Identification and Characterization of a Gene Conferring Resistance Against Barley Yellow Dwarf Virus in Maize

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Abbreviations

abbreviation	explanation
ABA	abscisic acid
ABP1	auxin binding protein 1
AGO	ARGONAUTE protein
bp	basepair
BYD	barley yellow dwarf disease
BYDV	Barley yellow dwarf virus
CF	pathway phase (stylet penetration oft he mesophyll)
CP	capsid/coat protein
DAS-ELISA	double antibody sandwich enzyme-linked immunosorbent assay
DCL	Dicer-like protein
DEG	differently expressed gene
dsRNA	double-stranded RNA
E1	phloem salivation phase
E2	phloem sap ingestion phase
EPG	electrical penetration graph
ETI	effector-triggered immunity
EX	mean virus titer per experimental unit
G	xylem sap ingestion
GO	Gene Ontology
GWAS	genome wide association study
H^2	broad sense heritability
HIF	heterozygous inbred family
hpi	hours post infection / inoculation
HR	hypersensitive response
ICTV	International Committee on the Taxonomy of Viruses
InDel	small insertion or deletion (<50bp)
IR	infection rate
JA	jasmonic acid
KASP™	Kompetitive allele-specific polymerase chain reaction
kbp	kilo basepair
KEGG	Kyoto Encyclopedia of Genes and Genomes
MAPKs	mitogen-activated protein kinases
Mbp	mega basepair
MP	movement protein
MRDD	Maize rough dwarf disease
mRNA	Messenger RNA
NAM	nested association mapping
NB-LRRs / NLRs	nucleotide-binding leucine-rich repeat proteins
NMD	nonsense-mediated decay
NO	nitric oxide

Np	nonprobing
NS	Nuclear speckle
ORF	open reading frame
PAMP	Pathogen-associated molecular pattern
PCD	programmed cell death
PCR	polymerase chain reaction
PR	pathogenesis-related
PRRs	pattern recognition receptors
PTI	PAMP-triggered immunity
qRT-PCR	Quantitative reverse transcriptase PCR
QTL	quantitative trait locus
R-gene	resistance genes
RdRp	RNA-dependent RNA polymerase
RISC	RNA-induced silencing complex
RLKs	receptor-like kinases
RLPs	receptor-like proteins
RNAi	RNA interference; post-transcriptional gene silencing
ROS	reactive oxygen species
RTP	readthrough protein
SA	salicylic acid
SCMV	Sugar cane mosaic virus
SNP	single nucleotide polymorphims
SnRK1	sucrose nonfermenting related protein kinase 1
ssRNA	single stranded RNA
SV	structural variation
TF	transcription factor
TMV	Tobacco mosaic virus
Upf1	Upstream frameshift 1
UPS	ubiquitin proteasome system
UTR	untranslated region
VOC	Volatile organic compound
VSR	viral suppressor of RNAi
YDVs	yellow dwarf viruses of cereals

1 General Introduction

1.1 *Zea mays* (maize, corn)

Maize (*Zea mays* L., also commonly known as corn) was domesticated more than 9,000 years ago in southern Mexico/Meso America (Awika 2011, Kennett et al. 2020). Today, growth areas cover temperate regions, the tropics, and subtropics (Erenstein et al. 2022).

In 2021, maize was grown at more than 205 million ha worldwide with a yield of more than 1.2 billion metric tons (faostat 2023). This accounts for approximately 12 % of the global production of primary crops (faostat analytical brief 41), making maize one of the economically most important crops worldwide.

Maize is a very versatile multi-purpose crop. Although it is primarily used as feed globally, maize is also an important food crop in Sub-Saharan Africa and Latin America (Erenstein et al. 2022, Shiferaw et al. 2011). In industrialized parts of the world, such as North America or Europe, maize is used for industrial purposes such as bio-ethanol production and is consumed directly or in highly processed forms (Erenstein et al. 2022).

Maize production is threatened by a variety of pests and diseases. More than thirty parasitic nematodes, at least eight bacterial diseases and three mollicute diseases, more than a hundred fungal and oomycete diseases are known to infect maize (Munkvold 2017). A recent study by Savary et al. (2019) estimates global yield losses for maize due to pests and pathogens to be 22.5 %, ranging between 19.5 % in South America and 41.1 % in the Indo-Gangetic Plain. Insect pests reduce maize production by directly attacking roots, leaves, stalks, ears and tassels but also grain during storage (Shiferaw et al. 2011). Wounds caused by insect feeding also favor entry of pathogens (Munkvold and Sobek 1999). Sap sucking pests, like aphids (*Aphididae*) and leafhoppers (*Cicadellidae*) cause limited economic damage through feeding (Meissle et al. 2010). However, they threaten crop production by spreading plant viruses (Fereres and Raccah 2015).

More than thirty viruses are known to infect maize, of which nine are known to be transmitted by aphids, nine by planthoppers, thirteen by leafhoppers and four by beetles or mites (Sastry et al. 2019, Munkvold 2017). Some of the maize-infecting viruses are attributed to significant yield losses by themselves or in concurrent infections with other viruses (Redingbaugh and Zambrano 2014).

1.2 Plant viruses

Viruses are obligate intracellular parasites that consist of a virus-encoded protein coat called capsid and a nucleic acid (Hull 2014b). A single, fully assembled virus particle is called virion. The nucleic acid can be DNA or RNA that is double- or single-stranded with a circular, linear or segmented arrangement (Hull 2014b, ICTV 2022a). Additionally, single stranded RNA (ssRNA) virus genomes may be either positive sense (+) or negative sense (−) (Hull 2014b).

The International Committee on the Taxonomy of Viruses (ICTV) lists approximately 2,000 different virus species that infect plants (ICTV 2022b). The evolutionary origins of plant viruses are polyphyletic (Hull 2014d, Koonin et al. 2015). However, they share some common features - most importantly, the ability to spread from cell to cell via plasmodesmata (Lefeuvre et al. 2019).

Plant viruses may be transmitted via seed, pollen, fungi, nematodes, parasitic plants, or wounding (for review see Hull 2014c, Jones and Naidu 2019, Lefeuvre et al. 2019). However, about 55% of plant-infecting viruses are transmitted by Hemipteran insects (Hogenhout et al. 2008). Aphids are the most important vectors, transmitting nearly 30 % of all plant virus species (Hogenhout et al. 2008).

Virus transmission through insect vectors consists of at least three step-wise processes: i) acquisition: the uptake of virus from an infected source, ii) the stable retention of acquired virions at requisite sites within the vector and iii) inoculation: the release of bound or retained virions from the insect vector and their delivery to a site of infection (Ng and Perry 2004).

Depending on their mode of transmission, insect-vectored plant viruses can be classified into four groups i) non-persistently transmitted, stylet-borne viruses; ii) semi-persistently transmitted, foregut-borne viruses; iii) persistently transmitted, circulative viruses; and iv) persistently transmitted, propagative viruses (Nault 1997). Non-persistent and semi-persistent transmission is characterized by very short acquisition and inoculation times of seconds to hours and vectors typically remain viruliferous for comparably short periods of time (Brault et al. 2010, Hogenhout et al. 2008, Ng and Perry 2004). In contrast, persistent transmission requires acquisition periods of hours to days, but vectors stay viruliferous for long periods of time (Brault et al. 2010, Hogenhout et al. 2008, Ng and Perry 2004). Following ingestion, viruses cross the gut epithelium, enter the haemocoel and circulate through the vector until they reach the accessory salivary gland cells, and are inoculated into plants with

salivary gland secretions (Gildow and Gray 1993). Virus particles have to cross several membranes during circulative transmission, leading to high vector specificity (Brault et al. 2010, Hogenhout et al. 2008). Depending on whether a virus replicates in the vector and is transmitted to vector offspring or not, circulative transmission is described as 'propagative' or 'nonpropagative' (Brault et al. 2010, Hogenhout et al. 2008, Nault, 1997, Ng and Perry 2004).
Once a virus entered a suitable host plant cell, the infection cycle is initiated. Core parts of the infection cycle of plant by a virus are: i) RNA translation, ii) genome replication, iii) virion formation, and iv) virion movement from cell-to-cell and systematically via the phloem (Garcia-Ruiz 2019).
Plant viruses have very small, condensed genomes that encode only for one to twelve proteins (Hull 2014b). These are replication, capsid (CP) and movement (MP) proteins (Garcia-Ruiz 2019, Hull 2014b). Additionally, viruses encode individual auxiliary proteins (e.g. for transmission by vectors) and proteins that target key components of antiviral immunity of the host plant (Garcia-Ruiz 2019, Hull 2014b).
However, the use of host factors is essential for viruses to complete their life cycle. Because viruses lack ribosomes, translation of viral proteins from RNA is dependent on the host cellular translation machinery (Garcia-Ruiz 2018). Next to viral MPs, virus movement through plasmodesmata also requires membranes, proteins, microtubules or actin filaments from the host plant cell (Garcia-Ruiz 2018).
During infection, viruses interfere with host plant's cellular machinery, disrupting host physiology and resulting in disease (for review see Osterbaan and Fuchs 2019, Pallas and García 2011). Additionally, plant viruses are also able to manipulate vectors and host plants to promote their own spread (for review see Blanc et al. 2016, Mauck et al. 2019, Ziegler-Graff 2020).
Climate change is predicted to affect the distribution and survival of plant viruses and their vectors, which are expected to increase in many geographic regions. Furthermore, the virulence and pathogenicity of plant viruses might also be affected, increasing the frequency and scale of disease outbreaks (Trebicki 2020).

Resistance mechanisms against plant viruses

To defend themselves against pathogen attack, plants rely on the innate immunity of each cell and on systemic signals emanating from infection sites (Jones and Dangl 2006). The

establishment of pathogen infection in general and virus infection in particular is genetically determined by the availability of pro-viral factors that are necessary for genome replication and virus particle movement, as well as by the balance between plant defense and viral suppression of defense responses (Garcia-Ruiz 2019). There is a constant evolutionary race of arms between hosts and pathogens involving defense mechanisms of host plants, over-coming of defense by pathogens, establishment of new defense mechanisms by host plants and so on. This is referred to as the "Zig-Zag Model" (Jones and Dangl 2006).

Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) forms the basal layer of defense against pathogens (Jones and Dangl 2006). During this process, conserved pathogen-associated proteins, carbohydrates, chitin or fragments of them are recognized by specific pattern recognition receptors (PRRs) at the cell surface (for review see Macho and Zipfel 2014, Palukaitis and Yeon 2020). In case of plant viruses, dsRNAs act as PAMPs activating PTI (Niehl et al. 2016). However, no other virus-derived PAMP than dsRNA has been identified yet, and antiviral PRRs remain to be described (Leonetti et al. 2021, Teixeira et al. 2019).

When PAMPs are recognized by PRRs, a signaling cascade is activated, leading to the biosynthesis of specific defense molecules and enabling plants to respond rapidly and efficiently to a wide range of pathogens (Jones and Dangl 2006; Macho and Zipfel 2014).

However, some pathogen species or strains of a pathogen species may suppress PTI by specific proteins called effectors (Jones and Dangl 2006). Most viral genomes also encode suppressors of PTI (Leonetti et al. 2021, Teixeira et al. 2019). In contrast, some genotypes of the host plant species are able to recognize pathogenic effectors directly or indirectly by intracellular receptors, activating resistance pathways referred to as effector-triggered immunity (ETI) (Jones and Dangl 2006). ETI is mediated by resistance genes (*R*-genes) that usually encode for nucleotide-binding leucine-rich repeat proteins, referred to as NB-LRRs or NLRs (Kourelis and van der Hoorn 2018, Palukaitis and Yeon 2020). ETI-mediated virus resistance is generally dominant and monogenic (Leonetti et al. 2021).

Pathogen detection by PRRs and NLRs during PTI and ETI initiates signaling cascades, including mitogen-activated protein kinases (MAPKs), G-proteins, ubiquitin, calcium ion influx, phytohormones, accumulation of reactive oxygen species (ROS) and nitric oxide (NO), transcription factors (TFs), and epigenetic modifications that regulate the expression of patho-genesis-related (PR) genes (Andersen et al. 2018). This leads to various responses: hypersensitive response (HR), cell wall modification, closure of stomata, callose deposition at

plasmodesmata or production of various defense molecules (e.g., chitinases, protease inhibitors, defensins, and phytoalexins) (Andersen et al. 2018, Calil and Fontes 2017).

The production of ROS is characteristic to both PTI and ETI. ROS are a group of highly reactive molecules derived from molecular oxygen (Mittler et al. 2022). ROS function as local and systemic signaling molecules in plant growth and development as well as in defense against pathogens (Mittler et al. 2022). On the one hand, ROS play an important role in plant defense against viruses by triggering defense mechanisms (for review see Hernández et al. 2016). On the other hand, there is evidence that some viruses are dependent on ROS for robust viral RNA replication (Hyodo et al. 2017). In both scenarios, plant viruses manipulate ROS levels in host cells to facilitate infection (Hyodo et al. 2017, Yang et al. 2018).

Similar to ROS, phytohormones like salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) regulate signaling during plant defense against viruses and other pathogens (Alazem et al. 2019, Andersen et al. 2018, Palukaitis and Yeon 2020, Zhao and Li 2021).

Besides regulation of transcription, plants use post-transcriptional regulation of mRNAs to shape the defense-related transcriptome (for review see Floris et al. 2009, Mazzucotelli et al. 2008). A specialized form of RNA degradation is nonsense-mediated decay (NMD). NMD contributes to plant innate immunity by controlling expression of defense-related genes like NLR-encoding genes or by targeting viral RNA directly (Garcia et al. 2014, Gloggnitzer et al. 2014).

Selective protein degradation is another layer of regulation of response to viruses and mediated by the ubiquitin proteasome system (UPS) (Dubiella and Serrano 2021). The UPS is involved in all steps of plant defense responses from pathogen perception to regulation of downstream signalling (Dubiella and Serrano 2021). Additionally, viral proteins may be degraded by the UPS as well (Dubiella and Serrano 2021).

Post-transcriptional gene silencing - also known as RNA interference (RNAi) - is a general mechanism of post-transcriptional gene regulation and relies on the generation of small double-stranded RNAs (dsRNAs) (Baulcombe 2004). RNAi is a primary defense mechanism of plants against virus infection (Leonetti et al. 2021). This process regulates host transcript abundance but may also degrade viral RNA (Baulcombe 2004, Leonetti et al. 2021). Similarly, dsRNAs of viral origin can tag host transcripts and, thus, may not only overcome defense mechanisms but also induce disease symptoms (Conti et al. 2017). Additionally, viruses possess a variety of co-evolving viral suppressors of RNAi (VSRs), which could enhance the

viral pathogenicity within susceptible hosts (for review see Baulcombe 2004, Lewsey et al. 2018).

Resistance against pathogens is usually considered being of either qualitative or quantitative nature. These terms are used to distinguish both the phenotypic expression of resistance and the type of inheritance that is typically associated (Niks et al. 2015). Qualitative resistance is typically conferred by dominant *R*-genes that tend to provide complete or near-complete resistance (Nelson et al. 2018). In contrast, quantitative pathogen resistance is controlled by multiple genes of small effect (Niks et al. 2015). Thus, populations show a continuum of phenotypic variation for this trait. Genomic regions harboring genes conferring quantitative resistance are known as quantitative trait loci (QTL).

Resistance genes against plant viruses

Until now, more than 300 genes conferring resistance against pathogens have been cloned and for many of them the resistance mechanism is known (Kourelis and van der Hoorn 2018). For example, BAK1 is a central PTI-regulator and also contributes to resistance against diverse RNA viruses (Kørner et al. 2013). An example of indirect recognition of an effector by an NLR is the *N* gene product of tobacco (Caplan et al. 2008, Whitham et al. 1994). The indirect recognition of the 50 kDa helicase (p50) domain of Tobacco mosaic virus (TMV) by *N* is mediated through the chloroplastic protein NRIP1 (Caplan et al. 2008).

In addition to PTI, ETI, and RNAi, plants may become resistant to pathogens through loss of susceptibility (Kourelis and van der Hoorn 2018). On the one hand, active loss of susceptibility is mediated by actively interrupting a key pathogenicity process. In the case of plant virus infection, host genes have been identified that target all stages of the viral replication cycle (reviewed in Garcia-Ruiz 2019). These are: i) viral RNA translation, ii) virus replication complex formation, iii) accumulation and activity of replication proteins, iv) RNA replication, v) mRNA stability, vi) virus movement within the host, and vii) virion assembly (Garcia-Ruiz 2019).

On the other hand, passive loss of susceptibility is mediated by loss-of-function-mutations in components of cellular pathways or by mutation in a host component, leading to the inability of the pathogen to manipulate the host. This is often a recessive trait, but may also involve dominant-negative alleles (Kourelis and van der Hoorn 2018).

Virus resistance genes in maize

Several loci conferring resistance toward viruses are known in maize (for review see Redinbaugh et al. 2018). Interestingly, many but not all of these loci form clusters on chromosomes 3, 6, and 10, respectively (Redinbaugh et al. 2018).

To date, only a few virus-resistance genes in maize are cloned. These are *Scmv1*, *Scmv2*, and *qMrdd1*. Two epistatically interacting major SCMV (sugar cane mosaic virus) resistance loci (*Scmv1* and *Scmv2*) are required to confer complete resistance against SCMV (Xing et al. 2006). *Scmv1* is located on the short arm of chromosome 6 and confers strong early resistance to SCMV (Melchinger et al. 1998). Liu et al. (2017) demonstrated that *ZmTrxh*, encoding an atypical h-type thioredoxin, is the causal gene of *Scmv1*. *ZmTrxh* possesses a strong molecular chaperone-like activity and suppresses accumulation of SCMV viral RNA (Liu et al. 2017). *ZmTrxh* alleles of resistant and susceptible genotypes share the identical coding and proximal promoter regions, but vary in the upstream regulatory regions (Liu et al. 2017). Additionally, the ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RbCS) was identified as interaction partner of *ZmABP1* (Leng et al. 2017).

Scmv2 is located on the long arm of chromosome 3 near the centromere (Melchinger et al. 1998, Xing et al. 2006). The casual gene of *Scmv2* is the auxin binding protein 1 (*ABP1*) (Leng et al. 2017). *ZmABP1* is active at later stages following SCMV infection (Leng et al. 2017, Xia et al. 1999, Xing et al. 2006) and its expression is greatly increased by SCMV infection in resistant genotypes (Leng et al. 2017). Thus, *Scmv2* is thought to reinforce resistance to viruses that escaped from resistance mechanisms of *ZmTrxh* (Leng et al. 2017). However, *Scmv2* alone does not provide any resistance (Xing et al. 2006). *ZmTrxh* and *ZmABP1* confer resistance without eliciting a phytohormone-mediated defense response (Leng et al. 2017, Liu et al. 2017).

Maize rough dwarf disease (MRDD) is caused by Fijiviruses MRDV, RBSDV, and MRCV (Milne and Lovisolo 1977). A major QTL, *qMrdd1,* was identified and explains 24.2 % to 39.3 % of the phenotypic variance of resistance to MRDD and confers recessive MRDD resistance (Tao et al. 2013). Recently, *qMrdd1* was found to be associated with Rab GDP dissociation inhibitor alpha (RabGDIα), *ZmGDIα*, which is the host susceptibility factor for RBSDV (Liu et al. 2020). Interaction of *ZmGDIα* and viral P7-1 protein is likely required for viral plasmodesmata targeting, cell-to-cell movement, and systemic spread (Liu et al. 2020). Insertion of a *helitron* transposon into *ZmGDIα* intron 10 induces alternative splicing to replace the wild-type exon

10 with a *helitron*-derived exon 10, causing the resistant allele. Compared to *ZmGDIα,* the gene product of *ZmGDIα-hel* binds viral P7-1 less effectively, resulting in reduction of MRDD disease severity index by ~30 % (Liu et al. 2020).

1.3 The barley yellow dwarf disease

Economic importance

The barley yellow dwarf disease (BYD) is one of the economically most important virus diseases of cereals worldwide (Choudhury et al. 2017, van den Eynde et al. 2020, Walls et al. 2019). The disease was first recognized in California in 1951 in barley (Oswald and Houston 1953). To date, more than 150 species of the family *Poaceae* are known to host BYD, including barley, wheat, oats, rye, triticale, sorghum, maize, rice and many wild grasses (Walls et al. 2019).

BYD is estimated to reduce global wheat production by 1 %, ranging from 0.35 % in China to 3.26 % in Northwest Europe (Savary et al. 2019). Locally, yield loss of cereal crops due to BYD of more than 80 % have been reported (Nancarrow et al. 2021). Yield losses are evoked by reduced numbers of i) tillers per plant, ii) seeds per tiller, and iii) seed weight (McKirdy et al. 2002; Perry et al. 2000, Riedell et al. 2003). Additionally, BYD negatively affects grain quality (Choudhury et al. 2019a, Peiris et al. 2019).

Symptoms

Symptoms of BYD in cereals differ between host species, virus strain, and environmental conditions but leaf discoloration and stunted growth are characteristic (Choudhury et al. 2017, Oswald and Houston 1953, Walls et al. 2019). In maize, red edges on leaves are a characteristic symptom of BYD (Beuve et al. 1999, Brown et al. 1984, Grüntzig et al. 1997, Horn et al. 2013, Osler et al. 1985, Pearson and Robb 1984, Stoner 1977). Furthermore, BYD was found to lead in maize to a reduction of i) plant height (Beuve et al. 1999), ii) total plant fresh weight (Panayotou 1977), iii) dry matter (Pearson and Robb 1984) and iv) grain yield (Beuve et al. 1999, Pearson and Robb 1984).

In barley, BYD has a negative influence on leaf and vascular bundle morphology and leads to decreased leaf width, vascular bundle area, sieve element area and xylem vessel area (Choudhury et al. 2018, Esau 1957a, Paulmann et al. 2018). BYD leads to premature necrosis

of the phloem which later spreads to the xylem and ground parenchyma (Esau 1957b, Panayotou 1978). Together with callose deposition at plasmodesmata (Esau 1957a), phloem degeneration potentially restricts the translocation of photosynthesis products, likely being the reason for increased carbohydrate content in leaves (Jensen 1972, Liu and Buchenauer 2005). Additionally, BYD infection damages chloroplasts and reduces chlorophyll content, leading to a reduction in photosynthesis and increased respiration (Jensen 1972, Jensen and van Sambeek 1972, Rong et al. 2018).

