATAT TCCTTTTAAT GGAAGAGGTG CCTGTTTTTC
                                                                 * **********

<HORVU6Hr1G047770   #930          ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
>CDS_47770          #888          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #145          ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
>L94_47770_2        #145          ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
>tolerant RILs      #142          ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
>susceptible RILs   #150          ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
                                  ........................................
consensus           #1001         ATGCTTGTAA TTTTTTCAGC TTTGTAACTA ACATCCTACT
                                  ********** ********** ********** **********

<HORVU6Hr1G047770   #970          TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
>CDS_47770          #928          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #185          TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
>L94_47770_2        #185          TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
>tolerant RILs      #182          TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
>susceptible RILs   #190          TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
                                  ........................................
consensus           #1041         TAGCTTACCT CGGTATGATT ATATGAGTAA CGTTCCCAGG
                                  ********** ********** ********** **********
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<HORVU6Hr1G047770   #1010          TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
>CDS_47770          #968           :CAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
>Vada_47770_2       #225           TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
>L94_47770_2        #225           TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
>tolerant RILs      #222           TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
>susceptible RILs   #230           TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
                                   ........................................
consensus           #1081          TCAGACGACT ACGGGTGCAG CAGCGAATCA GAACTGCTGA
                                   *

<HORVU6Hr1G047770   #1050          AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
>CDS_47770          #1008          AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
>Vada_47770_2       #265           AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
>L94_47770_2        #265           AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
>tolerant RILs      #262           AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
>susceptible RILs   #270           AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG
                                   ........................................
consensus           #1121          AAGCAGATGT TCCAGCAGTG AAGAAAATGA GATGCCGGAG


<HORVU6Hr1G047770   #1090          ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
>CDS_47770          #1048          ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACG:::::::
>Vada_47770_2       #305           ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
>L94_47770_2        #305           ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
>tolerant RILs      #302           ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
>susceptible RILs   #310           ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
                                   ........................................
consensus           #1161          ATGCCATCTA CCATTCCGTT TTTGCCTTAT ACGGTGACCG
                                                                       *******

<HORVU6Hr1G047770   #1130          ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
>CDS_47770          #1088          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #345           ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
>L94_47770_2        #345           ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
>tolerant RILs      #342           ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
>susceptible RILs   #350           ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
                                   ........................................
consensus           #1201          ACCCCCGGCC CTGAATTTTT TTATGTGCAA ACATGGCTTT
                                   ********** ********** ********** **********

<HORVU6Hr1G047770   #1170          TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
>CDS_47770          #1128          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #385           TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
>L94_47770_2        #385           TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
>tolerant RILs      #382           TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
>susceptible RILs   #390           TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
                                   ........................................
consensus           #1241          TTGAGCTCTT CTTGGTAATA TTCATTTCTT TTGTTTTGTA
                                   ********** ********** ********** **********

<HORVU6Hr1G047770   #1210          TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
>CDS_47770          #1168          ::::::::TC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
>Vada_47770_2       #425           TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
>L94_47770_2        #425           TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
>tolerant RILs      #422           TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
>susceptible RILs   #430           TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
                                   ........................................
consensus           #1281          TGAAGTAGTC ACCAAAGACA ATGAAGCAAC TCTATTTGAC
                                   ********

<HORVU6Hr1G047770   #1250          ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
>CDS_47770          #1208          ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
>Vada_47770_2       #465           ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
>L94_47770_2        #465           ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
>tolerant RILs      #462           ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
>susceptible RILs   #470           ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
                                   ........................................
consensus           #1321          ATCATTCTCT TTCATATCTG GGATAATCAT TTTTGGTGGC
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>Vada_47770_2       #505           TTGATAGCCC CAATAGTAAG TGCCAGCTTC ATCTCTCTGT
>L94_47770_2        #505           TTGATAGCCC CAATAGTAAG TGCCAGCTTC ATCTCTCTGT
>tolerant RILs      #502           TTGATAGCCC CAATAGTAAG TGCCAGCTTC ATCTCTCTGT
>susceptible RILs   #510           TTGATAGCCC CAATAGTAAG TGCCAGCTTC ATCTCTCTGT
                                   ........................................
consensus           #1361          TTGATAGCCC CAATAGTAAG TGCCAGCTTC ATCTCTCTGT
                                             ***** ********** **********

<HORVU6Hr1G047770   #1330          AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
>CDS_47770          #1288          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #545           AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
>L94_47770_2        #545           AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
>tolerant RILs      #542           AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
>susceptible RILs   #550           AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
                                   ........................................
consensus           #1401          AAATTTGTTG TTACTTGCTC CCTAAAACCA AAGACTATGT
                                   ********** ********** ********** **********

<HORVU6Hr1G047770   #1370          TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
>CDS_47770          #1328          :::::::::: :::::::::: :::::::::: ::::::::::
>Vada_47770_2       #585           TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
>L94_47770_2        #585           TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
>tolerant RILs      #582           TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
>susceptible RILs   #590           TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
                                   ........................................
consensus           #1441          TGGTAATTTA ATGTAGCTCT TCTAATATAT TATCTCTTCT
                                   ********** ********** ********** **********