Causative agent and transmission

BYD is caused by at least ten different viruses called barley yellow dwarf viruses (BYDVs) and cereal yellow dwarf viruses (CYDVs) (Choudhury et al. 2017, Miller et al. 2002, Miller and Lozier 2022, Walls et al. 2019) here collectively referred to as yellow dwarf viruses (YDVs). These viruses are genetically different but share similar features and a similar biology (for review see Choudhury et al. 2017, Miller et al. 2002, Miller and Lozier 2022, Walls et al. 2019). YDVs are phloem-limited viruses that are transmitted by different aphid vectors in a persistent and circulative manner, without replication in the vector or transmission to vector offspring (Miller et al. 2002).

Classification and characteristics of YDVs

Differences in vector specificity formed the basis of the first classification of YDVs (Rochow 1969, Rochow and Muller 1971). Later, as sequences became available, a classification based on sequence of CP and MP genes, BYDVs and CYDVs were assigned as genera Luteovirus and Polerovirus, respectively (D'Arcy et al. 2000).

However, the most recent classification (2022) by the ICTV based on the gene encoding RdRp categorizes BYDVs to the genus *Luteovirus* of family *Tombusviridae* in order *Tolivirales* (ICTV 2022c). CYDVs are placed in the far more distant genus *Polerovirus* of family *Solemoviridae* in order *Sobelivirales* (ICTV 2022c). Some YDVs still remain unassigned (ICTV 2022c).

Virions of Luteoviruses and Poleroviruses are 25 to 30 nm in diameter, hexagonal in outline and have no envelope (Hull 2014a, ICTV 2012). The capsid consists of a major CP of 21–23 kDa in size and smaller amounts of a readthrough protein (RTP), that encapsulate a single molecule of linear positive-sense single-stranded RNA ((+)ssRNA) (Hull 2014a, ICTV 2012). The genome

ranges from 5.6 kb to 6.0 kb in size and encodes for 6 or 7 proteins but does not contain a poly-A tail or 5'-cap (Hull 2014a, ICTV 2012, Miller and Lozier 2022).

Luteoviruses and Poleroviruses differ in the 5'-half of their genomes that contain open reading frames (ORFs) that encode for proteins involved in suppression of RNAi and viral replication (Miller et al. 2002). In Luteoviruses, ORF1 encodes a replication associated protein (P1) that likely lines the interior of virus replication vesicles (Miller and Lozier 2022). The RdRp (P1-2) is a C-terminal extended version of P1, containing the product of ORF 2 (Miller et al. 1988a). ORF 2 does not encode a separate gene product. In Poleroviruses, P1 is also involved in RNA replication but differs genetically from P1 of Luteoviruses (Shams-bakhsh and Symons 1997).

Poleroviruses contain an additional 5'-proximal ORF (ORF 0) that encodes a VSR, P0 (Almasi et al. 2015). Instead of ORF0, the VSR of Luteoviruses (P6) is encoded by an additional ORF (ORF6) at the 3'-end (Fusaro et al. 2017). However, VSR activity of P6 is weak (Fusaro et al. 2017). This is compensated by strong VSR activity of P4 in Luteoviruses but not in Poleroviruses (Fusaro et al. 2017).

Despite differences in VSR activities of P4, ORFs 3a, 3, 4 and 5 are highly homologous between Luteoviruses and Poleroviruses and unique to these groups (Miller et al. 2002, Miller and Lozier 2022). ORF3a encodes for a small membrane-spanning protein (P3a) that is associated to the Golgi apparatus and plasmodesmata and participates in viral systemic movement (Smirnova et al. 2015). The major CP is encoded by ORF3 (Miller et al. 1988a, Miller et al. 1988b, Tacke et al. 1989). Together with ORF5, ORF3 is also translated into the RTP via stop codon readthrough (Bahner et al. 1990, Miller et al. 1988a). The RTP contributes to viral cell-to-cell movement, symptom development, and is responsible for phloem restriction of YDVs (Peter et al. 2009, Xu et al. 2018).

The CP and RTP confer the highly specific aphid transmission properties (Chay et al. 1996), thus determine vector range. ORF4 lies entirely in ORF3 but in a different frame (Miller et al. 1988b). ORF4 encodes the MP (P4) that is necessary for systemic infection (Chay et al. 1996, Miller et al. 1988b). Recently, it has been demonstrated that the MP also disrupts mitosis of host plants, leading to dwarfing symptoms (Jin et al. 2020). The MP of BYDV-GAV interacts with 12 host proteins, including i) proteins that facilitate ROS production, ii) transcription factors related to those that control photo-morphogenesis and stress response, and iii) sucrose nonfermenting related protein kinase 1 (SnRK1) (Chen et al. 2021). Additionally,

the MP of BYDV-GAV was found to activate the TaATG6 mediated antiviral autophagy pathway in wheat (Shen et al. 2023).

Epidemiology

Because cereal crops are annual, YDVs have to move multiple times a year to alternative hosts (van den Eynde et al. 2020). In this context, cereal aphids act as mobile vectors that spread the viruses between host species (van den Eynde et al. 2020).

More than 25 aphid species are known to transmit YDVs (Halbert and Voegtlin 1995). However, each virus species is transmitted preferentially by specific aphid species (Miller et al. 2002, van den Eynde et al. 2020). *Rhopalosiphum padi* is the most prevalent aphid species in temperate regions and the main vector of BYDV-PAV (Aradottir and Crespo-Herrera 2021). Additionally, *R. padi* is also reported to transmit most other YDVs but usually with lower efficiency compared to BYDV-PAV (for review see Finley and Luck 2011, van den Eynde et al. 2020).

The aphids overwinter on their primary host, *Prunus padus* (bird cherry), where they reproduce sexually (Dixon 1971). New aphids hatch from eggs in spring and are YDV-free. Aleate (winged) individuals develop and migrate to the secondary hosts: cereals and grasses (Dixon 1971). Non-viruliferous *R. padi* are attracted by YDV-infected plants (Ingwell et al. 2012). The aphids acquire YDV from infected winter cereals by ingesting virus particles together with phloem sap when feeding (Gildow and Gray 1993, Ng and Perry 2004). YDV-carrying aphids transmit the virus to healthy plants when saliva is injected during feeding (Gildow and Gray 1993, Ng and Perry 2004).

Compared to small grain cereals, effects on plant performance and yield losses by BYD in maize are less severe (Beuve et al. 1999, Horn et al. 2013, Grüntzig et al. 1997). However, maize plays a central role in the infection cycle of YDVs. Maize serves as a “green bridge” when small grain cereals mature in summer (Brown et al. 1983, Haack et al. 1999, Rashidi et al. 2021). Maize plants are infected by aleate aphid vectors that migrate from ripening small grain cereals in early summer (Haack et al. 1999). *R. padi* colonies take up YDVs from infected maize before they migrate to newly sown winter cereals in autumn and the infection cycle is closed (Haack et al. 1999, Henry and Dedryver 1989).

Several studies suggest that climate change likely favors spread of *R. padi* and therefore incidence of BYDV-PAV in maize and small-grain cereals. For example, higher temperatures

cause anholocyclic overwintering in small-grain cereals and faster development of *R. padi*, leading to higher numbers of aphids in spring (Irwin and Thresh 1990). These large aphid populations infect maize plants at early development stages, when plants are highly susceptible to virus infections (Haack et al. 1999, Harrington et al. 2007, Leather 1980). Furthermore, higher temperatures in autumn and spring increase efficiency of BYDV-PAV transmission and prolong periods of aphid flight periods (Lowles et al. 1996, Smyrnioudis et al. 2000).

Control of BYD

Viruses have no own metabolism, making it difficult to control YDVs directly. To date, the application of RNAi constructs via spraying is the only possibility to target YDVs directly (Dalakouras et al. 2020). However, dsRNA application offers antiviral protection for only a few days and extensive research is necessary to exclude unwanted side effects (Dalakouras et al. 2020).

Spread of YDVs might be restricted by targeting the virus vectors via insecticides (McKirdy et al. 1996). However, this strategy faces some downsides. Firstly, the migration of vectors is difficult to predict and dependent on many factors (Holland et al. 2021, Jarošová et al. 2019). Secondly, insecticides may harm beneficial insects, have detrimental effects on biodiversity and ecosystem functioning (Chagnon et al. 2015, Serrão et al. 2022). Thirdly, because of negative effects on pollinators, the insecticides containing neonicotinoids are prohibited in the European Union (Regulation (EU) No. 485/2013). Finally, some aphid populations were found to be resistant towards pyrethroid insecticides (Foster et al. 2014, Walsh et al. 2020).

Optimizing planting date may help to reduce YDV pressure by escaping aphid migration (Walls et al. 2019). However, low temperatures at late planting dates of winter cereals or early planting dates of spring cereals might negatively affect seedlings (Walls et al. 2019).

Thus, the most promising approach to limit spread of BYD is the usage of YDV-resistant cultivars.

Resistance against YDVs

Today, four *R*-genes conferring YDV resistance or tolerance have been identified in barley and wheat, respectively (for review see Choudhury et al. 2017, Walls et al. 2019). In tolerant genotypes, the viruses are able to replicate and may reach high virus titers without causing

any visible symptoms. In contrast, resistant genotypes limit virus replication and/or spread within the plant (Cooper and Jones 1983).

In barley, YDV-tolerance genes are *ryd1*, *Ryd2*, *Ryd3*, and *Ryd4Hb* (for review see Choudhury et al. 2017, Walls et al. 2019). *ryd1* is a recessive gene and not very efficient (Suneson 1955). Therefore, *ryd1* is not used in barley breeding (Ordon et al. 2009). *Ryd2* and *Ryd3* originate from Ethiopian landraces (Niks et al. 2004, Rasmusson and Schaller 1959). *Ryd2* is located close to the centromere at the long arm of chromosome 3H and was introduced into commercial cultivars (Choudhury et al. 2017, Collins et al. 1996). *Ryd2* reduces BYDV-PAV and BYDV-MAV titer only in young plants but not in older plants (Riedel et al. 2011). This gene is also not effective against CYDV-RPV and dependent on ambient environmental conditions (Baltenberger et al. 1987, Šip et al. 2004).

Ryd3 is a dominant gene that maps to chromosome 6H (Niks et al. 2004). Pyramiding *Ryd2* and *Ryd3* confers stronger resistance towards BYDV-PAV in barley than one of these genes alone (Riedel et al. 2011).

Ryd4Hb was introgressed from *Hordeum bulbosum* to the long arm of chromosome 6H (Scholz et al. 2009). However, a recessive sublethality factor was also introduced, making it difficult to take advantage of *Ryd4Hb* (Scholz et al. 2009). *Ryd4Hb* is a dominant gene that likely indirectly conveys resistance to YDVs by preventing inoculation through decreased time of salivation of the aphid vectors (Schliephake et al. 2013, Scholz et al. 2009). However, nothing is known about the resistance mechanisms of *Ryd2* and *Ryd3.*

Additionally, several major QTL associated with resistance or tolerance to different YDV species were identified on chromosomes 1H, 2H, 4H, and 7H of barley (del Blanco et al. 2015, Kraakman et al. 2006, Scheurer et al. 2001, Toojinda et al. 2000). Recently, a QTL on chromosome 5H was discovered in a population originating from a cross between cultivated barley and *H. spontaneum* (Hu et al. 2019).

bdv1 is the only gene that is described from the primary gene pool of wheat and confers tolerance but not resistance to BYDV-MAV (Singh et al. 1993). *bdv1* is not efficient to all YDV species, or in all environments (Singh et al. 1993). The other YDV resistance genes in wheat – *Bdv2*, *Bdv3,* and *Bdv4* – were introgressed into wheat from *Thinopyrum intermedium* (for review see Choudhury et al. 2017, Zhang et al. 2009).

Bdv2 was introduced as a 7D-7Ai translocation (Brettell et al. 1988, Zhang et al. 1999) and is used in breeding programs (Zhang et al. 2009). It is suggested that *Bdv2* leads to a suppression

of BYDV-GAV replication or movement (Liu et al. 2005b). The serine/threonine kinase gene TiSTK1 is located in the *Bdv2* region and is specifically expressed during BYDV-GAV infection in *T. intermedium* and wheat-*T. intermedium* translocation lines carrying *Bdv2* (Zhang et al. 2011). TiSTK1 was proven to be causative for Bdv2-mediated BYDV-GAV resistance in wheat by transgenic approaches (Zhang et al. 2011). Biochemical assays indicated that the TiSTK1 protein localizes at the plasma membrane and interacts with the coat protein of BYDV-GAV (Zhang et al. 2011). *Bdv2*-mediated defense response to BYDV-GAV triggers expression of some resistance homologous genes, PTI-related genes, ABC transporter genes, pathogenesis-related protein genes, and genes in reactive oxygen species, phospholipid signaling, and jasmonic acid-signaling (Wang et al. 2013).

Bdv3 and *Bdv4* originate from translocations of different segments of the *T. intermedium* genome but are not used in breeding (Kong et al. 2009, Larkin et al. 1995, Sharma et al. 1995, Walls et al. 2019, Zhang et al. 2009). Recently, four novel QTL with additive effect were identified in Chinese wheat landraces and are located on chromosomes 2A, 2B, and 6A, respectively (Choudhury et al. 2019b).

YDV resistance in maize

YDV-tolerant and -resistant maize genotypes have been identified previously (Brown et al. 1984, Grüntzig and Fuchs 2000, Horn et al. 2013, Horn et al. 2014, Loi et al. 1986, Osler et al. 1985, Stoner 1977). However, tolerant genotypes are not able to break the transmission cycle of YDVs because they function as a virus reservoir and YDVs can be transmitted to other cereals. Thus, breeding for YDV-resistant maize is a feasible approach to break the infection cycle and reduce YDV-pressure on maize, wheat, and barley.

BYDV-resistance in maize shows high genotypic variance and high heritability (Horn et al. 2013, 2014, and 2015), making it a promising trait for breeding.

Using an association mapping population with 267 genotypes representing the world's maize gene pool, Horn et al. (2014) identified three single nucleotide polymorphisms (SNPs) on maize chromosome 10 that are significantly associated with BYDV-PAV resistance. All of them are located in the non-coding region of gene GRMZM2G018027 (Zm00001eb428020). For the trait virus titer (EX), these SNPs explain between 16 and 21 % of phenotypic variance each, and 25 % in a combined analysis (Horn et al. 2014). For the trait infection rate (IR), these SNPs explain between 11 % and 18 % each (Horn et al. 2014). In a simultaneous fit with two

additional SNPs identified on chromosomes 4 and 10, they explained 33 % of phenotypic variance of IR (Horn et al. 2014).

Using five connected linkage mapping populations, Horn et al. (2015) identified a QTL for the traits EX and IR on the distal end of chromosome 10 at the position of 46.90 cM. This QTL explained 45 % and 46 % of the observed phenotypic variance for the traits EX and IR, respectively (Horn et al. 2015). The QTL is flanked by molecular markers PUT-163a-60352819-2700 and SYN15407. The confidence interval of the QTL comprises 224 genes, including GRMZM2G018027 (Zm00001eb428020) (Horn et al. 2015).

Combining both studies, GRMZM2G018027 (Zm00001eb428020) is a promising candidate gene for BYDV-PAV resistance in maize. The best BLAST hit for GRMZM2G018027 in *Arabidopsis thaliana* is the gene *OXS3* (Horn et al. 2014). *OXS3* is expressed during response reactions to oxidative stress (Blanvillain et al. 2009) and likely improves resistance to Tobacco mosaic virus in *A. thaliana* by the production of H_2O_2 (Wang and Culver 2012).

1.4 Objectives

The aims of this thesis are:

i.) to verify if the resistance directly targets BYDV-PAV directly or the vector *R. padi,*

ii.) to gain insights into the resistance mechanism,

iii.) to characterize the degree of dominance of the BYDV-resistance gene in maize,

iv.) to identify the causative gene for BYDV-PAV resistance in maize, and

v.) to validate the candidate gene GRMZM2G018027 (Zm00001eb428020) as the causative gene for BYDV-PAV resistance in maize.

These findings may be used to develop markers for marker assisted breeding of BYDV-PAV resistant maize as well as a starting point for the investigation of the resistance mechanism.

1.5 References

Alazem M., Kim K.-H., Lin N.-S. (2019) Effects of abscisic acid and salicylic acid on gene expression in the antiviral RNA silencing pathway in *Arabidopsis*. *International Journal of Molecular Sciences*, 20.

Almasi R., Miller W.A., Ziegler-Graff V. (2015) Mild and severe cereal yellow dwarf viruses differ in silencing suppressor efficiency of the P0 protein. *Virus Research*, 208, 199–206.

Andersen E.J., Ali S., Byamukama E., Yen Y., Nepal M.P. (2018) Disease resistance mechanisms in plants. *Genes*, 9.

Aradottir G.I., Crespo-Herrera L. (2021) Host plant resistance in wheat to barley yellow dwarf viruses and their aphid vectors: a review. *Current Opinion in Insect Science*, 45, 59–68.

Awika J.M. (2011) Major cereal grains production and use around the world. In: *Advances in cereal science: implications to food processing and health promotion*, ACS Symposium Series. American Chemical Society, Washington, DC.

Bahner I., Lamb J., Mayo M.A., Hay R.T. (1990) Expression of the genome of potato leafroll virus: readthrough of the coat protein termination codon in vivo. *Journal of General Virology*, 71, 2251–2256.

Baltenberger D.E., Ohm H.W., Foster J. (1987) Reactions of oat, barley, and wheat to infection with barley yellow dwarf virus isolates. *Crop Science*, 27, 195–198.

Baulcombe D. (2004) RNA silencing in plants. *Nature*, 431, 356–363.

Beuve M., Naïbo B., Foulgocq L., Lapierre H. (1999) Irrigated hybrid maize crop yield losses due to barley yellow dwarf virus-PAV luteovirus. *Crop Science*, 39, 1830–1834.

Blanc S., Michalakis Y. (2016) Manipulation of hosts and vectors by plant viruses and impact of the environment. *Current Opinion in Insect Science*, 16, 36–43.

Blanvillain R., Kim J.H., Wu S., Lima A., Ow D.W. (2009) OXIDATIVE STRESS 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress. *The Plant Journal*, 57, 654–665.

Brault V., Uzest M., Monsion B., Jacquot E., Blanc S. (2010) Aphids as transport devices for plant viruses. *Comptes Rendus Biologies*, 333, 524–538.

Brettell R.I.S., Banks P.M., Cauderon Y., Chen X., Cheng Z.M., Larkin P.J., Waterhouse P.M. (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. *Annals of Applied Biology*, 113, 599–603.

Brown J.K., Wyatt S.D., Hazelwood D. (1984) Irrigated corn as a source of barley yellow dwarf virus and vector in Eastern Washington. *Phytopathology*, 74, 46–49.

Calil I.P., Fontes E.P.B. (2017) Plant immunity against viruses: antiviral immune receptors in focus. *Annals of Botany*, 119, 711–723.

Caplan J.L., Mamillapalli P., Burch-Smith T.M., Czymmek K., Dinesh-Kumar S.P. (2008) Chloroplastic protein NRIP1 mediates innate immune receptor recognition of a viral effector. *Cell*, 132, 449–462.

Chagnon M., Kreutzweiser D., Mitchell E.A.D., Morrissey C.A., Noome D.A., van der Sluijs J.P. (2015) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research International*, 22, 119–134.

Chay C.A., Gunasinge U.B., Dinesh-Kumar S.P., Miller W.A., Gray S.M. (1996) Aphid transmission and systemic plant infection determinants of barley yellow dwarf luteovirus-

PAV are contained in the coat protein readthrough domain and 17-kDa protein, respectively. *Virology*, 219, 57–65.

Chen S., Han X., Yang L., Li Q., Shi Y., Li H., Chen L., Sun B., Shi Y., Yang X. (2021) Identification and functional analyses of host factors interacting with the 17-kDa protein of barley yellow dwarf virus-GAV. *Scientific Reports*, 11, 8453.

Choudhury S., Hu H., Larkin P., Meinke H., Shabala S., Ahmed I., Zhou M. (2018) Agronomical, biochemical and histological response of resistant and susceptible wheat and barley under BYDV stress. *PeerJ*, 6, e4833.

Choudhury S., Hu H., Meinke H., Shabala S., Westmore G., Larkin P., Zhou M. (2017) Barley yellow dwarf viruses: infection mechanisms and breeding strategies. *Euphytica*, 213, 213-168.

Choudhury S., Larkin P., Meinke H., Hasanuzzaman M.D., Johnson P., Zhou M. (2019a) Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat. *Crop and Pasture Science*, 70, 16–25.

Choudhury S., Larkin P., Xu R., Hayden M., Forrest K., Meinke H., Hu H., Zhou M., Fan Y. (2019b) Genome wide association study reveals novel QTL for barley yellow dwarf virus resistance in wheat. *BMC genomics*, 20, 891.

Collins N.C., Paltridge N.G., Ford C.M., Symons R.H. (1996) The Yd2 gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. *Theoretical and Applied Genetics*, 92, 858–864.

Conti G., Zavallo D., Venturuzzi A.L., Rodriguez M.C., Crespi M., Asurmendi S. (2017) TMV induces RNA decay pathways to modulate gene silencing and disease symptoms. *The Plant Journal*, 89, 73–84.

Cooper J.I., Jones A.T. (1983) Responses of plants to viruses: proposals for the use of terms. *Phytopathology*, 73, 127–128.

Dalakouras A., Wassenegger M., Dadami E., Ganopoulos I., Pappas M.L., Papadopoulou K. (2020) Genetically modified organism-free RNA interference: exogenous application of RNA molecules in mlants. *Plant Physiology*, 182, 38–50.

Del Blanco I.A., Hegarty J., Gallagher L.W., Falk B.W., Brown-Guedira G., Pellerin E., Dubcovsky J. (2015) Mapping of QTL for tolerance to cereal yellow dwarf virus in two-rowed spring barley. *Crop Science*, 54, 1468–1475.

Dixon A.F.G. (1971) The life-cycle and host preferences of the bird cherry-oat aphid, *Rhopalosiphum padi* L., and their bearing on the theories of host alternation in aphids. *Annals of Applied Biology*, 68, 135-147.

Dubiella U., Serrano I. (2021) The ubiquitin proteasome system as a double agent in plant-virus interactions. *Plants*, 10, 928.

Erenstein O., Jaleta M., Sonder K., Mottaleb K., Prasanna B.M. (2022) Global maize production, consumption and trade: trends and R&D implications. *Food Security*, 14, 1295–1319.

Esau K. (1957a) Anatomic effects of barley yellow dwarf virus and maleic hydrazide on certain Gramineae. *Hilgardia*, 27, 15–69.

Esau K. (1957b) Phloem degeneration in *Gramineae* affected by the barley yellow-dwarf virus. *American Journal of Botany*, 44, 245–251.

Fereres A., Raccah B. (2015) Plant Virus Transmission by Insects. In *Enceclopedia of Life Sciences*, John Wiley & Sons, Ltd (Ed.).

Finlay K.J., Luck J.E. (2011) Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop–vector–virus pathosystem. *Agriculture, Ecosystems & Environment*, 144, 405–421.

Faostat (2023) https://www.fao.org/faostat/en/#data/QCL

FAOSTAT ANALYTICAL BRIEF 41: Agricultural production statistics 2000–2020. Available via https://www.fao.org/3/cb9180en/cb9180en.pdf

Floris M., Mahgoub H., Lanet E., Robaglia C., Menand B. (2009) Post-transcriptional regulation of gene expression in plants during abiotic stress. *International Journal of Molecular Sciences*, 10, 3168–3185.

Foster S.P., Paul V.L., Slater R., Warren A., Denholm I., Field L.M., Williamson M.S. (2014) A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, *Sitobion avenae*, is associated with resistance to pyrethroid insecticides. *Pest Management Science*, 70, 1249–1253.

Fusaro A.F., Barton D.A., Nakasugi K., Jackson C., Kalischuk M.L., Kawchuk L.M., Vaslin M.F.S., Correa R.L., Waterhouse P.M. (2017) The luteovirus P4 movement protein Is a suppressor of systemic RNA silencing. *Viruses*, 9.

Garcia D., Garcia S., Voinnet O. (2014) Nonsense-mediated decay serves as a general viral restriction mechanism in plants. *Cell Host & Microbe*, 16, 391–402.

Garcia-Ruiz H. (2018) Susceptibility genes to plant viruses. *Viruses*, 10.

Garcia-Ruiz H. (2019) Host factors against plant viruses. *Molecular Plant Pathology*, 20, 1588–1601.

Gildow F.E., Gray S.M. (1993) The aphid salivary gland basal Lammina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteoviruses. *Phytopathology*, 83, 1293–1302.

Gloggnitzer J., Akimcheva S., Srinivasan A., Kusenda B., Riehs N., Stampfl H., Bautor J., Dekrout B., Jonak C., Jiménez-Gómez J.M., Parker J.E., Riha K. (2014) Nonsense-mediated mRNA decay modulates immune receptor levels to regulate plant antibacterial defense. *Cell Host & Microbe*, 16, 376–390.

Grüntzig M., Fuchs E., Werner M. (1997) Occurence and influence of barley yellow dwarf luteovirus (BYDV) on growth and yield of maize. *Nachrichtenblatt des deutschen Pflanzenschutzdienstes*, 49, 45–50.

Grüntzig, M., Fuchs E. (2000) Occurence of luteoviruses of cereals in Zea mays L. *Journal of Plant Diseases and Protection*, 107, 523–538.

Haack L., Courbon R., Riault G., Tanguy S., Le Vilain D., Henry M., Dedryver C.A. (1999) A plant and field study of BYDV-PAV and-MAV distribution on maize in France. *Journal of Plant Diseases and Protection*, 106, 297–303.

Halbert S., Voegtlin D. (1995) Biology and taxonomy of vectors of barley yellow dwarf viruses. In: *Barley yellow dwarf: 40 years of progress*, pp. 9–28. Eds C. J. D'Arcy and P. A. Burnett. St Paul: APS Press.

Harrington R., Clark S.J., Welham S.J., Verrier P.J., Denholm C.H., Hullé M., Maurice D., Rounsevell, M.D., Cocu N. (2007) Environmental change and the phenology of European aphids. *Global Change Biology*, 13, 1550–1564.

Henry M., Dedryver C.A. (1989) Fluctuations in cereal aphid populations on maize (*Zea mays*) in western France in relation to the epidemiology of barley yellow dwarf virus (BYDV). *Journal of Applied Entomology*, 107, 401–410.

Hernández J.A., Gullner G., Clemente-Moreno M.J., Künstler A., Juhász C., Díaz-Vivancos P., Király L. (2016) Oxidative stress and antioxidative responses in plant–virus interactions. *Physiological and Molecular Plant Pathology*, 94, 134–148.

Hogenhout S.A., Ammar E.-D., Whitfield A.E., Redinbaugh M.G. (2008) Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*, 46, 327–359.

Holland J.M., McHugh N.M., Salinari F. (2021) Field specific monitoring of cereal yellow dwarf virus aphid vectors and factors influencing their immigration within fields. *Pest Management Science*, 77, 4100–4108.

Horn F., Habekuß A., Stich B. (2013) Natural variation for BYDV resistance in maize. *Maydica*, 58.

Horn F., Habekuß A., Stich B. (2014) Genes involved in barley yellow dwarf virus resistance of maize. *Theoretical and Applied Genetics*, 127, 2575–2584.

Horn F., Habekuß A., Stich B. (2015) Linkage mapping of barley yellow dwarf virus resistance in connected populations of maize. *BMC Plant Biology*.

Hu H., Choudhury S., Shabala S., Gupta S., Zhou M. (2019) Genomic regions on chromosome 5H containing a novel QTL conferring barley yellow dwarf virus-PAV (BYDV-PAV) tolerance in barley. *Scientific Reports*, 9, 11298.

Hull R. (2014a) Appendix A - Profiles of Families and Genera of Plant Viruses. In: *Plant Virology*, pp. 973–1042. Ed R. Hull. Elsevier.

Hull R. (2014b) Chapter 1 - Introduction. In: *Plant Virology*, pp. 3–14. Ed R. Hull. Elsevier.

Hull R. (2014c) Chapter 12 - Plant to Plant Movement. In: *Plant Virology*, pp. 669–751. Ed R. Hull. Elsevier.

Hull R. (2014d) Chapter 8 - Origins and Evolution of Plant Viruses. In *Plant Virology*, pp. 423–476. Ed R. Hull: Elsevier.

Hyodo K., Hashimoto K., Kuchitsu K., Suzuki N., Okuno T. (2017) Harnessing host ROS-generating machinery for the robust genome replication of a plant RNA virus. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E1282-E1290.

ICTV (2012) Luteoviridae, In: ICTV Ninth Report, URL https://ictv.global/report_9th/RNApos/Luteoviridae

ICTV (2022a) The ICTV report on virus classification and taxon nomenclature – virus properties, URL https://ictv.global/report/chapter/information/information/virus_properties

ICTV (2022b) Virus metadata resource, URL https://ictv.global/vmr

ICTV (2022c) Virus taxonomy: 2023 Release, URL https://ictv.global/taxonomy

Ingwell L.L., Eigenbrode S.D., Bosque-Pérez N.A. (2012) Plant viruses alter insect behavior to enhance their spread. *Scientific Reports*, 2, 578.

Irwin M.E., Thresh J.M. (1990) Epidemiology of barley yellow dwarf: a study in ecological complexity. *Annual Review of Phytopathology*, 28, 393–424.

Jarošová J., Żelazny W.R., Kundu J.K. (2019) Patterns and predictions of barley yellow dwarf virus vector migrations in Central Europe. *Plant Disease*, 103, 2057–2064.

Jensen S.G. (1972) Metabolism and carbohydrate composition in barley yellow dwarf virus-infected wheat. *Phytopathology*, 62, 587–592.

Jensen S.G., van Sambeek J.W. (1972) Differential effects of barley yellow dwarf virus on the physiology of tissues of hard red spring wheat. *Phytopathology*, 62, 290–293.

Jin, Huaibing, Du, Zhiqiang, Zhang Y., Antal J., Xia, Zongliang, Wang Y., Yang G., Zhao X., Han X., Cheng Y., Shen Q., Zhang K., Elder R.E., Benko Z., Fenyvuesvolgyi C., Li G., Rebello D., Li J., Bao S., Zhao R.Y., Wang D. (2020) A distinct class of plant and animal viral proteins that disrupt mitosis by directly interrupting the mitotic entry switch Wee1-Cdc25-Cdk1. *Science Advances*, 6.

Jones J.D.G., Dangl J.L. (2006) The plant immune system. *Nature*, 444, 323–329.

Jones R.A., Naidu R.A. (2019) Global dimensions of plant virus diseases: current status and future perspectives. *Annual Review of Virology*, 6, 387–409.

Kennett D.J., Prufer K.M., Culleton B.J., George R.J., Robinson M., Trask W.R., Buckley G.M., Moes E., Kate E.J., Harper T.K…, O'Donnell L., Ray E.E., Hill Ethan C., Alsgaard A., Merriman C., Meredith C., Edgar H.J.H., Awe J.J., Gutierrez S.M. (2020) Early isotopic evidence for maize as a staple grain in the Americas. *Science Advances*, 6.

Kong L., Anderson J., Ohm H. (2009) Segregation distortion in common wheat of a segment of *Thinopyrum intermedium* chromosome 7E carrying *Bdv3* and development of a *Bdv3* marker. *Plant Breeding*, 128, 591–597.

Koonin E.V., Dolja V.V., Krupovic M. (2015) Origins and evolution of viruses of eukaryotes: The ultimate modularity. *Virology*, 479-480, 2–25.

Kørner C.J., Klauser D., Niehl A., Domínguez-Ferreras A., Chinchilla D., Boller T., Heinlein M., Hann D.R. (2013) The immunity regulator *BAK1* contributes to resistance against diverse RNA viruses. *Molecular Plant-Microbe Interactions*, 26, 1271–1280.

Kourelis J., van der Hoorn R.A.L. (2018) Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *The Plant cell*, 30, 285–299.

Kraakman A.T.W., Martínez F., Mussiraliev B., van Eeuwijk F.A., Niks R.E. (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. *Molecular Breeding*, 17, 41–58.

Larkin P.J., Banks P.M., Lagudah E.S., Appels R., Xiao C., Zhiyong X., Ohm, H.W., McIntosh R.A. (1995) Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome,* 38, 385-94.

Leather S.R. (1980) Egg survival in the bird cherry-oat aphid, *Rhopalosiphum padi*. *Entomologia experimentalis et applicata*, 27, 96–97.

Lefeuvre P., Martin D.P., Elena S.F., Shepherd D.N., Roumagnac P., Varsani A. (2019) Evolution and ecology of plant viruses. *Nature Reviews. Microbiology*, 17, 632–644.

Leng P., Ji Q., Asp T., Frei U.K., Ingvardsen C.R., Xing Y., Studer B., Redinbaugh M., Jones M., Gajjar P., Liu S., Li F., Pan G., Xu M., Lübberstedt T. (2017) Auxin Binding Protein 1 reinforces resistance to sugarcane mosaic virus in maize. *Molecular Plant*, 10, 1357–1360.

Leonetti P., Stuttmann J., Pantaleo V. (2021) Regulation of plant antiviral defense genes via host RNA-silencing mechanisms. *Virology Journal*, 18, 194.

Lewsey M., Palukaitis P., Carr J.P. (2018) Plant-virus interactions: defence and counter-defence. *Annual Plant Reviews*, 134–176.

Liu Q., Deng S., Liu B., Tao Y., Ai H., Liu J., Zhang Y., Zhao Y., Xu M. (2020) A helitron-induced RabGDIα variant causes quantitative recessive resistance to maize rough dwarf disease. *Nature Communications*, 11, 495.

Liu Q., Liu H., Gong Y., Tao Y., Jiang L., Zuo W., Yang Q., Ye J., Lai J., Wu J., Lübberstedt T., Xu M. (2017) An atypical thioredoxin imparts early resistance to sugarcane mosaic virus in maize. *Molecular Plant*, 10, 483–497.

Liu X.D., Zhang Z.Y., Liu Y., Xin Z.Y. (2005) Molecular evidence of barley yellow dwarf virus replication/movement suppressed by the resistance gene *Bdv2*. *Acta Genetica Sinica*, 32, 942–947.

Liu Y., Buchenauer H. (2005) Effect of infections with barley yellow dwarf virus and Fusarium spp. on assimilation of $^{14}CO_2$ by flag leaves and translocation of photosynthates in wheat. *Journal of Plant Diseases and Protection*, 112, 529–543.

Loi N., Osler R., Snidaro M., Ardigo A., Lorenzoni C. (1986) Tolerance to BYDV (barley yellow dwarf virus) in inbred and hybrids of maize. *Maydica*, 31, 307–314.

Lowles A.J., Tatcheel G.M., Harrington R., Clark S.J. (1996) The effect of temperature and inoculation access period on the transmission of barley yellow dwarf virus by *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.). *Annals of Applied Biology*, 128, 45–53.

Macho A.P., Zipfel C. (2014) Plant PRRs and the activation of innate immune signaling. *Molecular Cell*, 54, 263–272.

Mauck K.E., Kenney J., Chesnais Q. (2019) Progress and challenges in identifying molecular mechanisms underlying host and vector manipulation by plant viruses. *Current Opinion in Insect Science*, 33, 7–18.

Mazzucotelli E., Mastrangelo A.M., Crosatti C., Guerra D., Stanca A.M., Cattivelli L. (2008) Abiotic stress response in plants: When post-transcriptional and post-translational regulations control transcription. *Plant Science*, 174, 420–431.

McKirdy S.J., Jones R.A.C. (1996) Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of Barley yellow dwarf luteovirus in cereals. *Plant Disease*, 80, 895–901.

McKirdy S.J., Jones R.A.C., Nutter F.W. (2002) Quantification of yield losses caused by barley yellow dwarf virus in wheat and oats. *Plant Disease*, 86, 769–773.

Meissle M., Mouron P., Musa T., Bigler F., Pons X., Vasileiadis V.P., Otto S., Antichi D., Kiss J., Pálinkás Z., Dorner Z., van der Weide R., Groten J., Czembor E., Adamczyk J., Thibord J.-B., Melander B., Cordsen Nielsen G., Poulsen R.T., Zimmermann O., Verschwele A., Oldenburg E. (2010) Pests, pesticide use and alternative options in European maize production: current status and future prospects. *Journal of Applied Entomology*, 134, 357–375.

Melchinger A.E., Kuntze L., Gumber R.K., Lübberstedt T., Fuchs E. (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. *Theoretical and Applied Genetics*, 96, 1151–1161.

Miller W.A., Liu S., Beckett R. (2002) Barley yellow dwarf virus: Luteoviridae or Tombusviridae? *Molecular Plant Pathology*, 3, 177–183.

Miller W.A., Lozier Z. (2022) Yellow dwarf viruses of cereals: taxonomy and molecular mechanisms. *Annual Review of Phytopathology*, 60, 121–141.

Miller W.A., Waterhouse P.M., Gerlach W.L. (1988a) Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acids Research*, 16, 6097–6111.

Miller W.A., Waterhouse P.M., Kortt A.A., Gerlach W.L. (1988b) Sequence identification of the barley yellow dwarf virus coat protein gene. *Virology*, 165, 306–309.

Milne R.G., Lovisolo O. (1977) Maize rough dwarf and related viruses. *Advances in Virus Research*, 21, 267–341.

Mittler R., Zandalinas S.I., Fichman Y., Breusegem F. (2022) Reactive oxygen species signaling in plant stress responses. *Nature Reviews. Molecular Cell Biology*, 23, 663–679.

Munkvold, Sobek (1999) European corn borer (*Lepidoptera: Pyralidae*) larvae as vectors of *Fusarium moniliforme*, causing kernel rot and symptomless infection of maize kernels. *Journal of Economic Entomology*, 92, 503–509.

Munkvold G.P. (2017) Diseases of Corn (syn. Maize) (Zea mays L.) https://www.apsnet.org/edcenter/resources/commonnames/Pages/Corn.aspx

Nancarrow N., Aftab M., Hollaway G., Rodoni B., Trębicki P. (2021) Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: A three-year field study in South-Eastern Australia. *Microorganisms*, 9.

Nault L.R. (1997) Arthropod transmission of plant viruses: a new synthesis. *Annals of the Entomological Society of America*, 90, 521–541.

Nelson R., Wiesner-Hanks T., Wisser R., Balint-Kurti P. (2018) Navigating complexity to breed disease-resistant crops. *Nature Reviews. Genetics*, 19, 21–33.

Ng J.C.K., Perry K.L. (2004) Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, 5, 505–511.

Niehl A., Wyrsch I., Boller T., Heinlein M. (2016) Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. *New Phytologist*, 211, 1008–1019.

Niks R.E., Habekuss A., Bekele B., Ordon F. (2004) A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus. *Theoretical and Applied Genetics*, 109, 1536–1543.

Niks R.E., Qi X., Marcel T.C. (2015) Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. *Annual Review of Phytopathology*, 53, 445–470.

Ordon F., Habekuß A., Kastirr U., Rabenstein F., Kühne T. (2009) Virus resistance in cereals: sources of resistance, genetics and breeding. *Journal of Phytopathology*, 157, 535–545.

Osler R., Loi N., Lorenzoni C., Snidaro M., Refatti E. (1985) Barley yellow dwarf virus infections in maize (*Zea mays* L) inbreds and hybreds in Northern Italy. *Maydica*, 30, 285-229.

Osterbaan L.J., Fuchs M. (2019) Dynamic interactions between plant viruses and their hosts for symptom development. *Journal of Plant Pathology*, 101, 885–895.

Oswald J.W., Houston B.E. (1953) The yellow-dwarf virus disease of cereal crops. *Phytopathology*, 43, 128–136.

Pallas V., García J.A. (2011) How do plant viruses induce disease? Interactions and interference with host components. *The Journal of General Virology*, 92, 2691–2705.

Palukaitis P., Yoon J.-Y. (2020) R-gene mediated defense against viruses. *Current Opinion in Virology*, 45, 1–7.

Panayotou P.C. (1977) Effect of barley yellow dwarf on several varieties of maize. *Plant Disease Reporter*, 61, 815–819.

Panayotou P.C. (1978) Degenerative changes induced by barley yellow dwarf virus on certain tissues of several cereals. *Phytopathologia Mediterranea*, 17, 23–29.

Paulmann M.K., Kunert G., Zimmermann, Theis N., Ludwig A., Meichsner D., Oelmüller R., Gershenzon J., Habekuss A., Ordon F., Furch A.C., Will T. (2018) Barley yellow dwarf virus infection leads to higher chemical defense signals and lower electrophysiological reactions in susceptible compared to tolerant barley genotypes. *Frontiers in Plant Science*, 9.

Pearson M.N., Robb S.M. (1984) The occurrence and effects of barley yellow dwarf virus in maize in SW England. *Plant Pathology*, 33, 503–512.

Peiris K.H.S., Bowden R.L., Todd T.C., Bockus W.W., Davis M.A., Dowell F.E. (2019) Effects of barley yellow dwarf disease on wheat grain quality traits. *Cereal Chemistry*, 96, 754–764.

Perry K.L., Kolb F.L., Sammons B., Lawson C., Cisar G., Ohm H. (2000) Yield effects of barley yellow dwarf virus in soft red winter wheat. *Phytopathology*, 90, 1043–1048.

Peter K.A., Gildow F., Palukaitis P., Gray S.M. (2009) The C-terminus of the polerovirus p5 readthrough domain limits virus infection to the phloem. *Journal of Virology*, 83, 5419–5429.

Rashidi M., Cruzado R.K., Hutchinson P.J.S., Bosque-Pérez N.A., Marshall J.M., Rashed A. (2021) Grassy weeds and corn as potential sources of barley yellow dwarf virus spread into winter wheat. *Plant Disease*, 105, 444–449.

Rasmusson D.C., Schaller C.W. (1959) The inheritance of resistance in barley to the yellow-dwarf virus 1. *Agronomy Journal*, 51, 661–664.

Redinbaugh M.G., Lübberstedt T., Leng P., Xu M. (2018) The Genetics and Genomics of Virus Resistance in Maize. In: Bennetzen, J., Flint-Garcia, S., Hirsch, C., Tuberosa, R. (eds) *The Maize Genome, Compendium of Plant Genomes*, pp. 185–200.

Redinbaugh M.G., Zambrano Mendoza J.L. (2014) Control of virus diseases in maize. In: Lobenstein G, Katis N (eds) *Control of plant virus disease: seed-propogated crops. Advances in Virus Research vol 90*, pp 391–429.

Riedel C., Habekuss A., Schliephake E., Niks R., Broer I., Ordon F. (2011) Pyramiding of *Ryd2* and *Ryd3* conferring tolerance to a German isolate of barley yellow dwarf virus-PAV (BYDV-PAV-ASL-1) leads to quantitative resistance against this isolate. *Theoretical and Applied Genetics*, 123, 69–76.

Riedell W.E., Kieckhefer R.W., Langham M.A.C., Hesler L.S. (2003) Root and shoot responses to bird cherry-oat aphids and barley yellow dwarf virus in spring wheat. *Crop Science*, 43, 1380–1386.

Rochow W.F. (1969) Biological properties of four isolates of barley yellow dwarf virus. *Phytopathology*, 59.

Rochow W.F., Muller I. (1971) A fifth variant of barley yellow dwarf virus in New York. *Plant Disease Reporter*, 55, 874–877.

Rong W., Wang X., Wang X., Massart S., Zhang Z. (2018) Molecular and ultrastructural mechanisms underlying yellow dwarf symptom formation in wheat after infection of barley yellow dwarf virus. *International Journal of Molecular Sciences*, 19.

Sastry K.S. (2019) Zea mays (corn or maize). In: *Encyclopedia of plant viruses and viroids*. 1st Ed 2824-2853.

Savary S., Willocquet L., Pethybridge S.J., Esker P., McRoberts N., Nelson A. (2019) The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3, 430–439.

Scheurer K.S., Friedt W., Huth W., Waugh R., Ordon F. (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (Hordeum vulgare L.). *Theoretical and Applied Genetics*, 103, 1074–1083.

Schliephake E., Habekuß A., Scholz M., Ordon F. (2013) Barley yellow dwarf virus transmission and feeding behaviour of *Rhopalosiphum padi* on *Hordeum bulbosum* clones. *Entomologia experimentalis et applicata*, 146, 347–356.