<HORVU6Hr1G047770   #1410          TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
>CDS_47770          #1368          :::::::::: :::::::::: ::::CTTGAA CTGAAATTAG
>Vada_47770_2       #625           TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
>L94_47770_2        #625           TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
>tolerant RILs      #622           TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
>susceptible RILs   #630           TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
                                   ........................................
consensus           #1481          TAAAACAATG ATTATTTGGT GAAGCTTGAA CTGAAATTAG
                                   ********** ********** ****

<HORVU6Hr1G047770   #1450          GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
>CDS_47770          #1408          GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
>Vada_47770_2       #665           GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
>L94_47770_2        #665           GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
>tolerant RILs      #662           GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
>susceptible RILs   #670           GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT
                                   ........................................
consensus           #1521          GGCTTGGTGG TACCTCTTAT GAAGATTTTA TACGGAACAT


<HORVU6Hr1G047770   #1490          TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
>CDS_47770          #1448          TTATTTGCCT CTTCAGTTAA G::::::::: :::::::::
>Vada_47770_2       #705           TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
>L94_47770_2        #705           TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
>tolerant RILs      #702           TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
>susceptible RILs   #710           TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
                                   ........................................
consensus           #1561          TTATTTGCCT CTTCAGTTAA GGTGATTTAA AGAAACTTGT
                                                ********* **********
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<HORVU6Hr1G047770   #1530          CCTCTTACAG AATTATCCAG CAATGTTTAG AATAATAACT
>CDS_47770          #1488          :::::::::: :::::::::: :::::::::: ::::::::::
<Vada_47770_3       >#1>                          CGACGTTGTA AA:ACGACCT
<L94_47770_3       >#1>                           CGACGTTGTA AA:ACGACCT
>tolerant RILs      >#1>                      CN: CGACGTTGTA AA:ACGACCT
<susceptible RILs   >#1>                  CGTCA: CGACGTTGTA AA:ACGACCT
>Vada_47770_2       #745           CCTCTTACAG AATTATCCAG CA
>L94_47770_2        #745           CCTCTTACAG AATTATCCAG CA
>tolerant RILs      #742           CCTCTTACAG AATTATCCAG CAATGTTTAG AATAATA
>susceptible RILs   #750           CCTCTTACAG AATTATCCAG CAATGTTTAG AA
                                   ..........................................
consensus           #1601          CCTCTTACAG AATTATCCAG CAACGTTGTA AA:ACGACCT
                                   ********** ********** ********** **********

<HORVU6Hr1G047770   #1570          TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
>CDS_47770          #1528          :::::::::: :::::::::: ::::TCAGGT AGATCCCATA
<Vada_47770_3       #21            TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
<L94_47770         #21            TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
>tolerant RILs      #24            TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
<susceptible RILs   #27            TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
                                   ..........................................
consensus           #1641          TGATACTTCT TGGCTGTCCA TTAGTCAGGT AGATCCCATA
                                   ********** ********** ****

<HORVU6Hr1G047770   #1610          GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG
>CDS_47770          #1568          GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG
<Vada_47770_3       #61            GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG
<L94_47770         #61            GTGGCATCCT TT
>tolerant RILs      #64            GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG
<susceptible RILs   #67            GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG
                                   ..........................................
consensus           #1681          GTGGCATCCT TTTCAGGTGG TGCAGTTGGT GTAATTTCAG


<HORVU6Hr1G047770   #1650          CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA
>CDS_47770          #1608          CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA
<Vada_47770_3       #101           CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA
>tolerant RILs      #104           CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA
<susceptible RILs   #107           CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA
                                   ..........................................
consensus           #1721          CCTTAATGTT GGTTGAAGTG AAAAATGTGA GGCAGCAAGA


<HORVU6Hr1G047770   #1690          AAAGAACAGA TGCACATATT GTCATGGGAC AGGTATGAGT
>CDS_47770          #1648          AAAGAACAGA TGCACATATT GTCATGGGAC AGGTATGAGT
<Vada_47770_3       #141           AAAGAAC
>tolerant RILs      #144           AAAGAACAAW T
<susceptible RILs   #147           AAAGAAC
                                   ..........................................
consensus           #1761          AAAGAACAGA TGCACATATT GTCATGGGAC AGGTATGAGT
                                          *+

<HORVU6Hr1G047770   #1730          GAATTCGAGC TCGGTA:::: :::::::::: ::::::::::
>CDS_47770          #1688          GAATTCGAGC TCGGTANNNN NNNNNNNNNN NNNNNNNNNN
                                   ..........................................
consensus           #1801          GAATTCGAGC TCGGTANNNN NNNNNNNNNN NNNNNNNNNN
                                             **** ********** **********
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Eidesstattliche Erklärung

Ich erkläre:

Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.
Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.
Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Sandra Färber


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Federal Research Centre for Cultivated Plants