Scholz M., Ruge-Wehling B., Habekuss A., Schrader O., Pendinen G., Fischer K., Wehling P. (2009) *Ryd4(Hb)*: a novel resistance gene introgressed from *Hordeum bulbosum* into barley and conferring complete and dominant resistance to the barley yellow dwarf virus. *Theoretical and Applied Genetics*, 119, 837–849.

Serrão J.E., Plata-Rueda A., Martínez L.C., Zanuncio J.C. (2022) Side-effects of pesticides on non-target insects in agriculture: a mini-review. *The Science of Nature*, 109, 17.

Shams-bakhsh M., Symons R.H. (1997) Barley yellow dwarf virus-PAV RNA does not have a VPg. *Archives of Virology*, 142, 2529-2535.

Sharma H., Ohm H., Goulart L., Lister R., Appels R., Benlhabib O. (1995) Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. *Genome*, 38, 406–413

Shen C., Wei C., Wu Y. (2023) Barley yellow dwarf Virus-GAV movement protein activating wheat TaATG6-Mediated antiviral autophagy pathway. *Plant Physiology and Biochemistry*, 200, 107771.

Shiferaw B., Prasanna B.M., Hellin J., Bänziger M. (2011) Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, 3, 307–327.

Singh R.P., Burnett P.A., Albarrán M., Rajaram S. (1993) *Bdv1*: a gene for tolerance to barley yellow dwarf virus in bread wheats. *Crop Science*, 33, 231–234.

Šip V., Chrpová J., Vacke J., Ovesná J. (2004) Possibility of exploiting the *Yd2* resistance to BYDV in spring barley breeding. *Plant Breeding*, 123, 24–29.

Smirnova E., Firth A.E., Miller W.A., Scheidecker D., Brault V., Reinbold C., Rakotondrafara A.M., Chung B.Y.-W., Ziegler-Graff V. (2015) Discovery of a small non-AUG-initiated ORF in Poleroviruses and Luteoviruses that is required for long-distance movement. *PLoS Pathogens*, 11, e1004868.

Smyrnioudis I.N., Harrington R., Katis N., Clark S.J. (2000) The effect of drought stress and temperature on spread of barley yellow dwarf virus (BYDV). *Agricultural and Forest Entomology*, 2, 161–166.

Stoner W.N. (1977) Barley yellow dwarf virus infection in maize. *Phytopathology*, 67, 975–981.

Suneson C.A. (1955) Breeding for resistance to yellow dwarf virus in barley. *Agronomy Journal*, 47, 283.

Tacke E., Sarkar S., Salamini F., Rohde W. (1989) Cloning of the gene for the capsid protein of potato leafroll virus. *Archives of Virology*, 105, 153–163.

Tao Y., Liu Q., Wang H., Zhang Y., Huang X., Wang B., Lai J., Ye J., Liu B., Xu M. (2013) Identification and fine-mapping of a QTL, *qMrdd1*, that confers recessive resistance to maize rough dwarf disease. *BMC Plant Biology*, 13.

Teixeira R.M., Ferreira M.A., Raimundo G.A.S., Loriato V.A.P., Reis P.A.B., Fontes E.P.B. (2019) Virus perception at the cell surface: revisiting the roles of receptor-like kinases as viral pattern recognition receptors. *Molecular Plant Pathology*, 20, 1196–1202.

Toojinda T., Broers L.H., Chen X.M., Hayes P.M., Kleinhofs A., Korte J., Kudrna D., Leung H., Line R.F., Powell W., Ramsey L., Vivar H., Waugh R. (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (Hordeum vulgare). *Theoretical and Applied Genetics*, 101, 580–589.

Trebicki P. (2020) Climate change and plant virus epidemiology. *Virus Research*, 286, 198059.

van den Eynde R., van Leeuwen T., Haesaert G. (2020) Identifying drivers of spatio-temporal dynamics in barley yellow dwarf virus epidemiology as a critical factor in disease control. *Pest Management Science*, 76, 2548–2556.

Walls J., Rajotte E., Rosa C. (2019) The past, present, and future of barley yellow dwarf management. *Agriculture*, 9, 23.

Walsh L.E., Ferrari E., Foster S.P., Gaffney M.T. (2020) Evidence of pyrethroid tolerance in the bird cherry-oat aphid *Rhopalosiphum padi* in Ireland. *Outlooks on Pest Management*, 31, 5–9.

Wang X., Culver J.N. (2012) DNA binding specificity of ATAF2, a NAC domain transcription factor targeted for degradation by tobacco mosaic virus. *BMC Plant Biology*, 12.

Wang X., Liu Y., Chen L., Zhao D., Wang X., Zhang Z. (2013) Wheat resistome in response to barley yellow dwarf virus infection. *Functional & Integrative Genomics*, 13, 155–165.

Whitham S., Deng, Dinesh-Kumar, S. P., Choi D., Hehl R., Corr, Catherine, Baker, Barbara (1994) The product of the tobacco mosaic virus resistance gene N: similarity to Toll and the interleukin-1 receptor. *Cell*, 78, 1101–1115.

Xia X., Melchinger A.E., Kuntze L., Lübberstedt T. (1999) Quantitative trait loci mapping of resistance to sugarcane mosaic virus in maize. *Phytopathology*, 89, 660–667.

Xing Y., Ingvardsen C., Salomon R., Lübberstedt T. (2006) Analysis of sugarcane mosaic virus resistance in maize in an isogenic dihybrid crossing scheme and implications for breeding potyvirus-resistant maize hybrids. *Genome*, 49, 1274–1282.

Xu Y., Da Silva W.L., Qian Y., Gray S.M. (2018) An aromatic amino acid and associated helix in the C-terminus of the potato leafroll virus minor capsid protein regulate systemic infection and symptom expression. *PLoS Pathogens*, 14, e1007451.

Yang M., Li Z., Zhang K., Zhang X., Zhang Y., Wang X., Han C., Yu J., Xu K., Li D. (2018) Barley stripe mosaic virus γb interacts with glycolate oxidase and inhibits peroxisomal ROS production to facilitate virus infection. *Molecular Plant*, 11, 338–341.

Zhang Z., Chen L., Wang X., Xu H., Zhu X., Gao L., Xin Z. (2011) A serine/threonine kinase gene derived from *Thinopyrum intermedium* confers wheat resistance to BYDV infection. *Aspects of Applied Biology*, 110.

Zhang Z., Lin Z., Xin Z. (2009) Research progress in BYDV resistance genes derived from wheat and its wild relatives. *Journal of Genetics and Genomics*, 36, 567–573.

Zhang Z., Xin Z., Ma Y., Chen X., Xu Q., Lin Z. (1999) Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. *Science in China*, 42, 663–668.

Zhao S., Li Y. (2021) Current understanding of the interplays between host hormones and plant viral infections. *PLoS Pathogens*, 17, e1009242.

Ziegler-Graff V. (2020) Molecular insights into host and vector manipulation by plant viruses. *Viruses*, 12.

2 Manuscript 1: Identification of Candidate Genes for BYDV-PAV Resistance in Maize

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2.1 Abstract

Barley yellow dwarf is one of the economically most important virus diseases of cereals worldwide, causing yield losses of up to 80 %. However, means to control BYD are limited and the use of genetically resistant cultivars is the most economic and environmentally friendly approach. Maize plays a central role in the BYD infection cycle, serving as a reservoir for BYD-causing viruses and their vectors in summer. Growing BYD resistant maize varieties would reduce BYD pressure on maize and other cereals. Using two biparental mapping populations, we were able to reduce a previously published QTL for BYDV-PAV resistance in maize to a size of ~0.3 Mbp, comprising nine genes. Association mapping and gene expression analysis further reduced the number of candidate genes for BYDV-PAV resistance in maize to two: Zm00001eb428010 and Zm00001eb428020. Predicted functions of these genes suggest that they confer BYDV-PAV resistance either via interfering with virus replication or induction of ROS signaling. The sequence of one of these genes, Zm00001eb428010, is affected by a 54 bp deletion in the 5`-UTR and a protein altering variant in BYDV-PAV resistant maize inbreds but not BYDV-PAV susceptible and BYDV-PAV tolerant inbreds. This suggests that altered abundance and/or properties of the proteins that are encoded by Zm00001eb428010 may lead to BYDV-PAV resistance.

2.2 Introduction

World food consumption heavily relies on cereals. The three most important food crops in the world are rice, wheat, and maize (corn), accounting for an estimated 42 % of the world's human food calorie intake and 37 % of protein intake (Erenstein et al. 2022). With expected increase of world population (United Nations 2022), there is an increase of demand on cereals but global cereal production is threatened by climate change effects, herbivore pests and several diseases caused by fungi, bacteria and viruses (Rivero et al. 2022, Savary et al. 2019).

Barley Yellow Dwarf (BYD) is one of the economically most important diseases in small grain cereals (Choudhury et al. 2017, van den Eynde et al. 2020). This disease can infect all members of grass family (*Poaceae*), causing yield losses in cereals of up to 80 % and negatively affecting grain quality (Choudhury et al. 2019, Nancarrow et al. 2021, Peiris et al. 2019). BYD is caused by at least ten different phloem-limited single stranded positive sense RNA viruses called Barley Yellow Dwarf Viruses (BYDV) and Cereal Yellow Dwarf Viruses (CYDV) (Walls et al. 2019, Miller and Lozier 2022). Here, we refer to all BYD-causing viruses as YDVs.

YDVs are transmitted by more than 25 aphid species worldwide (Halbert and Voegtlin 1995). BYDV-PAV is the most prevalent YDV-species worldwide and mainly transmitted by *Rhopalosiphum padi* (Aradottir and Crespo-Herrera 2021). Several studies suggest that climate change will promote spread of *R. padi* and therefore spread of BYDV-PAV (for review see Irwin and Thresh 1990, Moreno-Delafuente et al. 2020).

Viruses possess no own metabolism, making difficult to target YDVs directly for control. The use of insecticides to limit the spread of aphid vectors not desirable because the application of insecticides is costly and not environmentally friendly (Chagnon et al. 2015, Serrão et al. 2022). Due to their harmful effects on beneficial insects, neonicotinoids - a very efficient and previously widely used class of insecticides (Simon-Delso et al. 2015) - are banned from application in the field in the European Union (Regulation (EU) No. 485/2013). Moreover, there is evidence for resistance against pyrethroid insecticides in some *R. padi* populations (Walsh et al. 2020). Thus, employment of genetically resistant cereal cultivars is the most promising approach to limit spread of YDVs.

However, breeding for YDV resistance is hampered by the unavailability of reliable high-throughput phenotyping methods. Visual symptom scoring is difficult. On the one hand, BYD symptoms are influenced by the environment and might be confused with symptoms of other diseases, nutrient deficiency, waterlogging or mechanical injury (Grüntzig et al. 1997). On the other hand, YDV-tolerant genotypes show no symptoms but the virus is able to replicate and move systematically in these plants. Thus, YDV-tolerant plants act as a virus reservoir and are a source for infection of other cereals. In contrast, resistant plants inhibit virus replication and/or systemic movement.

A more reliable alternative to visual symptom scoring is the quantification of the virus titer in plants by quantitative real time PCR (qRT-PCR), double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) or tissue blot immunoassay (TBIA). However, these

methods are laborious and time-consuming. Thus, the use of genetic information may help to accelerate the breeding process for YDV resistant varieties.

To date, no gene that confers complete YDV resistance has been identified in cereals so far. Only a limited number of Quantitative Trait Loci (QTL) are known. An even smaller number is used in breeding programs. These QTL often mediate rather tolerance than true resistance (for review see Choudhury et al. 2017, Jarošová et al. 2016, Walls et al. 2019).

Four genes - r*yd1, Ryd2, Ryd3*, and *Ryd4Hb*- are known to convey tolerance or resistance to YDVs in barley, but only *Ryd2* was introduced into commercial cultivars (Choudhury et al. 2017, Jarošová et al. 2016, Walls et al. 2019). *Ryd2* reduces the virus titer of BYDV-PAV and BYDV-MAV in young plants but does not convey resistance against all YDV species and is not effective in adult plants (Baltenberger et al. 1987, Riedel et al. 2011, Šip et al. 2004). *Ryd3* is a major gene for BYDV-PAV resistance (Niks et al. 2004). Despite presence of *Ryd3* seems to prevent BYDV-PAV replication, 20% of the plants carrying the *Ryd3* gene developed symptoms and had virus concentrations similar to those of susceptible accessions (Niks et al. 2004). *Ryd4Hb* was introgressed into barley from the wild relative *Hordeum bulbosum* but is not used in breeding programs because of linkage drag with a recessive sublethality factor (Scholz et al. 2009). Additional QTL for tolerance to BYD-PAV and BYDV-RPV were mapped on chromosomes 1H, 2H, 4H, and 7H of barley (del Blanco et al. 2015, Kraakman et al. 2006, Scheurer et al. 2001, Toojinda et al. 2000).

In wheat, no efficient resistance gene or QTL against YDVs is known from the primary gene pool. Only one gene – *bdv1* – confers tolerance to BYDV-MAV (Singh et al. 1993) and was introduced to commercial breeding. However, YDV resistance was found in the tertiary gene pool of wheat. Three YDV resistance genes – *Bdv2*, *Bdv3,* and *Bdv4* – were introgressed from *Thinopyrum intermedium* into wheat via translocation lines (for review see Zhang et al. 2009). However, the only gene used in wheat breeding is *Bdv2*, which conveys a broad-spectrum resistance to YDVs (Brettell et al. 1988, Zhang et al. 1999).

Maize plays an important role in the YDV transmission cycle serving as a “green bridge” between harvest of small-grain cereals in early summer and sowing of winter cereals in autumn (Brown et al. 1983, Haack et al. 1999, Rashidi et al. 2021). Cultivation of YDV-resistant maize may reduce YDV pressure on maize and small-grain cereals like wheat and barley. BYDV-PAV resistance in maize shows high genotypic variance and high heritability (Horn et al. 2013, 2014, and 2015), making it a promising target for breeding efforts.

Recently, a QTL for BYDV-PAV resistance was discovered in maize on the distal end of chromosome 10 (Horn et al. 2014 and 2015). In a genome wide association study (GWAS), Horn et al. (2014) identified three single nucleotide polymorphisms (SNPs) in gene GRMZM2G018027 (Zm00001eb428020) that are associated with BYDV-PAV resistance. These SNPs explained between 16 % and 21 % of the phenotypic variance of the trait virus titer (EX) as well as between 11 % and 18 % of the phenotypic variance of the trait infection rate (IR). Similarly, in another study employing five connected linkage mapping populations, Horn et al (2015) identified a QTL on the distal end of chromosome 10 that explained 45 % of the phenotypic variance for EX and IR.

The goals of this study were i) to identify the causative gene for BYDV-PAV resistance in maize, ii) to identify SNPs and/or structural variations in the gene sequences that may cause differences in susceptibility to BYDV-PAV of maize inbreds, and iii) to analyze effect of BYDV-PAV infection on gene expression of susceptible, tolerant, and resistant maize inbreds. These findings may be used to develop markers for marker assisted breeding of BYDV-PAV resistant maize as well as a starting point for the investigation of the resistance mechanism.

2.3 Methods

Plant cultivation and aphid rearing

Maize plants (*Zea mays*) were grown in a greenhouse (16h light, 20°C / 8h darkness, 16°C) for phenotyping of segregating material. all other experiments were conducted in a climate chamber (16h light, 24°C / 8h darkness, 22 °C).

BYDV-PAV carrying and virus-free *Rhopalosiphum padi* were reared on BYDV-susceptible barley cv. ‘Rubina’ at room temperature under artificial light conditions. Viruferous and virus-free aphids were checked regularly for presence of BYDV-PAV using the DAS-ELISA method with in-house polyclonal antisera for BYDV-PAV from the Julius Kühn-Institute as described by Horn et al. (2013).

Mapping of the BYDV-PAV resistance gene

Our study was based on heterogenous inbred family (HIF) populations developed from recombinant inbred lines (RILs) which were derived from crosses of BYDV-PAV tolerant inbred line P092 with BYDV-PAV resistant inbred lines FAP1360A and Ky226 (Horn et al. 2015). RILs

were selected that were heterozygous for the QTL interval but homozygous for the rest of the genome. RILs were selfed and their offspring were genotyped using total of 39 SNP-based Kompetitive allele-specific polymerase chain reaction (KASP™) marker (see below). We selected genotypes that were homozygous recombinant in the QTL for phenotyping (see below). This selection resulted in 83 homozygous genotypes derived from P092 x FAP1360A (population A) and 102 homozygous recombinants derived from Ky226 x P092 (population B). These individuals were selfed to generate seeds for replicated infection experiments as described below. Heterozygous HIF offspring were subjected to another round of selfing.

DNA extraction

DNA extraction was conducted using an in-house protocol. In brief, about 25 to 50 mg frozen plant material was homogenized using Tissue Lyzer II (Qiagen, Hilden, Germany) and 150 µl extraction buffer was added. After centrifugation (10 min, 4 °C, 4.000 RCF), 75 µl supernatant was transferred to a new plate containing 60 µl isopropanol, gently mixed and centrifuged (10 min, 4 °C, 4.000 RCF). The supernatant was discarded, the pellet was washed with 150 µl ethanol (70 %) and eluted in 100 µl TE buffer. DNA concentration of random samples was checked with a nanophotometer

KASP™ marker design and genotyping

KASP™ marker were designed in several rounds based on different sources of information on SNPs. No matter of the source, SNP information was filtered for identical alleles of Ky226 and FAP1360A but a different allele of P092. In the first rounds, marker information from Horn et al. (2015) was employed. Sequences flanking the SNPs at least 50 bp upstream and downstream were retrieved from the maize genetics and genomics database (https://www.maizegdb.org/) using reference version 4 of the B73 genome. Later, information from targeted sequencing of parental inbred lines was used for marker design. SNPs were preferred that have identical sequences of P092, Ky226, and FAP1360A in the 50 bp flanking regions. We aimed to spread markers evenly across the QTL confidence interval. Sequences were sent to the manufacturer LGC Genomics Ltd. (Hoddesdon, Herts, UK) who designed the markers.

Genotyping was conducted as recommended by the manufacturer. For each sample, 2,5 µl DNA were mixed with 2,5 µl KASP™ master mix (KASP V4.0 2X Master mix, Low Rox) and

0,07 µl KASP assay mix being specific for each SNP. An ABI Quantstudio 5 (Applied Biosystems) was used for analysis.

Inoculation and quantification of virus titer

The above described HIF populations were evaluated for IR and EX in four replications per genotype, where an experimental unit comprised eight to ten plants of one genotype.

When the maize plants reached the two-leaf stage, BYDV-PAV carrying *R. padi* were collected from the barley plants used for rearing. The aphids were distributed evenly across the maize plants in a way that approximately ten aphids per plant were applied. After one week, plants were treated with insecticide. Six weeks after the start of the inoculation, leaf material from the sixth leaf of each plant was harvested separately and the virus titer was determined using the DAS-ELISA method as described by Horn et al. (2013).

Plants with a virus titer < 0.5 were classified as resistant. The infection rate (IR) was calculated as the percentage of plants of one experimental unit with virus titer ≥ 0.5. EX was calculated as mean virus titer per experimental unit. An experimental unit comprised eight or ten plants of one genotype.

Phenotypic data analyses and association mapping analyses

Estimated marginal means of EX and IR from all repetitions of an experiment were calculated using a mixed linear model with genotype and repetition as fixed effects.

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

where Y_{ij} was the phenotypic observation for the ith genotype for the jth replicate, μ the general mean, g_i the effect of the ith genotype, r_j the effect of the jth replication, and e_{ij} the residual.

With the same model but with genotype as random effect, genotypic σ_g^2 and error variance σ_e^2 were calculated. Broad-sense heritability (H^2) was calculated based on the formula

$$H^2 = \sigma_g^2 / (\sigma_g^2 + (\sigma_e^2/n))$$

where n was the number of replications.

An association analyses of the traits, EX and IR, were performed for each of the 39 SNP-based KASP^{TM} marker using the model

$$Y_{ik} = \mu + s_k + m_i + e_{ik}$$

were s_k is the effect of the subpopulation and m_i is the effect of the marker. P-values were calculated with ANOVA.

Phenotypic data for BYDV-PAV resistance of an association panel were obtained from Horn et al. (2014). HapMap_3.2.1 genotypic data (Bukowski et al. 2018) corresponding to the 0.3 Mbp QTL confidence interval were retrieved from PANZEA (https://www.panzea.org/genotypes). Ambiguous data points were removed and marker were filtered for minor allele frequency > 0.025 and missing values < 20 %. Association analysis was conducted as described by Horn et al. (2014) using the Q matrix from Flint-Garcia et al. (2005) and K matrix from Horn et al. (2014).

Analyses were conducted using R version 3.6.3 (R Core Team 2020, https://www.R-project.org/) with packages "lme4" version 1.1-23 (Bates et al. 2015), "emmeans" version 1.5.1 (Lenth 2020), "car" version 3.0-10 (Fox and Weisberg 2019) and RStudio version 1.3.1073 (RStudio Team 2020, http://www.rstudio.com/).

Gene annotation

Protein sequences and information on gene annotation were retrieved from the maize genetics and genomics database (https://www.maizegdb.org/). Protein sequences were loaded into the webpage-based tool InterPro (https://www.ebi.ac.uk/interpro/; Paysan-Lafosse et al. 2022) to predict functional protein domains.

Degree of dominance of the resistance gene

Six sub-populations of HIFs were created to estimate the degree of dominance. Each of these sub-populations consisted of one genotype that was homozygous for the allele of P092 at marker SYN4811 and a sibling that was homozygous for the allele of Ky226 or FAP1360A. Additionally, one sibling was included that was heterozygous at marker SYN481. Alternatively, two offspring from crosses between the homozygous siblings were used to create a heterozygous genotype.

Plants were inoculated with BYDV-PAV carrying *R. padi* in two replications. Virus titer was measured and EX and IR per genotype were calculated (see previous section). Mean EX and IR per group (homozygous resistant, homozygous susceptible or heterozygous) were calculated. The degree of dominance was calculated using the formula

$$D = Aa – m / AA - m$$

where Aa is the phenotypic value of heterozygous plants, AA is the phenotypic value of plants that were homozygous for the allele of the BYDV-PAV susceptible parent P092, and m is the intermediate phenotypic value of AA and plants that were homozygous for the allele of the BYDV-PAV resistant parent.

Genomic characterization of maize inbreds

Probe design

Probes for target enrichment sequencing of founder inbred lines were designed for the QTL confidence interval identified by Horn et al. (2014) plus 1 Mbp to the distal end of the chromosome. At the time of probe design, reference sequences of seven maize inbred lines were available. These were B73 (Zm00001d.2), CML247 (Zm00024a.1), EP1 (Zm00010a.1), F7 (Zm00011a.1), Mo17 (Zm00014a.1), PH207 (Zm00008a.1), and W22 (Zm00004a.1). Sequences were retrieved from the maize genetics and genomics database (https://www.maizegdb.org/) and send to the probe design team of the manufacturer (Roche/Nimblegen). After masking of repetitive sequences, 2 million probes optimized for PacBio sequencing were designed, allowing up to three matches to the reference genome of B73 version 4 (Zm00001d.2).

DNA extraction and sequencing

DNA was extracted using NucleoMag Plant Kit (Macherey Nagel) following manufacturer's instructions including optional steps. DNA concentration and quality were assessed with a nanophotometer, a Qubit fluorometer (Invitrogen) with a Qubit dsDNA HS Assay Kit and a Fragment Analyzer (Advanced Analytical Technologies).

Sample preparation was conducted following PacBio protocol “Multiplex Genomic DNA Target Capture Using SeqCap EZ Libraries” (PN 100-893-500 version 03). In brief, genomic DNA was fragmented using gTUBES (Covaris), end-repaired and A-tailed using a KAPA HyperPlus Kit

(Roche), barcoded and adapters were ligated. DNA fragments were then amplified using a universal primer (Sigma-Aldrich) and Takara LA Taq DNA polymerase hot-start version (Takara). PCR fragments were selected for fragment length greater than 4.5 kbp with a BluePippin™ automated DNA size selection device (Sage Science), pooled, hybridized with SeqCap EZ Prime Developer Probes (Roche) and captured using HyperCap Target Enrichment Kit (Roche) and Dynabeads M-270 Streptavidin (Invitrogen). Captured DNA fragments were amplified using a universal primer and Takara LA Taq DNA polymerase hot-start version (Takara). SMRTbell™ library preparation was performed following the manufacturer's instructions. Sequencing was conducted on a Sequel II platform (PacBio).

Data processing, SNP calling and prediction of SVs

Reads were demultiplexed with python package demultiplex and trimmed with bbmap (sourceforge.net/projects/bbmap/). Trimmed reads were used to make a reference guided assembly of the QTL confidence interval with RaGOO (Alonge et al. 2019). Trimmed reads were mapped to B73 reference sequence version 5 (Zm00001eb) using minimap2 (Li et al. 2018) with parameter -ax asm20 and coverage was calculated with samtools depth and custom awk and python scripts. From reads that mapped into the QTL interval, single nucleotide polymorphisms (SNPs) and insertions/deletions of less than 50 bp length (InDels) were called using freebayes (Garrison et al. 2012). A custom python script was used to transform the vcf-files of all genotypes into a single matrix with variants per genotype. SNPs and InDels were subjected to variant effect prediction using Variant Effect Predictor tool from Gramene (https://ensembl.gramene.org/Oryza_sativa/Tools/VEP#) that employs the SIFT algorithm (Ng and Henikoff 2003). Insertions and deletions of 50 bp or more were defined as structural variations (SVs) and were called by re-mapping reads with cuteSV (Jiang et al. 2020).

Analysis of gene expression

RNA extraction and sequencing

Two independent experiments were conducted to analyze gene expression. In both experiments, plants of inbred lines FAP1360A, P092, and W64A were treated with BYDV-PAV carrying *R. padi*, virus-free *R. padi* or without aphids as control. Approximately ten BYDV-PAV carrying aphids per plant were applied when plants reached two leaf stage. After one week, all plants including controls were sprayed with insecticide “Careo” (Substral Celaflor).

In experiment 1 samples were taken 24 and 96 hours post infection (hpi). An experimental unit consisted of 4-8 plants of an inbred x treatment x timepoint of sampling combination. All plants of an experimental unit were pooled and the experiment was conducted in four replications. In experiment 2, 2-4 single plants per inbred x treatment combination were tested individually. Samples were taken two weeks after inoculation.

Leaves were harvested, frozen immediately in liquid nitrogen, and stored at -80 °C until further analysis. RNA was extracted with TRIzol (Ambion by Life Technologies) and Direct-Zol RNA MiniPrep Kit (Zymo Research) (experiment 1) or RNeasy Plant Mini Kit (Qiagen) (experiment 2) following the manufacturer's recommendations. All samples were treated with RNase-free DnaseI (ThermoFisher Scientific). RNA concentration was quantified using a Qubit fluorometer (Invitrogen) and a Qubit RNA HS Assay kit (Life Technologies, Eugene, USA) and RNA quality was assessed with a nanophotometer. Extracted RNA was send to Novogene Corporation Inc. (Sacramento, USA; experiment 1) or BGI Tech Solutions Co., Limited (Hong Kong; experiment 2). The high RNA quality of samples was confirmed and paired end sequencing of 150 bp reads was conducted on an Illumina or DNBseq™ platform, respectively. BYDV infection status was confirmed via DAS-ELISA six weeks after inoculation from the sixth leaf (experiment 1) or two weeks after inoculation from the youngest fully developed leaf, which was also used for RNA extraction (experiment 2).

RNA-Seq data processing

DNBSeq reads were filtered by BGI, including removing adaptor sequences, contamination and low-quality reads from raw reads using SOAPnuke software.

Quality control of raw and filtered reads of experiments 1 and 2 was conducted using FastQC version 0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Read trimming and adapter sequence removal using Trimmomatic v0.39 (Bolger et al. 2014). Unpaired reads were discarded. Exon and splice site information was retrieved from reference genome B73 reference sequence version 5 using HISAT2 version 2.1.0 (Kim et al. 2019). Reads were aligned to the reference genome B73 version 5 using HISAT2 version 2.1.0 (Kim et al. 2019). SamTools version1.6 (Li et al. 2009, Danecek et al. 2021) was used to index, sort, and filter mapped reads. Duplicates were removed. Reads per gene were counted with HTSeq version 0.11.1 (Anders et al. 2014, Putri et al. 2022).

Analysis of differently expressed genes (DEGs)

Analysis of differently expressed genes was conducted with edgeR version 3.28.1 (Robinson et al. 2010). Comparisons were made for aphid infested plants versus control (Aphid vs Ctrl), BYDV-PAV infected plants versus control (BYDV vs Ctrl), and BYDV-PAV infected plants versus aphid infested plants (BYDV vs Aphid) using "makeContrasts" function.

The lists of DEGs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using ShinyGO 0.76.3 (Ge et al. 2020, http://bioinformatics.sdstate.edu/go/). Pathway databases "KEGG", "GO Biological Process", "GO Cellular Component" and "GO Molecular Function" were used and parameters were set to FDR = 0.05, Pathway size: min = 2 and max = 2000, and redundancy was removed. No background gene list was provided because ShinyGO 0.76.3 employs protein coding genes as default.

2.4 Results

Mapping of the BYDV-PAV resistance gene

We used two connected populations of heterozygous inbred families (HIF) for fine mapping of the BYDV resistance gene. Only HIFs that were heterozygous for the QTL confidence interval but homozygous in the remaining parts of the genome were used for further analyses.

We calculated the degree of dominance using six subpopulations. Across all subpopulations and replications, the degree of dominance was -0,44 for EX and -0,18 for IR.

For fine mapping of the BYDV-PAV resistance in maize, homozygous genotypes were selected that were recombinant in the QTL confidence interval. This selection procedure resulted in 83 plants originating from cross P092 x FAP1360A (population A) and 102 plants from cross Ky226 x P092 (population B). These were subjected to phenotyping for BYDV-PAV resistance.

Broad sense heritability (H^2) was calculated 0.89 for EX and 0.82 for IR in a combined analysis of both populations. Analyzing both populations separately, H^2 of population A was with 0.79 for EX and 0.70 for IR lower, compared to 0.92 for EX and 0.85 for IR in population B.

Estimated marginal means ranged from 0.11 to 1.65 for the trait EX and -0.05 to 1.14 for the trait IR. For both traits, estimated marginal means looked normally distributed (Figure 2.1), Density curves separated by population suggest that BYDV-PAV resistance might be differently

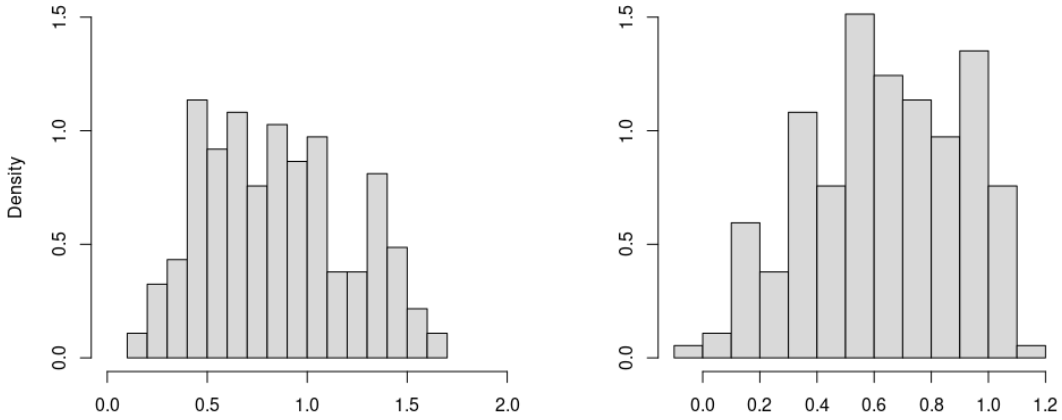

Figure 2.1: Histogram of estimated marginal means of virus titer (EX, left) and infection rate (IR, right). One-hundred and eighty-five homozygous offspring from two heterozygous inbred families were infected with BYDV-PAV and virus titer in the sixth was analyzed six weeks after infection.

strong in the two populations and effects overlap (Figure 2.2). Thus, we involved the population effect in our linkage analyses of BYDV-PAV titer and genetic marker data.

We conducted several rounds of linkage mapping of genetic marker data with traits EX and IR. In each round, new homozygous inbreds were added that were recombinant in the QTL confidence interval, as well as new genetic marker data that provided a higher resolution of the QTL. Finally, we considered marker PZE-110080306 and the newly developed marker BYDV-M20 as flanking marker of the QTL interval as these are the first marker for which -log10 p-values started to decline (Figure 2.3).

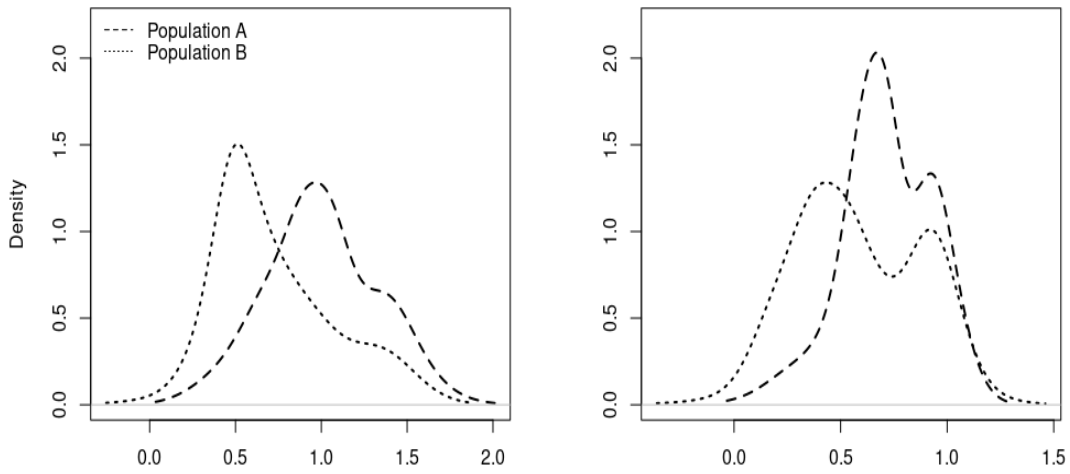

Figure 2.2: Density curves of estimated marginal means of virus titer (EX, left) and infection rate (IR, right), separated by population. One-hundred and eighty-five homozygous offspring from two heterozygous inbred families were infected with BYDV-PAV and virus titer in the sixth was analyzed six weeks after infection.

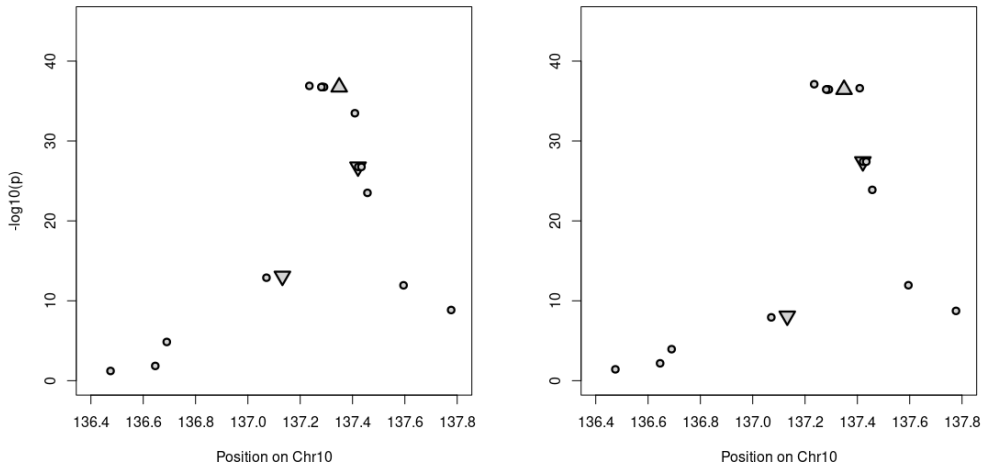

Figure 2.3: Manhattan plots of the association between BYDV-PAV resistance in 185 maize inbreds and genetic marker. Left: trait virus titer (EX), right: trait infection rate (IR). Marker positions are given based on the reference sequence of B73, version 5. Up-facing triangle: marker SYN4811. Down-facing triangles: flanking marker PZE-110080306 (left) and BYDV-M20 (right).

Thus, the QTL interval was reduced from 8 Mbp (Horn et al. 2015) to ~0.3 Mbp. The QTL is located chromosome 10 at 137131915..137421072 bp, comprising nine genes, including Zm00001eb428020, a candidate gene for BYDV-PAV resistance (Horn et al. 2014), two transcription factors (Zm00001eb427970 and Zm00001eb427980), a putative WAK-related receptor-like protein kinase family protein (Zm00001eb427960), a putative RING zinc finger domain superfamily protein (Zm00001eb427950), a DNA2/NAM7 helicase-like protein (Zm00001eb428010) (Table 2.1).

Table 2.1: Genes in the 0.3 Mbp QTL confidence interval for BYDV-PAV resistance in maize on chromosome 10. Coordinates are given based on the reference sequence of B73, version 5.

Gene ID	Start	End	Description / suggested function
Zm00001eb427940	137133463	137134445	unknown
Zm00001eb427950	137197560	137198870	RING zinc finger domain superfamily protein
Zm00001eb427960	137214761	137217464	WAK-related receptor-like protein kinase family protein
Zm00001eb427970	137229874	137233357	ABI3-VP1-transcription factor 2
Zm00001eb427980	137263651	137266456	Transcription factor bHLH28 like
Zm00001eb427990	137278582	137280136	unknown
Zm00001eb428000	137280991	137284148	unknown
Zm00001eb428010	137285187	137290824	DNA2/NAM7 helicase-like protein
Zm00001eb428020	137348959	137349907	response to oxidative stress, response to cadmium ion

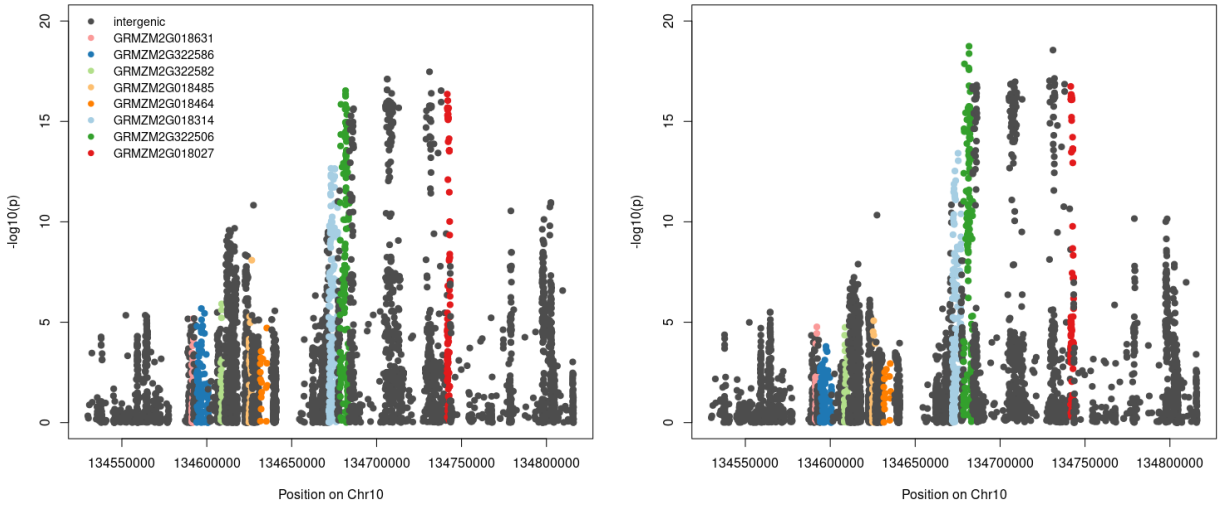

Figure 2.4: Manhattan plots for the association analysis of BYDV-PAV resistance and HapMap3.2.1 marker. The association of genetic marker with EX (left) and IR (right) is shown. Variant positions and gene names are given based on B73 reference genome version 3.

To further reduce the number of candidate genes, we used BYDV-PAV phenotyping data from an association mapping panel described by Horn et al. (2014). Genotypic data were retrieved from HapMap3.2.1 (Bukowski et al. 2018). Highest association for BYDV-PAV resistance was found for markers located in genes GRMZM2G322506 (Zm00001eb428010) and GRMZM2G018027 (Zm00001eb428020) and intergenic space in between these two genes (Figure 2.4).

Analysis of sequence variation in the QTL interval

Targeted long read sequencing of the five founder inbred lines FAP1360A, Ky226, P092, D408, and W64A resulted in 1,579,826 raw reads and 8,421,284,727 bases sequenced. Reads were filtered, mapped against B73 reference genome version 5 (Zm00001eb) and assembled to contigs. The total length of contigs was between 9,747,441 and 14,948,168 bp per maize inbred.

Sequences that mapped to the 0.3 Mbp QTL confidence interval were subjected to analysis of SNPs, InDels, and SVs. The three BYDV-PAV resistant inbreds had similar numbers of variants when compared to reference B73. We counted 1972, 1911, and 1869 SNPs and InDels for D408, FAP1360A, and Ky226, respectively. BYDV-PAV susceptible W64A had slightly less variants (1797) and for BYDV-PAV tolerant P092 the lowest number of variants (1139) compared to B73 was detected.

SNPs and InDels were subjected to variant effect prediction. More than 94 % of SNPs and InDels were predicted to be modifiers such as upstream and downstream gene variants, intron variants, intergenic variants, and 5'- and 3'-UTR variants (Table 2.1). The SIFT algorithm predicted a high impact for 19 SNPs and InDels (Table 2.2). However, of those only one SNP, which leads to a frameshift in gene Zm00001eb428000, was shared by the three BYDV-resistant founder inbreds but not by P092 and W64A (Supplementary table 2.1). Additionally, six protein altering variants were detected. One protein altering variant in gene Zm00001eb428010 was shared by the three BYDV-PAV resistant inbreds but not by P092 and W64A (Supplementary table 2.1). The other five variants were located in gene Zm00001eb427970, of which three were shared between D408 and FAP1360A, and two were unique to Ky226 (Supplementary table 2.1).

Table 2.2: Variant effect prediction of SNPs and InDels in the 0.3 Mbp QTL confidence interval on chromosome 10 of five maize inbreds. Individual SNPs and InDels may have multiple effects, depending on the transcripts that are affected.

Impact	Consequence	Count
High	stop gained	2
	start lost	1
	stop gained, frameshift variant	1
	frameshift variant	12
	splice acceptor variant, coding sequence variant	1
	splice acceptor variant, intron variant	2
Moderate	protein altering variant	6
	inframe deletion	6
	inframe insertion	4
	missense variant, splice region variant	1
	missense variant	159
Low	splice region variant, intron variant	11
	splice region variant, synonymous variant	1
	stop retained variant	1
	synonymous variant	85
Modifier	5'- UTR variant	63
	3'- UTR variant	148
	intron variant	244
	upstream gene variant	1688
	downstream gene variant	1258
	intergenic variant	1699

In addition, 34 SVs were detected in the 0.3 Mbp QTL confidence interval, the majority of them (24) being unique to one inbred (Supplementary table 2.2). Only nine SVs were located in a gene (Supplementary table 2.2). Remarkably, the three BYDV-PAV resistant inbreds shared in Zm00001eb428010 a 54 bp deletion located in the 5' UTR, a 91 bp insertion in intron 6 and a 362 bp deletion in intron 7. These were not present in susceptible and tolerant inbreds, respectively (Supplementary table 2.2).

Only SNPs and InDels with low (synonymous variants) or modifier effect (intron or upstream/down-stream gene variants) but no SNPs with predicted high or protein altering effect or SVs were detected for the BYDV-PAV resistance candidate gene Zm00001eb428020 (Supplementary table 2.1 and Supplementary table 2.2).

Gene expression

Two independent experiments were conducted to analyze the effect of BYDV-PAV infection on genome-wide gene expression in maize. Samples were taken 24 hours past infection (hpi) and 96 hpi in experiment 1 as well as two weeks after inoculation in experiment 2. Differently expressed genes (DEGs) were identified for following comparisons: aphid infested plants versus control (Aphid vs Ctrl), BYDV-PAV infected plants versus control (BYDV vs Ctrl), and BYDV-PAV infected plants versus aphid infested plants (BYDV vs Aphid).

Only a small number of genes was differently expressed in experiment 1 (Table 2.3). P092 had the most DEGs with a total of 111 across all time points and comparisons. A total of 88 DEGs were found for FAP1360A but no DEGs were found for W64A (Table 2.3). Also, we did not find any DEGs in FAP1360A for the comparison BYDV vs Aphid as well as in P092 for Aphid vs Ctrl (Table 2.3). Most DEGs were found in the up-regulated group at 96 hpi in both FAP1360A and P092 (Table 2.3). Remarkably, the 25 downregulated genes in BYDV vs Ctrl of FAP1360A at 24 hpi were enriched for nucleotide and nucleoside biosynthesis and metabolism processes.

In the second experiment, a considerably higher number of DEGs was detected (Table 2.4). *R. padi* infestation and BYDV-PAV infection had a low effect on gene expression in FAP1360A in comparison to P092 and W64A. We found eight to 19 times more DEGs in P092 and 13 to 204 times more DEGs in W64A than in FAP1360A, respectively. Interestingly, there were no DEGs in BYDV vs Aphid in FAP1360A (Table 2.4). In contrast, BYDV vs Aphid was the comparison with most DEGs in P092 and W64A for up-regulated and down-regulated genes, respectively. Remarkably, among downregulated genes in P092 in BYDV vs Aphid, KEGG

pathways ‘Phagosome’ (zma04145) and ‘Spliceosome’ (zma03040) were enriched 2.8-fold and 1.9-fold, respectively.

Table 2.3: Counts of differently expressed genes (DEGs) in experiment 1 at 24 hpi and 96 hpi

Inbred	Comparison	24 hpi		96 hpi	
		up	down	up	down
FAP1360A	BYDV vs Control	4	25	26	0
	BYDV vs Aphid	0	0	0	0
	Aphid vs Control	0	1	31	1
P092	BYDV vs Control	0	1	61	5
	BYDV vs Aphid	0	1	43	0
	Aphid vs Control	0	0	0	0
W64A	BYDV vs Control	0	0	0	0
	BYDV vs Aphid	0	0	0	0
	Aphid vs Control	0	0	0	0

In the second experiment, a considerably higher number of DEGs was detected (Table 2.4). *R. padi* infestation and BYDV-PAV infection had a low effect on gene expression in FAP1360A in comparison to P092 and W64A. We found eight to 19 times more DEGs in P092 and 13 to 204 times more DEGs in W64A than in FAP1360A, respectively. Interestingly, there were no DEGs in BYDV vs Aphid in FAP1360A (Table 2.4). In contrast, BYDV vs Aphid was the comparison with most DEGs in P092 and W64A for up-regulated and down-regulated genes, respectively. Remarkably, among downregulated genes in P092 in BYDV vs Aphid, KEGG pathways ‘Phagosome’ (zma04145) and ‘Spliceosome’ (zma03040) were enriched 2.8-fold and 1.9-fold, respectively.

Only two genes of the 0.3 Mbp QTL confidence interval, Zm00001eb428010 and Zm00001eb428020, were expressed in both experiments. Additionally, Zm00001eb428000 was expressed in experiment 2 but with lower abundance than Zm00001eb428010 and Zm00001eb428020. None of these three genes was differently expressed in any genotype under any condition.

Table 2.4: Counts of differently expressed genes (DEGs) in experiment 2.

Genotype	Comparison	up	down
	BYDV vs Control	347	290
FAP1360A	BYDV vs Aphid	0	0
	Aphid vs Control	18	36
	BYDV vs Control	2904	3509
P092	BYDV vs Aphid	3058	3546
	Aphid vs Control	350	383
	BYDV vs Control	4577	4880
W64A	BYDV vs Aphid	5137	5010
	Aphid vs Control	3671	4001

2.5 Discussion

Genes in the QTL for BYDV-PAV resistance in maize

We identified a 0.3 Mbp QTL confidence interval for BYDV-PAV resistance in maize comprising nine annotated genes in the fifth version of the B73 reference genome (Zm00001eb) (Table 2.1). The putative function of these nine genes suggest that some of them might be involved in virus defense-related processes and thus convey resistance against BYDV-PAV in maize. Genes in the QTL confidence interval and their putative functions are the following:

Zm00001eb427950 is annotated as RING-H2 finger protein ATL2L and associated GO terms include protein ubiquitination (GO:0016567) and ubiquitin protein ligase activity (GO:0061630), among others. The ubiquitin proteasome system (UPS) is a rapid, adaptive mechanism for selective protein degradation and plays complex roles during virus infection in plants (reviewed in Dubiella and Serrano 2021).

Zm00001eb427960 is a putative wall associated kinase (WAK)-related receptor-like protein kinase family protein characterized by a serine-threonine/tyrosine-protein kinase as catalytic domain. Transcripts of several WAKs and serine/threonine-protein kinases are significantly up-regulated during *Psathyrostachys huashanica* (*Poaceae*) infection with BYDV-GAV (Shen et al. 2020) and a cytosolic serine/threonine/tyrosine kinase was found to suppress replication of *Hordeiviruses* barley stripe mosaic virus (BSMV) and lychnis ringspot virus (LRSV) in *Nicotiana benthamiana* (Zhang et al. 2021). The serine/threonine kinase gene TiSTK1 was identified as the causative YDV resistance gene of *Bdv2* in wheat (Zhang et al. 2011).

Transcription factors (TFs) are key components of plant defense signaling (Viswanath et al. 2023). Zm00001eb427980 contains a MYC-type basic helix-loop-helix (bHLH) domain. Shen et al. (2020) found that TFs, including bHLH type TFs, are widely involved in the defense response of *P. huashanica* to BYDV-GAV.

Proteins encoded by Zm00001eb428000 and Zm00001eb428010 contain two AAA domains. GO-terms for both genes are RNA binding (GO:0003723) and helicase activity (GO:0004386). AAA domain containing proteins possess diverse functions, including disassembly of SNARE proteins, protein quality control, DNA replication, ribosome assembly, and viral replication (Khan et al. 2022). Proteins encoded by Zm00001eb428010 and Zm00001eb428000 are predicted to belong to the DNA2/NAM7-like helicase family. Nam7, also known as Upstream frameshift 1 (Upf1), targets plant and animal viruses for nonsense-mediated mRNA decay (NMD) (for review see May and Simon 2021). However, many viruses escape Upf1-mediated decay through *cis*-acting RNA sequences and *trans*-acting viral proteins (May and Simon 2021).

Horn et al. (2014) identified three SNPs in Zm00001eb428020 (GRMZM2G018027) that were significantly associated with EX and IR and proposed this gene as a candidate gene for BYDV-PAV resistance in maize. Zm00001eb428020 is associated with GO terms 'response to oxidative stress' (GO:0006979) and 'response to cadmium ion' (GO:0046686) in the molecular function category and 'nuclear speck' (GO:0016607) in the cellular component category.

Nuclear speckles (NS) are nuclear membraneless bodies that are enriched in splicing factors (Hasenson and Shav-Tal 2020). Fungal effectors are able to induce susceptibility of host plants by inducing alternative splicing of host transcripts at NS (Tang et al. 2022). The same process is suspected for oomycete effectors (Wang et al. 2015).

The best BLAST hit for Zm00001eb428020 in *Arabidopsis thaliana* is the gene *OXS3* (Horn et al. 2014). *OXS3* is expressed during response reactions to oxidative stress (Blanvillain et al. 2009) and likely improves resistance to Tobacco mosaic virus in *A. thaliana* by the production of hydrogen-peroxide (Wang and Culver 2012).

Until now, no function has been suggested for genes Zm00001eb427940, Zm00001eb427970, and Zm00001eb427990.

Changes in gene expression following BYDV-PAV infection

We performed two independent experiments to investigate changes in gene expression in maize caused by BYDV-PAV infection. In experiment 1, samples were taken 24 hpi and 96 hpi,

representing processes at early infection stages. In contrast to other gene expression studies on YDV infection in cereals or virus infection in maize (Cao et al. 2019, Li et al. 2018, Rong et al. 2018, Shen et al. 2020, Wang et al. 2013, Zhou et al. 2016), only a low number of DEGs was detected (Table 2.3). We suspect that early reactions to BYDV-PAV infection are limited to the phloem cells that are penetrated by aphids during feeding and maybe a few adjacent cells. Using whole leaves might lead to 'dilution effects' that prevent detection of DEGs because unaffected cells outnumber infected cells. Thus, single cell sequencing might be a more feasible approach.

Experiment 2 represents processes in the plant at a later infection stage in systemic leaves. Virus titer was proportional to the number of DEGs. Very low virus titer and numbers of DEGs were detected in the BYDV-PAV resistant inbred FAP1360A when compared to BYDV-PAV tolerant P092 and BYDV-PAV susceptible W64A. Together with the fact that no DEGs were found in BYDV vs Aphid (Table 2.4), this leads to the conclusion that the BYDV-PAV resistance gene may act at early stages after infection, hampering virus replication and/or movement, enabling the plant to grow relatively unaffected.

Less genes of BYDV-PAV tolerant inbred P092 were differently expressed than genes of BYDV-PAV susceptible W64A (Table 2.4), potentially reflecting the lack of symptom formation (Horn et al. 2013, this study). Consistently, DEGs of the BYDV-PAV susceptible genotype P092 were not enriched for genes related to chloroplasts or photosynthesis. This might be a starting point to answer the question why BYDV-PAV is able to replicate and spread in P092 but does not cause visible symptoms.

In both experiments, Zm00001eb428010 and Zm00001eb428020 were the only two genes in the 0.3 Mbp QTL confidence interval that were expressed, indicating that either one of them is the causative agent for BYDV-PAV resistance in maize. However, neither Zm00001eb428010 nor Zm00001eb428020 were differently expressed, suggesting that BYDV-PAV resistance in maize might be of passive nature or mechanisms act at time points that were not covered by our experiments or act only in cells that are penetrated during aphid salivation.

Another explanation is that resistance gene product regulation happens at protein level and not at gene expression level. Protein abundance might be shaped by post-transcriptional gene regulation (for review see Prall et al. 2019). Protein substrate specificity and kinetics might be influenced by changes in amino acid sequence evoked through SNPs or alternative splicing. Indeed, it has been shown that the transcriptome of maize is subjected alternative splicing

during viral infection (Du et al. 2020, Zhou et al. 2022). Two isoforms of Zm00001eb428010 are published for B73 and up to four isoforms are published for the 25 NAM parents (Hufford et al. 2021). Additionally, we identified one small InDel in Zm00001eb428010 that is predicted to have a protein altering effect in all BYDV-PAV resistant inbreds but not in the tolerant and susceptible inbreds (Supplementary table 2.1). However, further work is necessary to identify isoforms of Zm00001eb428010 that are expressed in different inbreds under BYDV-PAV infection and analyze their protein substrate specify and kinetics.

Sequence variations in genes of the QTL

A number of other studies found associations between SVs and agronomic traits, tolerance against abiotic stress or disease resistance (for review see Lye and Purugganan 2019, Zanini et al. 2022).

In this study, we identified 34 SVs in the QTL confidence interval, the majority of them are insertions or deletions and located in intergenic region and/or were unique to one genotype (Supplementary table 2.2), suggesting that these variants are not responsible for the differences in BYDV-PAV susceptibility of the maize inbreds.

However, one gene in the QTL interval (Zm00001eb428010) was affected by three SVs (Supplementary table 2.2). These SVs were shared between all three BYDV-PAV resistant inbreds but not present in susceptible and tolerant inbreds (Supplementary table 2.2). The relatively small size of the SVs in Zm00001eb428010 (51 bp, 91 bp, and 362 bp) is in accordance with findings by Hufford et al. (2021). Almost half of the SVs found among the 25 NAM parents and B73 were smaller than 5 kbp, and a quarter were smaller than 500 bp (Hufford et al. 2021).

Two SVs are located in intronic regions of Zm00001eb428010 and one SV is a 54 bp deletion that is located in the 5`-UTR. 5`- and 3`-UTR possess *cis*-acting elements for post-transcriptional control that regulate mRNA stability, transport, and translation efficiency as well as the functioning and subcellular localization of the translated proteins (Mignone et al. 2002). Some 5`-UTR are known to influence translation efficiency (Srivastava et al. 2018, Yamasaki et al. 2018). Thus, the deletion in the 5`-UTR may influence protein abundance. This is supported by the fact that we could not detect differential expression of Zm00001eb428010 upon BYDV-PAV infection.

Association study

To further reduce the number of candidate genes, we conducted an association study using BYDV-PAV resistance data from an association mapping population (Horn et al. 2014) and HapMap3.2.1 genotypic data (Bukowski et al. 2018) for the 0.3 Mbp QTL confidence interval. These analyses showed strong associations of BYDV-PAV resistance with marker located in genes Zm00001eb428010 and Zm00001eb428020 but not with marker located in other genes of the 0.3 Mbp QTL confidence interval. This confirms that either Zm00001eb428010 or Zm00001eb428020 confers BYDV-PAV resistance in maize.

2.6 Conclusion

Combining biparental mapping, an association study, and analysis of gene expression, we identified two candidate genes for BYDV-PAV resistance in maize: Zm00001eb428010 and Zm00001eb428020. The predicted functions of these genes suggest a rather unspecific resistance mechanism, potentially by interfering with virus replication or induction of ROS signaling. Expression of Zm00001eb428010 and Zm00001eb428020 was not influenced by BYDV-PAV infection in any inbred. However, sequence variants of Zm00001eb428010 that are present in BYDV-PAV resistant inbreds but in BYDV-PAV susceptible or BYDV-PAV tolerant inbreds suggest that abundance and/or properties of the proteins that are encoded by Zm00001eb428010 may lead to BYDV-PAV resistance. Zm00001eb428010 and Zm00001eb428020 are located at the distal end of maize chromosome 10, a genomic region that contains multiple overlapping QTL for resistance to diverse viruses. This suggests that the BYDV-PAV resistance gene may be efficient to other viruses of maize as well.

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2.8 References

Alonge M., Soyk S., Ramakrishnan S., Wang X., Goodwin S., Sedlazeck F.J., Lippman Z.B., Schatz M.C. (2019) RaGOO: fast and accurate reference-guided scaffolding of draft genomes. *Genome Biology*, 20, 224.

Anders S., Pyl P.T., Huber W. (2015) HTSeq - a Python framework to work with high-throughput sequencing data. *Bioinformatics*, 31, 166–169.

Aradottir G.I., Crespo-Herrera L. (2021) Host plant resistance in wheat to barley yellow dwarf viruses and their aphid vectors: a review. *Current Opinion in Insect Science*, 45, 59–68.

Baltenberger D.E., Ohm H.W., Foster J. (1987) Reactions of oat, barley, and wheat to infection with barley yellow dwarf virus isolates. *Crop Science*, 27, 195–198.

Bates D., Maechler M., Bolker B., Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Blanvillain R., Kim J.H., Wu S., Lima A., Ow D.W. (2009) OXIDATIVE STRESS 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress. *The Plant Journal*, 57, 654–665.

Bolger A.M., Lohse M., Usadel B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114–2120.

Brettell R.I.S., Banks P.M., Cauderon Y., Chen X., Cheng Z.M., Larkin P.J., Waterhouse P.M. (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. *Annals of Applied Biology*, 113, 599–603.

Brown J.K., Wyatt S.D., Hazelwood D. (1984) Irrigated corn as a source of barley yellow dwarf virus and vector in eastern Washington. *Phytopathology*, 74, 46–49.

Bukowski R., Guo X., Lu Y., Zou C., He B., Rong Z., Wang B., et al. (2018) Construction of the third-generation *Zea mays* haplotype map. *GigaScience*, 7.

Cao N., Zhan B., Zhou X. (2019) Nitric oxide as a downstream signaling molecule in Brassinosteroid-mediated virus susceptibility to maize chlorotic mottle virus in maize. *Viruses*, 11.

Chagnon M., Kreutzweiser D., Mitchell E.A.D., Morrissey C.A., Noome D.A., van der Sluijs J.P. (2015) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research International*, 22, 119–134.

Choudhury S., Hu H., Meinke H., Shabala S., Westmore G., Larkin P., Zhou M. (2017) Barley yellow dwarf viruses: infection mechanisms and breeding strategies. *Euphytica*, 213, 213-168.

Choudhury S., Larkin P., Meinke H., Hasanuzzaman M.D., Johnson P., Zhou M. (2019) Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat. *Crop and Pasture Science*, 70, 16.

Danecek P., Bonfield J.K., Liddle J., Marshall J., Ohan V., Pollard M.O., Whitwham A., Keane T., McCarthy S.A., Davies R.M., Li H. (2021) Twelve years of SAMtools and BCFtools. *GigaScience*, 10.

Del Blanco I.A., Hegarty J., Gallagher L.W., Falk B.W., Brown-Guedira G., Pellerin E., Dubcovsky J. (2015) Mapping of QTL for tolerance to cereal yellow dwarf virus in two-rowed spring barley. *Crop Science*, 54, 1468–1475.

Du K., Jiang T., Chen H., Murphy A.M., Carr J.P., Du Z., Li X., Fan Z., Zhou T. (2020) Viral perturbation of alternative splicing of a host transcript benefits infection. *Plant Physiology*, 184, 1514–1531.

Dubiella U., Serrano I. (2021) The ubiquitin proteasome system as a double agent in plant-virus interactions. *Plants*, 10.

Erenstein O., Jaleta M., Sonder K., Mottaleb K., Prasanna B.M. (2022) Global maize production, consumption and trade: trends and R&D implications. *Food Security*, 14, 1295–1319.

Flint-Garcia S.A., Thuillet A.C., Yu J., Pressoir G., Romero S.M., Mitchell S.E., Doebley J., Kresovich S., Goodman M.M., Buckler E.S. (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant Journal*, 44, 1054–1064.

Fox J., Weisberg S. (2019) An {R} companion to applied regression. URL https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Garrison E., Marth G. (2012) Haplotype-based variant detection from short-read sequencing. *arXiv: Genomics*.

Ge S.X., Jung D., Yao R. (2020) ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*, 36, 2628–2629.

Grüntzig M., Fuchs E., Werner M. (1997) Occurence and influence of barley yellow dwarf luteovirus (BYDV) on growth and yield of maize. *Nachrichtenblatt des deutschen Pflanzenschutzdienstes*, 49, 45–50.

Haack L., Courbon R., Riault G., Tanguy S., Le Vilain D., Henry M., Dedryver C.A. (1999) A plant and field study of BYDV-PAV and-MAV distribution on maize in France. *Journal of Plant Diseases and Protection*, 297–303.

Halbert S., Voegtlin D. (1995) Biology and taxonomy of vectors of barley yellow dwarf viruses. In: *Barley yellow dwarf: 40 years of progress*, pp. 9–28. Eds C. J. D'Arcy and P. A. Burnett. St Paul: APS Press.

Hasenson S.E., Shav-Tal Y. (2020) Speculating on the roles of nuclear speckles: How RNA-protein nuclear assemblies affect gene expression. *BioEssays*, 42.

Horn F., Habekuß A., Stich B. (2013) Natural variation for BYDV resistance in maize. *Maydica*, 58.

Horn F., Habekuß A., Stich B. (2014) Genes involved in barley yellow dwarf virus resistance of maize. *Theoretical and Applied Genetics*, 127, 2575–2584.

Horn F., Habekuß A., Stich B. (2015) Linkage mapping of barley yellow dwarf virus resistance in connected populations of maize. *BMC Plant Biology*.

Hufford M.B., Seetharam A.S., Woodhouse M.R., Chougule K.M., Ou S., Liu J., Ricci W.A., et al. (2021) De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. *Science*, 373, 655–662.

Irwin M.E., Thresh J.M. (1990) Epidemiology of barley yellow dwarf: A study in ecological complexity. *Annual Review of Phytopathology*, 28, 393–424.

Jarošová J., Beoni E., Kundu J.K. (2016) Barley yellow dwarf virus resistance in cereals: Approaches, strategies and prospects. *Field Crops Research*, 198, 200–214.

Jiang T., Liu Y., Jiang Y., Li J., Gao Y., Cui Z., Liu Y., Liu B., Wang Y. (2020) Long-read-based human genomic structural variation detection with cuteSV. *Genome Biology*, 21, 189.

Khan Yousuf A., White K. Ian, Brunger Axel T. (2022) The AAA+ superfamily: a review of the structural and mechanistic principles of these molecular machines. *Critical Reviews in Biochemistry and Molecular Biology*, 57, 156–187.

Kim D., Paggi J.M., Park C., Bennett C., Salzberg S.L. (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*, 37, 907–915.

Kraakman A.T.W., Martínez F., Mussiraliev B., van Eeuwijk F.A., Niks R.E. (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. *Molecular Breeding*, 17, 41–58.

Lenth R. (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means: R package version 1.5.1. URL https://CRAN.R-project.org/package=emmeans.

Li A., Li G., Zhao Y., Meng Z., Zhao M., Li C., Zhang Y., Li P., Ma C.-L., Xia H., Zhao S., Hou L., Zhao C., Wang X. (2018) Combined small RNA and gene expression analysis revealed roles of miRNAs in maize response to rice black-streaked dwarf virus infection. *Scientific Reports*, 8, 13502.

Li H. (2018) Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, 34, 3094–3100.

Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics*, 25, 2078–2079.

Lye Z.N., Purugganan M.D. (2019) Copy number variation in domestication. *Trends in Plant Science*, 24, 352–365.

May J.P., Simon A.E. (2021) Targeting of viral RNAs by Upf1-mediated RNA decay pathways. *Current Opinion in Virology*, 47, 1–8.

Mignone F., Gissi C., Liuni S., Pesole G. (2002) Untranslated regions of mRNAs. *Genome Biology*, 3.

Miller W.A., Lozier Z. (2022) Yellow dwarf viruses of cereals: taxonomy and molecular mechanisms. *Annual Review of Phytopathology*, 60, 121–141.

Moreno-Delafuente A., Viñuela E., Fereres A., Medina P., Trębicki P. (2020) Simultaneous increase in CO_2 and temperature alters wheat growth and aphid performance differently depending on virus infection. *Insects*, 11.

Nancarrow N., Aftab M., Hollaway G., Rodoni B., Trębicki P. (2021) Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: A three-year field study in South-Eastern Australia. *Microorganisms*, 9.

Ng P.C., Henikoff S. (2003) SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Research*, 31, 3812–3814.

Niks R.E., Habekuss A., Bekele B., Ordon F. (2004) A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus. *Theoretical and Applied Genetics*, 109, 1536–1543.

Paysan-Lafosse T., Blum M., Chuguransky S., Grego T., Pinto B.L., Salazar G.A., Bileschi M.L., Bork P., Bridge A., Colwell L., Gough J., Haft D.H., Letunić I., Marchler-Bauer A., Mi H., Da Natale, Orengo C.A., Pandurangan A.P., Rivoire C., Sigrist CJA, Sillitoe I., Thanki N., Thomas P.D., Tosatto S.C., Wu C.H., Bateman A. (2022) InterPro in 2022. *Nucleic Acids Research*, 51, D418-D427

Peiris K.H.S., Bowden R.L., Todd T.C., Bockus W.W., Davis M.A., Dowell F.E. (2019) Effects of barley yellow dwarf disease on wheat grain quality traits. *Cereal Chemistry*, 96, 754–764.

Prall W., Sharma B., Gregory B.D. (2019) Transcription is just the beginning of gene expression regulation: the functional significance of RNA-binding proteins to post-transcriptional processes in plants. *Palnt & Cell Physiology*, 60, 1939–1952.

Putri G.H., Anders S., Pyl P.T., Pimanda J.E., Zanini F. (2022) Analysing high-throughput sequencing data in Python with HTSeq 2.0. *Bioinformatics*, 38, 2943–2945.

R Core Team (2020) R: A language and environment for statistical computing. URL https://www.R-project.org/.

Rashidi M., Cruzado R.K., Hutchinson P.J.S., Bosque-Pérez N.A., Marshall J.M., Rashed A. (2021) Grassy weeds and corn as potential sources of barley yellow dwarf virus spread into winter wheat. *Plant Disease*, 105, 444–449.

Riedel C., Habekuss A., Schliephake E., Niks R., Broer I., Ordon F. (2011) Pyramiding of *Ryd2* and *Ryd3* conferring tolerance to a German isolate of Barley yellow dwarf virus-PAV (BYDV-PAV-ASL-1) leads to quantitative resistance against this isolate. *Theoretical and Applied Genetics*, 123, 69–76.

Rivero R.M., Mittler R., Blumwald E., Zandalinas S.I. (2022) Developing climate-resilient crops: improving plant tolerance to stress combination. *The Plant Journal*, 109, 373–389.

Robinson M.D., McCarthy D.J., Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26, 139–140.

Rong W., Wang X., Wang X., Massart S., Zhang Z. (2018) Molecular and ultrastructural mechanisms underlying yellow dwarf symptom formation in wheat after infection of barley yellow dwarf virus. *International Journal of Molecular Sciences*, 19.

Savary S., Willocquet L., Pethybridge S.J., Esker P., McRoberts N., Nelson A. (2019) The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3, 430–439.

Scheurer K.S., Friedt W., Huth W., Waugh R., Ordon F. (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (Hordeum vulgare L.). *Theoretical and Applied Genetics*, 103, 1074–1083.

Scholz M., Ruge-Wehling B., Habekuss A., Schrader O., Pendinen G., Fischer K., Wehling P. (2009) *Ryd4(Hb)*: a novel resistance gene introgressed from *Hordeum bulbosum* into barley and conferring complete and dominant resistance to the barley yellow dwarf virus. *Theoretical and Applied Genetics*, 119, 837–849.

Serrão J.E., Plata-Rueda A., Martínez L.C., Zanuncio J.C. (2022) Side-effects of pesticides on non-target insects in agriculture: a mini-review. *Die Naturwissenschaften*, 109, 17.

Shen C., Wei C., Li J., Zhang X., Wu Y. (2020) Integrated single-molecule long-read sequencing and Illumina sequencing reveal the resistance mechanism of *Psathyrostachys huashanica* in response to barley yellow dwarf virus-GAV. *Phytopathology Research*, 2.

Simon-Delso N., Amaral-Rogers V., Belzunces L.P., Bonmatin J.M., Chagnon M., Downs C., Furlan L., Gibbons D.W., Giorio C., Girolami V., Goulson D., Kreutzweiser D.P., Krupke C.H., Liess M., Long E., McField M., Mineau P., Mitchell E.A.D., Morrissey C.A., Noome D.A., Pisa L., Settele J., Stark J.D., Tapparo A., van Dyck H., van Praagh J., van der Sluijs J.P., Whitehorn P.R., Wiemers M. (2015) Systemic insecticides (neonicotinoids and fipronil):

trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research International*, 22, 5–34.

Singh R.P., Burnett P.A., Albarrán M., Rajaram S. (1993) *Bdv1*: A gene for tolerance to barley yellow dwarf virus in bread wheats. *Crop Science*, 33, 231–234.

Šip V., Chrpová J., Vacke J., Ovesná J. (2004) Possibility of exploiting the *Yd2* resistance to BYDV in spring barley breeding. *Plant Breeding*, 123, 24–29.

Srivastava A.K., Lu Y., Zinta G., Lang Z., Zhu J.-K. (2018) UTR-dependent control of gene expression in plants. *Trends in Plant Science*, 23, 248–259.

Tang C., Xu Q., Zhao J., Yue M., Wang J., Wang X., Kang Z., Wang X. (2022) A rust fungus effector directly binds plant pre-mRNA splice site to reprogram alternative splicing and suppress host immunity. *Plant Biotechnology Journal*, 20, 1167–1181.

Toojinda T., Broers L.H., Chen X.M., Hayes P.M., Kleinhofs A., Korte J., Kudrna D., Leung H., Line R.F., Powell W., Ramsey L., Vivar H., Waugh R. (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). *Theoretical and Applied Genetics*, 101, 580–589.

United Nations – Department of economic and social affairs (2022) World Population Prospects 2022: Summary of Results. 152 pp. United Nations, New York.

van den Eynde R., van Leeuwen T., Haesaert G. (2020) Identifying drivers of spatio-temporal dynamics in barley yellow dwarf virus epidemiology as a critical factor in disease control. *Pest Management Science*, 76, 2548–2556.

Viswanath K.K., Kuo S.-Y., Tu C.-W., Hsu Y.-H., Huang Y.-W., Hu C.-C. (2023) The role of plant transcription factors in the fight against plant viruses. *International Journal of Molecular Sciences*, 24.

Walls J., Rajotte E., Rosa C. (2019) The past, present, and future of barley yellow dwarf management. *Agriculture*, 9, 23.

Walsh L.E., Ferrari E., Foster S.P., Gaffney M.T. (2020) Evidence of pyrethroid tolerance in the bird cherry-oat aphid *Rhopalosiphum padi* in Ireland. *Outlooks on Pest Management*, 31, 5–9.

Wang X., Culver J.N. (2012) DNA binding specificity of ATAF2, a NAC domain transcription factor targeted for degradation by tobacco mosaic virus. *BMC Plant Biology*, 12.

Wang X., Boevink P., McLellan H., Armstrong M., Bukharova T., Qin Z., Birch P.R.J. (2015) A host KH RNA-binding protein Is a susceptibility factor targeted by an RXLR effector to promote late blight disease. *Molecular Plant*, 8, 1385–1395.

Wang X., Liu Y., Chen L., Zhao D., Wang X., Zhang Z. (2013) Wheat resistome in response to barley yellow dwarf virus infection. *Functional & Integrative Genomics*, 13, 155–165.

Yamasaki S., Suzuki A., Yamano Y., Kawabe H., Ueno D., Demura T., Kato K. (2018) Identification of 5'-untranslated regions that function as effective translational enhancers in monocotyledonous plant cells using a novel method of genome-wide analysis. *Plant Biotechnology*, 35, 365–373.

Zanini S., Bayer P.E., Wells R., Snowdon R., Batley J., Rajeev V., Nguyen H.T., Edwards D., Golicz A.A. (2021) Pangenomics in crop improvement - from coding structural variations to finding regulatory variants with pangenome graphs. *The Plant Genome*, 15.

Zhang Z., Chen L., Wang X., Xu H., Zhu X., Gao L., Xin Z. (2011) A serine/threonine kinase gene derived from *Thinopyrum intermedium* confers wheat resistance to BYDV infection. *Aspects of Applied Biology*, 110.

Zhang Z., Lin Z., Xin Z. (2009) Research progress in BYDV resistance genes derived from wheat and its wild relatives. *Journal of Genetics and Genomics*, 36, 567–573.

Zhang X., Wang X., Xu K., Jiang Z., Dong K., Xie X., Zhang H., Yue N., Zhang Y., Wang X.-B., Han C., Yu J., Li D. (2021) The serine/threonine/tyrosine kinase *STY46* defends against hordeivirus infection by phosphorylating γb protein. *Plant Physiology*, 186, 715–730.

Zhang Z., Xin Z., Ma Y., Chen X., Xu Q., Lin Z. (1999) Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. *Science in China*, 42, 663–668.

Zhou Y., Lu Q., Zhang J., Zhang S., Weng J., Di H., Zhang L., Li X., Liang Y., Dong L., Zeng X., Liu X., Guo P., Zhang H., Li X., Wang Z. (2022) Genome-wide profiling of alternative splicing and gene fusion during rice black-streaked dwarf virus stress in maize (*Zea mays* L.). *Genes*, 13.

Zhou Y., Xu Z., Duan C., Chen Y., Meng Q., Wu J., Hao Z., Wang Z., Li M., Yong H., Zhang D., Zhang S., Weng J., Li X. (2016) Dual transcriptome analysis reveals insights into the response to rice black-streaked dwarf virus in maize. *Journal of Experimental Botany,* 67, 4593–4609

2.9 Supplementary material

Supplementary table 2.1: SNPs and InDels (< 50 bp) of five maize inbreds with high impact and selected moderate impact consequences in the 0.3 Mbp QTL confidence interval on chromosome 10.

Location	Gene	Inbred	Impact	Consequence
137132252-137132263	Zm00001eb427930	FAP1360A	high	frameshift variant
137132285-137132296	Zm00001eb427930	FAP1360A	high	frameshift variant
137132357-137132362	Zm00001eb427930	FAP1360A	high	frameshift variant
137134227-137134229	Zm00001eb427940	FAP1360A, W64A	high	stop gained
137134415-137134415	Zm00001eb427940	W64A	high	stop gained
137198057-137198068	Zm00001eb427950	D408, W64A	high	splice acceptor variant, coding sequence variant
137215832-137215843	Zm00001eb427960	W64A	high	splice acceptor variant, intron variant
137216497-137216501	Zm00001eb427960	W64A	high	frameshift variant
137231759-137231768	Zm00001eb427970	D408, FAP1360A	moderate	protein altering variant
137231849-137231853	Zm00001eb427970	Ky226_DNA	moderate	protein altering variant
137232026-137232029	Zm00001eb427970	D408, FAP1360A	moderate	protein altering variant
137232639-137232669	Zm00001eb427970	Ky226_DNA	moderate	protein altering variant
137232639-137232669	Zm00001eb427970	Ky226	high	frameshift variant
137232649-137232669	Zm00001eb427970	W64A	high	frameshift variant
137232655-137232667	Zm00001eb427970	FAP1360A	high	frameshift variant
137232747-137232755	Zm00001eb427970	D408, FAP1360A	moderate	protein altering variant
137265234-137265242	Zm00001eb427980	W64A	high	frameshift variant
137280016-137280026	Zm00001eb427990	P092, W64A	high	frameshift variant
137281005-137281005	Zm00001eb428000	W64A	high	start lost
137281019-137281020	Zm00001eb428000	D408, FAP1360A, Ky226	high	frameshift variant
137283154-137283160	Zm00001eb428000	Ky226	high	frameshift variant
137283470-137283474	Zm00001eb428000	W64A	high	splice acceptor variant, intron variant
137285376-137285385	Zm00001eb428010	FAP1360A	high	stop gained, frameshift variant
137287706-137287718	Zm00001eb428010	D408, FAP1360A, Ky226	moderate	protein altering variant
137288545-137288548	Zm00001eb428010	D408, FAP1360A, Ky226	moderate	missense variant, splice region variant
137290395-137290403	Zm00001eb428010	FAP1360A	high	frameshift variant

Supplementary table 2.2: SVs of five maize inbreds in the 0.3 Mbp QTL confidence interval on chromosome 10 compared to the B73 reference sequence. DEL: deletion, INS: insertion, DUP: duplication. The size is given in bp

			D408		FAP1360A		Ky226		P092		W64A	
Position	Gene	Type	size	reads	size	reads	size	reads	size	reads	size	reads
137134930	intergenic	DEL	-69	12	-69	10	-	-	-	-	-	-
137196587	intergenic	DEL	-68	99	-69	50	-	-	-68	67	-	-
137217634	intergenic	DEL	-	-	-	-	-	-	-71	13	-	-
137222506	intergenic	INS	525	695	522	434	-	-	-	-	-	-
137223407	intergenic	INS	-	-	-	-	367	36	-	-	-	-
137231044	Zm00001eb427970 exon 2	DUP	-	-	-	-	586	33	-	-	-	-
137233263	Zm00001eb427970 exon 2	DEL	-	-	-	-	-35	52	-	-	-	-
137233396	Zm00001eb427970 promoter	DEL	-	-	-	-	-	-	-45	31	-	-
137233519	intergenic	INS	298	70	299	32	-	-	-	-	-	-
137234264	intergenic	INS	-	-	-	-	713	10	-	-	-	-
137234364	intergenic	INS	-	-	-	-	-	-	126	31	-	-
137235051	intergenic	INS	-	-	-	-	-	-	-	-	30	7
137236873	intergenic	INS	332	22	-	-	-	-	-	-	-	-
137246382	intergenic	INS	-	-	-	-	48	49	-	-	-	-
137246779	intergenic	DEL	-	-	-	-	-44	40	-	-	-	-
137269727	intergenic	DEL	-998	86	-998	51	-	-	-	-	-	-
137276225	intergenic	DUP	-	-	-	-	917	26	-	-	-	-
137277113	intergenic	INS	-	-	-	-	189	27	-	-	-	-
137277265	intergenic	DEL	-	-	-	-	-84	22	-	-	-	-
137278264	intergenic	INS	-	-	-	-	-	-	-	-	366	235
137279395	Zm00001eb427990 intron 1	DEL	-	-	-	-	-	-	-35	207	-	-
137279469	Zm00001eb427990 intron 1	INS	-	-	-	-	-	-	-	-	187	488
137283830	Zm00001eb428000 intron 5	INS	1623	20	-	-	-	-	-	-	-	-
137284303	intergenic	INS	-	-	-	-	-	-	-	-	260	235
137284911	intergenic	DEL	-	-	-	-	-	-	-	-	-50	290
137285231	Zm00001eb428010 5' UTR	DEL	-54	552	-54	239	-54	175	-	-	-	-
137289267	Zm00001eb428010 intron 6	INS	91	77	91	44	91	25	-	-	-	-
137289894	Zm00001eb428010 intron 7	DEL	-362	84	-362	50	-361	29	-	-	-	-
137293457	intergenic	DEL	-	-	-18838	109	-18838	66	-	-	-18840	131
137293458	intergenic	DEL	-18838	206	-	-	-	-	-	-	-	-
137316117	intergenic	DEL	-187	32	-187	23	-187	14	-	-	-	-
137316902	intergenic	DEL	-2766	202	-2767	117	-2766	67	-	-	-	-
137320044	intergenic	DEL	-17947	172	-17947	95	-17947	61	-	-	-	-
137385285	intergenic	DEL	-17869	65	-17869	41	-17869	25	-	-	-	-

3 Manuscript 2: Characterization of the Resistance Mechanism Against BYDV-PAV in Maize

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3.1 Abstract

Arthropods threaten crop production by feeding and transmission of, most importantly viruses. BYDV-PAV is the most prevalent virus species causing the barley yellow dwarf disease, one of the economically most important virus diseases of cereals worldwide. Maize plays a central role in BYDV-PAV epidemiology, serving as a 'green bridge' for BYDV-PAV and its vector *Rhopalosiphum padi* in summer. Some studies report that incidence of persistently transmitted viruses may be reduced in plants that are resistant to their insect vectors. In contrast, our choice tests showed that *R. padi* is not repelled by BYDV-PAV resistant maize plants. Differences in phloem architecture suggested that aphids feeding on BYDV-PAV resistant maize may have difficulties in reaching the phloem or establishing a stable feeding site. However, monitoring of aphid feeding behavior on maize inbreds that differed in their BYDV-PAV susceptibility revealed no correlation between *R. padi* feeding and BYDV-PAV resistance. Furthermore, we could not confirm the generation of reactive oxygen species (ROS), a typical reaction of plants during aphid infestation and infection of some viruses. Thus, we conclude that the BYDV-PAV resistance mechanisms in maize acts directly on the virus and not on its vector, *R. padi.*

3.2 Introduction

Arthropods are a double-threat in crop production. Firstly, they damage the crop plants during feeding, leading to reduced plant productivity. About 18 to 20 % of annual crop production worldwide is destructed by arthropods (Sharma et al. 2017). Secondly, arthropods transmit a variety of diseases, most importantly viruses (Eigenbrode et al. 2018). Approximately 30 % of all plant viruses described to date are transmitted by aphids, making them the most important virus vectors, followed by leafhoppers, whiteflies, and thrips (Brault et al. 2010).

Virus-transmitting insects usually feed on saps of the vascular bundle, either phloem or xylem, and have therefore developed specialized mouthparts, the stylets (Leybourne and Aradottir 2022). To reach the nutrient source, the insects have to cross many cell layers and different tissues. Healthy, insect-resistant plants possess a variety of resistance factors located on the leaf surface or in leaf tissues to prevent insect feeding (Alvarez et al. 2006). Resistance to insect vectors may hinder virus transmission into healthy plants and therefore can be considered as indirect virus resistance (Rodríguez-López et al. 2011). The electrical penetration graph (EPG) technique allows to study the different stylet activities produced by aphids and other piercing-sucking insects in plant tissues (Tjallingii and Hogen Esch 1993, Jiménez et al. 2021, Leybourne and Aradottir 2022). Differences in insect feeding activities between resistant and susceptible plants can lead to conclusions where insect resistance factors are located (Alvarez et al. 2006).

Volatile organic compounds (VOCs) and visual cues of host plants may attract or repel insects (Jiménez-Martínez et al. 2004). Physical barriers like epidermal waxes, leaf glandular trichomes, fortified cell walls, and acyl-sucroses on the leaf surface lead to piercing-sucking insects spending more time in non-probing activities (Np) and longer time to start probing (Alvarez et al. 2006, Rodríguez-López et al. 2011). The position within the leave and anatomy of the vasculature may also influence the accessibility of the phloem and, hence, insect acceptance of the plant as a food source (Leybourne et al. 2019).

Restricted phloem accessibility is the most common and unspecific aphid resistance mechanism in plants (Leybourne and Aradottir 2022). Additionally, plants are able to sense herbivore or pathogen attack by specific receptors, and activate molecular mechanisms that induce defense reactions (for review see Castro et al. 2021, Jones and Dangl 2006).

The generation of reactive oxygen species (ROS) during pathogen and herbivore attack is a central process linked to plant defense response (for review see Castro et al. 2021, Goggin and Fischer 2022, Mittler et al. 2022). ROS are a group of highly reactive molecules derived from molecular oxygen. They function as signaling molecules and are essential for multiple biological processes but they are also toxic byproducts of aerobic metabolism. Therefore, balancing ROS production, scavenging, and transport is crucial to living organisms (Castro et al. 2021, Mittler et al. 2022). Biotic and abiotic stresses can disrupt this homeostasis, leading to a stress-specific accumulation of different ROS in various subcellular compartments (Mittler et al. 2022). Aphid-responsive hydrogen peroxide (H_2O_2) accumulation is widely conserved

across plant species (Goggin and Fischer 2022). Recently, saliva proteins have been identified that act as effectors that trigger ROS accumulation in host plants (reviewed in Goggin and Fischer 2022). Additionally, ROS-accumulation is also involved in response mechanisms during plant virus infection (for review see Hernández et al. 2016).

The barley yellow dwarf (BYD) disease is caused by at least ten different phloem-limited viruses of barley yellow dwarf viruses (BYDVs) and cereal yellow dwarf viruses (CYDVs). Here, we refer to BYDVs and CYDVs as yellow dwarf viruses of cereals (YDVs). Symptoms of the BYD disease are stunting and discoloration of leaves (Choudhury et al. 2017, Oswald and Houston 1953, Walls et al. 2019). Results from barley indicate that infection with YDVs has a negative effect on leaf and vascular bundle morphology and leads to decreased leaf width, vascular bundle area, sieve element area and xylem vessel area (Choudhury et al. 2018, Esau 1957, Paulmann et al. 2018).

YDVs are transmitted by at least 25 different aphid species (Halbert and Voegtlin 1995). BYDV-PAV is the most prevalent YDV species in temperate regions and predominantly transmitted by *Rhopalosiphum padi* (Aradottir and Crespo-Herrera 2021). BYDV-PAV is one of the economically most important viruses in cereals causing up to 80 % yield loss (Choudhury et al. 2017, Nancarrow et al. 2021, van den Eynde et al. 2020).

Aphids of *R. padi* overwinter on their primary host, *Prunus padus*, where they reproduce sexually (Dixon 1971). New, YDV-free aphids hatch from eggs in spring and aleate (winged) individuals develop that migrate to the secondary hosts: cereals and grasses (Dixon 1971). The aphids acquire YDV from infected winter cereals by ingesting virus particles together with phloem sap when feeding (Gildow and Gray 1993, Ng and Perry 2004). YDV-carrying aphids move preferentially to uninfected plants and transmit the virus when saliva is injected during feeding (Gildow and Gray, 1993, Ingwell et al. 2012, Ng and Perry 2004). Maize plays a central role in the infection cycle of YDVs. Maize plants are infected by alate aphid vectors that migrate from ripening small grain cereals in early summer (Haack et al. 1999). *R. padi* colonies oversummer in maize and transfer YDVs when migrating to newly sown winter cereals in autumn and the infection cycle is closed (Haack et al. 1999, Henry and Dedryver 1989).

Maize inbreds were identified that are resistant to BYDV-PAV (Brown et al. 1984, Grüntzig and Fuchs 2000, Horn et al. 2013 and 2015, Loi et al. 1986, Osler et al. 1985, Stoner 1977). Additionally, a candidate gene for BYDV-PAV resistance in maize was identified that potentially confers resistance via H_2O_2 generation (Horn et al. 2014). However, it is not known

if and how the resistance mechanism targets the virus directly or indirectly via interfering with virus transmission by the vector, *R. padi*.

The aims of this study were to i) evaluate if BYDV-PAV resistance in maize is due to direct virus resistance or indirectly to vector resistance, ii) identify the location of possible *R. padi* resistance factors in or on the maize leaves, iii) to analyze if a general pathogen defense mechanism, the generation of ROS, is involved in BYDV-PAV resistance, and iv) characterize the influence of BYDV-PAV infection on the vasculature of susceptible, tolerant, and resistant maize inbreds.

3.3 Methods

Plant cultivation and aphid rearing

Five maize inbreds – namely BYDV-PAV resistant D408, FAP1360A, and Ky226; BYDV-PAV tolerant P092; and BYDV-susceptible W64A – were cultivated in a greenhouse (16 h light, 20 °C / 8 h darkness, 16 °C), if not stated otherwise. These inbreds are the founders of the connected segregating mapping populations that were used by Horn et al. (2015) to identify the QTL for BYDV-PAV resistance in maize.

BYDV-PAV carrying and virus-free aphids of species *R. padi* clone R07 were reared on BYDV-susceptible barley cv. 'Rubina' at room temperature under artificial daylight conditions. Viruliferous and virus-free aphids were checked regularly for presence of BYDV-PAV using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method with in-house polyclonal antisera for BYDV-PAV at the Julius Kühn-Institute as described by Horn et al. (2013).

Electrical penetration graph (EPG)

EPG was conducted according to Tjallingii and Hogen Esch (1993). Randomly selected adult apterous BYDV-PAV-carrying *R. padi* were starved for at least one hour and then attached to a thin gold wire (2 cm length) connected to a copper wire using water-based silver conductive paint (EPG-Systems, The Netherlands). Then, the copper wires were connected to the EPG device placed in a Faraday cage. Plants of the five maize inbreds were distributed randomly to the positions in the EPG amplifier device. Aphids were placed on the lower side of the

youngest mature leaf of plants in the two- or three-leaf stage and the aphid feeding behavior was monitored for eight hours.

Recording of EPG waveforms was conducted using two GIGA-8 EPG amplifier devices and EPG stylet$^+$ software (EPG Systems, The Netherlands). EPG waveform identification was performed according to Tjallingii and Hogen Esch (1993). EPG parameter analysis was conducted by an Excel-based analysis tool (version 10.8, Schliephake et al. 2013). Five parameters were selected for further analysis: i) the duration of the absence of contact of the aphid's stylet to the plant (termed non-probing, Np); ii) the duration of stylet pathway activities inside the apoplast including short cell penetrations and penetration difficulties (CF); iii) the duration of saliva injection into the phloem (E1); iv) the duration of ingestion of phloem sap from sieve elements (E2); and v) the duration of xylem sap ingestion. Additionally, the proportion of aphids that had reached E1 and E2 was calculated in hour-wise intervals.

For every test, new plants and aphids were used. A total of eight to 19 individual plants per maize inbred were used.

Choice test

Leaves of maize inbreds D408, FAP 1360A, Ky226, P092, and W64A of plants in two or three leaf stage were placed in a choice arena as described by Hewer et al. (2010). Leaves were placed in equal distances from the middle of the arena in a randomized order. Thirty BYDV-PAV carrying *R. padi* were placed in the middle of the arena. Upper and lower sides of the leaves were photographed after 24 h and the number of aphids on the leaves was counted. The experiment was replicated 17 times.

Quantification of reactive oxygen species (ROS)

Maize inbreds FAP1360A (BYDV-PAV resistant), P092 (BYDV-PAV tolerant), and W64A (BYDV-PAV susceptible) were cultivated in a climate chamber (16 h light, 24 °C / 8 h darkness, 22 °C). When plants reached two-leaf stage, they were treated with i) BYDV-PAV carrying aphids, ii) virus-free aphids or iii) left untreated as control. Samples were taken at the start of the experiment, 6, 12, 24, 48, 96 hours and 6 weeks (end of the experiment) after inoculation. Aphids were gently removed with a fine paint brush. The youngest fully developed leaves of eight plants per inbred and treatment were pooled, frozen immediately in liquid nitrogen and stored at -80 °C until further usage. Leaves were homogenized and weighted under deep-

frozen conditions. The amount of ROS was determined using the method of Jambunathan (2010).

Inoculation success was confirmed on a per plant basis via the DAS-ELISA method six weeks after inoculation using the sixth leaf of each plant.

Morphology

Maize inbreds FAP1360A, P092, and W64A were grown in a climate chamber (16 h light, 24 °C / 8 h darkness, 22 °C). One half of the plants was treated with BYDV-PAV carrying *R. padi* at two leaf stage while the other half was left untreated as control. Cross-sections of the middle of the eighth leaf were examined microscopically and vascular bundle diameter, diameter of ten sieve elements, sieve element number and diameter of the xylem were quantified. Additionally, leaf length, leaf width, and plant height were recorded. The virus titer of inoculated and control plants was analyzed on a per plant basis six weeks after via the DAS-ELISA method. The experiment was conducted in three replicates with 16 plants per inbred x treatment combination.

Statistics

The data collected in the choice test were analyzed using the following mixed linear model with genotype of the inbred and repetition as fixed effects.

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

where Y_{ij} was the phenotypic observation for the ith inbred in the jth replicate, μ the general mean, g_i the effect of the ith inbred, r_j the effect of the jth replication, and e_{ij} the residual. Because the replication was not significant, the final model

$$Y_i = \mu + g_i + e_i$$

was applied. Residuals were checked graphically for normal distribution. This was true and ANOVA and Tukey HSD test were applied for further analyses.

Analyses of EPG data was conducted using the following mixed linear model

$$Y_{ijk} = \mu + g_i + r_j + d_k/p_l + e_{ijkl}$$

where Y_{ijk} was the phenotypic observation for the ith inbred for the jth replicate at the kth position in the GIGA-8 EPG amplifier device, μ the general mean, g_i the effect of the ith inbred, r_j the effect of the jth replication, p_l the effect of the kth position in the device nested in device d_k and e_{ijkl} the residual. A replication represents one day of measurements, that is 8 positions per device times two devices. Because the factors replication and position in the GIGA-8 EPG amplifier device and the device were not significant, the final model

$$Y_i = \mu + g_i + e_i$$

was applied. Residuals were checked graphically for normal distribution. This was not the case and Kruskal-Wallis and Dunn post-hoc test with Bonferroni correction for multiple testing were applied for further analyses.

The data collected in the ROS experiment were analyzed using the following mixed linear model

$$Y_{ijkl} = \mu + g_i * t_j + h_k + r_l + e_{ijkl}$$

where Y_{ijkl} was the observed concentration of H_2O_2 equivalents, μ the general mean, g_i the effect of the ith inbred, t_j the effect of the jth treatment, h_k the effect of the kth time of sampling, r_l the effect of the lth replication, and e_{ijkl} the residual.

Analyses of data collected from microscopic observation of leaves of BYDV-infected plants was conducted using the following mixed linear model

$$Y_{ijk} = \mu + g_i * t_j + r_k + e_{ijk}$$

where Y_{ijk} was the phenotypic observation for the ith inbred and the jth treatment for the kth replicate, μ the general mean, g_i the effect of the ith inbred, t_j the effect of the jth treatment, r_k the effect of the kth replicate, and e_{ijk} the residual.

Analyses were conducted using R version 3.6.3 (R Core Team 2020, https://www.R-project.org/) with packages "lme4" version 1.1-23 (Bates et al. 2015), "dplyr" version 1.0.2, "dunn.test" version 1.3.5, "reshape2" version 1.4.4, and RStudio version 1.3.1073 (RStudio Team 2020, http://www.rstudio.com/.)

3.4 Results

Choice test

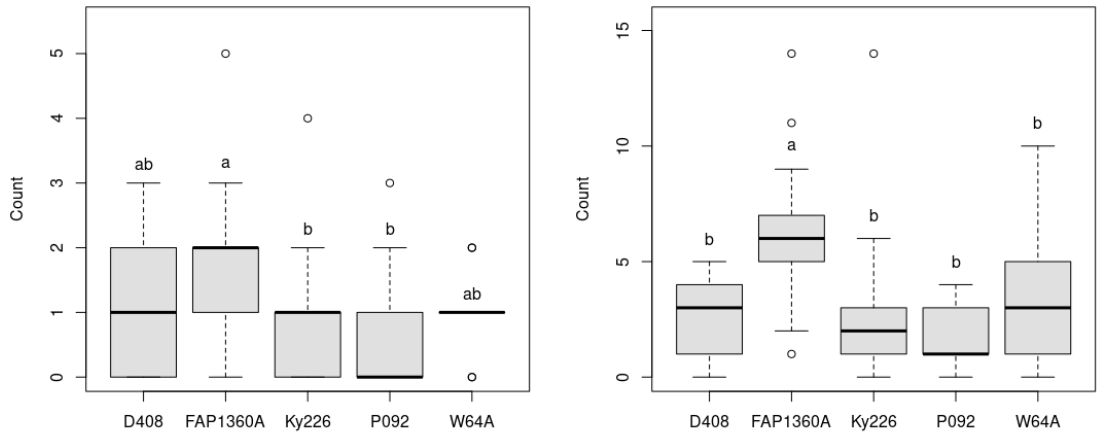


Figure 3.1: Boxplot of aphid counts in the choice experiment. Viruliferous *R. padi* were placed in a choice arena containing BYDV-PAV free inbreds D408 (resistant), FAP1360A (resistant), Ky226 (resistant), P092 (tolerant), and W64A (susceptible). Aphids were counted on the upper leaf sides after 2 h (left) and on both sides of the leaves after 24 h (right). Please note the different scales of the y-axes. Letters above boxes indicate grouping by Tukey post-hoc test (α = 0.05).

To test aphid preferences when they were allowed to chose freely between different maize inbreds, BYDV-PAV carrying aphids of *R. padi* were offered detached leaves of inbreds D408, FAP1360A, Ky226, P092, and W64A. A significant (α = 0.05) preference of *R. padi* for BYDV-PAV resistant inbred FAP1360A over other inbreds was visible after 2 h (Figure 3.1). This preference for FAP1360A became even more pronounced after 24 h (Figure 3.1).

EPG

We conducted an EPG analysis to test if BYDV-PAV susceptibility of maize inbreds correlates with differences feeding activities of *R. padi* on these inbreds. Regardless of the maize inbred, BYDV-PAV carrying *R. padi* spend the majority of time of the 8-hour EPG experiment with penetrating the leaf (phases CF, G, E1, and E2; Figure 3.2). Non-probing (Np) activities without

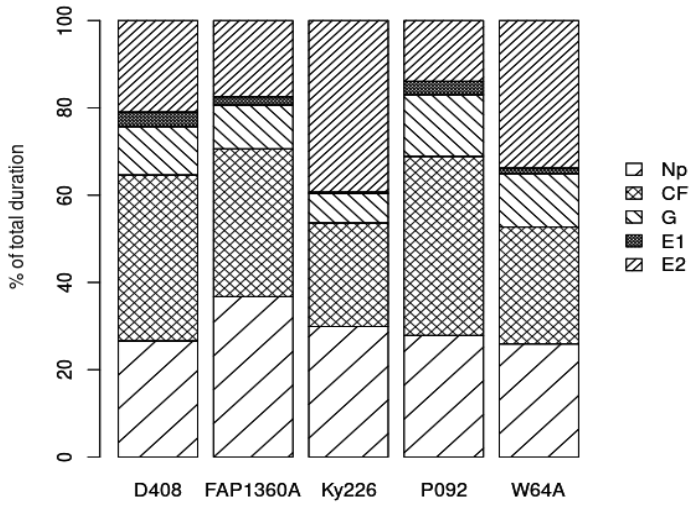


Figure 3.2: Proportion of EPG-waveforms of viruliferous *R. padi* feeding on five BYDV-PAV free maize inbreds. Aphid feeding was monitored for eight hours. Np: non-probing (no leaf penetration), CF: pathway phase (transition of the insect stylets through epidermis and mesophyll cells including difficulties in stylet penetrations), G: xylem sap ingestion, E1: phloem salivation phase, E2: phloem sap ingestion.

stylet penetration of the leaf lasted between one quarter (aphids feeding on W64A) and one third (aphids feeding on FAP1360A) of total duration (Figure 3.2). The phloem salivation phase (E1) was the shortest phase. Aphids spend between 0.3 % and 3.3 % of total duration in E1 on leaves of Ky266 and D408, respectively (Figure 3.2). The phase of phloem sap ingestion (E2), which indicates host plant acceptance, varied between 13.9 % (P092) and 39.3 % (Ky226) of total duration (Figure 3.2).

Comparing the total duration of *R. padi* feeding phases over the eight hours of the experiment, we observed significant differences between maize inbreds for the duration of CF (pathway phase, Kruskal-Wallis: $p = 0.0053$) and E1 ($p = 0.0064$) (Table 3.1). The aphids spend least time in CF feeding on plants BYDV-PAV susceptible inbred W64A (6700 s) (Table 3.1). That differed significantly ($\alpha = 0.01$) from aphids feeding on BYDV-PAV tolerant inbred P092 (10700 s) and BYDV-PAV resistant inbred D408 (10101 s), respectively (Table 3.1 and Supplementary table 3.1). Duration of E1 was shortest for aphids feeding on Ky226 (100 s), which significantly differed ($\alpha = 0.01$) from E1 of aphids feeding on D408 (877 s) and P092 (803 s), respectively (Table 3.1 and Supplementary table 3.1). However, the length of phases Np (non-probing), G (xylem sap ingestion), and E2 did not significantly differ between *R. padi* feeding on the various maize inbreds (Table 3.1).

Table 3.1: Duration of EPG-waveforms of viruliferous *R. padi* feeding on five BYDV-PAV free maize inbreds. Aphid feeding was monitored for eight hours. Data are given as medians of all replications. Durations are given in seconds. Np: non-probing (no leaf penetration), CF: pathway phase (transition of the insect stylets through epidermis and mesophyll cells including difficulties in stylet penetrations), G: xylem sap ingestion, E1: phloem salivation phase, E2: phloem sap ingestion. P-value: probability of difference between maize inbreds.

Inbred	Np	CF	G	E1	E2
D408	7002	10011	2921	877	5520
FAP1360A	9861	9086	2699	508	4686
Ky226	8596	6828	1928	101	11307
P092	7260	10700	3685	803	3634
W64A	6481	6700	3059	320	8453
P-value	0.4393	0.0053	0.9117	0.0064	0.1201

Data were further analysed in intervals of one hour to investigate the temporal aspect. Significant (α = 0.05) differences of EPG-waveforms between aphids feeding on D408, FAP1360A, Ky226, P092, and W64A were observed for E1 in hour 5 and CF, E1, as well as E2 in hour 4, respectively (Table 3.2). The interval with most significant differences (3 of 4 EPG-waveforms) was hour 4 (Table 3.2). Three out of four significant (α = 0.05) differences were found in phloem-related EPG-waveforms E1 and E2 (Table 3.2). Pairwise inbred-inbred comparisons revealed that only in hour 5 E1 was significantly (α = 0.05) longer in P092 than in Ky226 but not in hour 4 (Supplementary table 3.2). In hour 4, E2 was significantly (α = 0.05) longer in W64A compared to D408, FAP1360A, and P092 but no distinct inbred was identified for CF and E1 (Supplementary table 3.2).

Table 3.2: Hour-wise Kruskal-Wallis test on significant differences of EPG parameters between BYDV-PAV susceptible, tolerant, and resistant maize inbreds

Hour	Np	CF	G	E1	E2
1	0.3611	0.6163	0.5981	0.7657	0.9369
2	0.4908	0.0530	0.6491	0.0693	0.4782
3	0.7921	0.4689	0.2401	0.3085	0.0935
4	0.1383	0.0008	0.4571	0.0263	0.0010
5	0.6344	0.3419	0.0971	0.0188	0.7984
6	0.8365	0.6678	0.6970	0.8032	0.6872
7	0.8702	0.4767	0.3812	0.5309	0.6961
8	0.0939	0.5420	0.6834	0.1580	0.0973

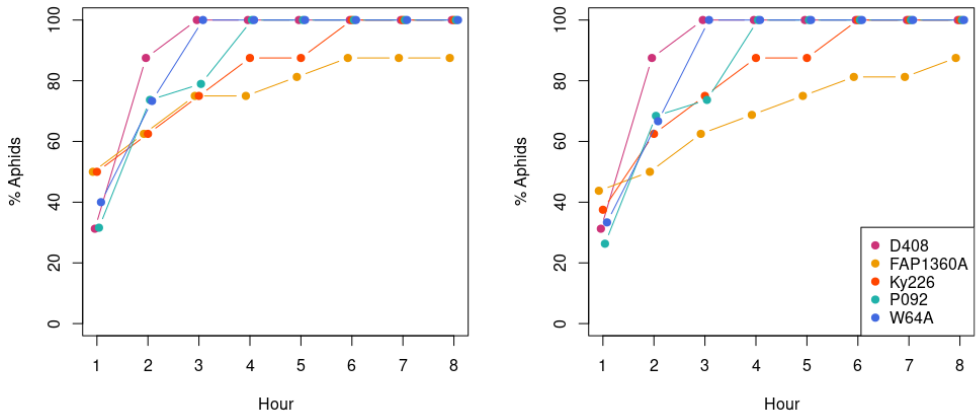


Figure 3.3: Percentage of *R. padi* aphids that reached phloem salivation phase (E1, left) and phloem sap ingestion phase (E2, right) on five maize inbreds.

Twenty-six to forty-four percent of aphids feeding on the different maize inbreds reached E1 within the first hour (Figure 3.3). However, 12.5 % of aphids feeding on FAP1360A failed to reach the phloem within the eight-hour observation period of the experiment (Figure 3.3).

ROS

Concentrations of ROS varied between 38 µmol g^{-1} FW and 898 µmol g^{-1} FW (Figure 3.4). The maize inbred, the time point of sampling, and the replication had a significant influence on the measured ROS concentration (Table 3.3). However, the treatment (BYDV-PAV infection, aphid infestation or control) did not significantly affect ROS concentrations – neither across the whole time of the experiment nor at single time points (Table 3.3).

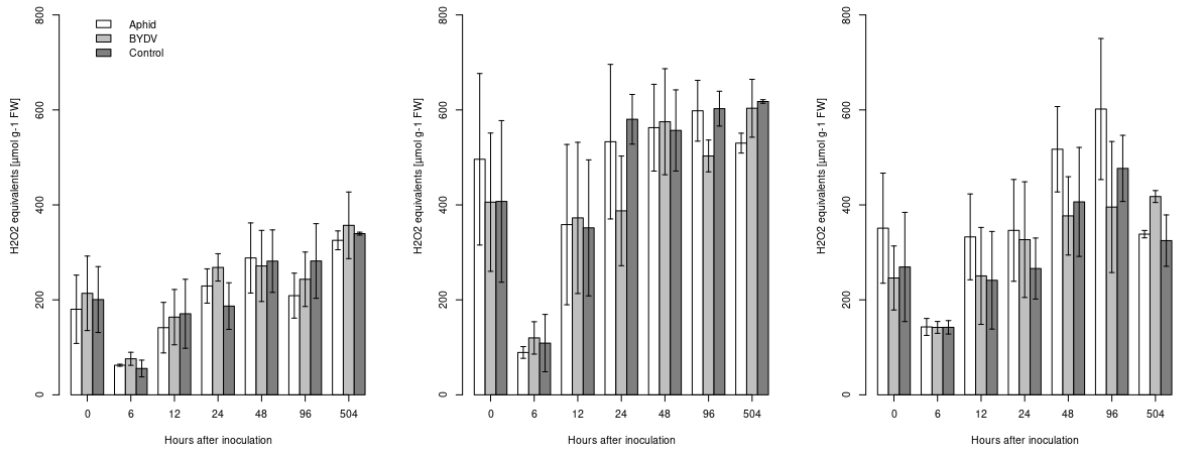


Figure 3.4: ROS levels in *R. padi* infested, BYDV-PAV infected, and control plants of maize inbreds FAP1360A (left), P092 (middle), and W64A(right). Error bars represent standard error of the mean.

Table 3.3: ANOVA of factors that influence ROS levels in Rhopalosiphum padi infested, BYDV-PAV infected, and control plants of maize inbreds FAP1360A, P092, and W64A. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

	0 h	6 h	12 h	24 h	48 h	96 h	504 h	total
Inbred	**	*	**	**	**	***	***	***
Treatment	ns	ns	ns	ns	ns	ns	ns	ns
Replication	***	ns	***	*	ns	*	ns	***
Inbred x treatment	ns	ns	ns	ns	ns	ns	ns	ns
Time point	-	-	-	-	-	-	-	***

ROS concentrations of the three maize inbreds were significantly ($\alpha = 0.05$) different from each other (Table 3.3) with FAP1360A having the lowest ROS concentrations (average of 219 µmol g^{-1} FW across all time points and treatments) and P092 having highest ROS concentrations (average of 462 µmol g^{-1} FW). ROS concentrations of W64A were one and a half times higher than FAP1360A (average of 338 µmol g^{-1} FW).

ROS concentrations measured in replications A and B were significantly ($\alpha = 0.05$) lower than in replications C and D. This was true for all time points. ROS concentrations measured at time points 0 h and 12 h were both significantly ($\alpha = 0.05$) lower than concentrations measured at 48 h and 96 h, respectively.

Morphology

The diameter of the vascular bundles varied between 140 µm and 310 µm (Figure 3.5 and Supplementary figure 3.1). The size of sieve element cells was between 8 µm and 17 µm and the xylem sizes varied between 42 µm and 81 µm (Figure 3.5 and Supplementary figure 3.1). We counted 21 to 48 sieve element cells per vascular bundle (Figure 3.5 and Supplementary figure 3.1).

The genotype of the maize inbred significantly influenced the diameter of the vascular bundle, sieve element cells, and xylem as well the number of sieve element cells in both main and marginal veins (Table 3.4 and Supplementary table 3.3). These parameters were highest in P092 and lowest in FAP1360A (Figure 3.5 and Supplementary figure 3.1). However, xylem diameter of the marginal veins was highest in W64A (Supplementary figure 3.1).

BYDV-PAV infection significantly influenced the number of sieve element cells, diameter of the vascular bundle and sieve element cells as well in both midrib and marginal veins but not the xylem diameter (Table 3.4 and Supplementary table 3.3)

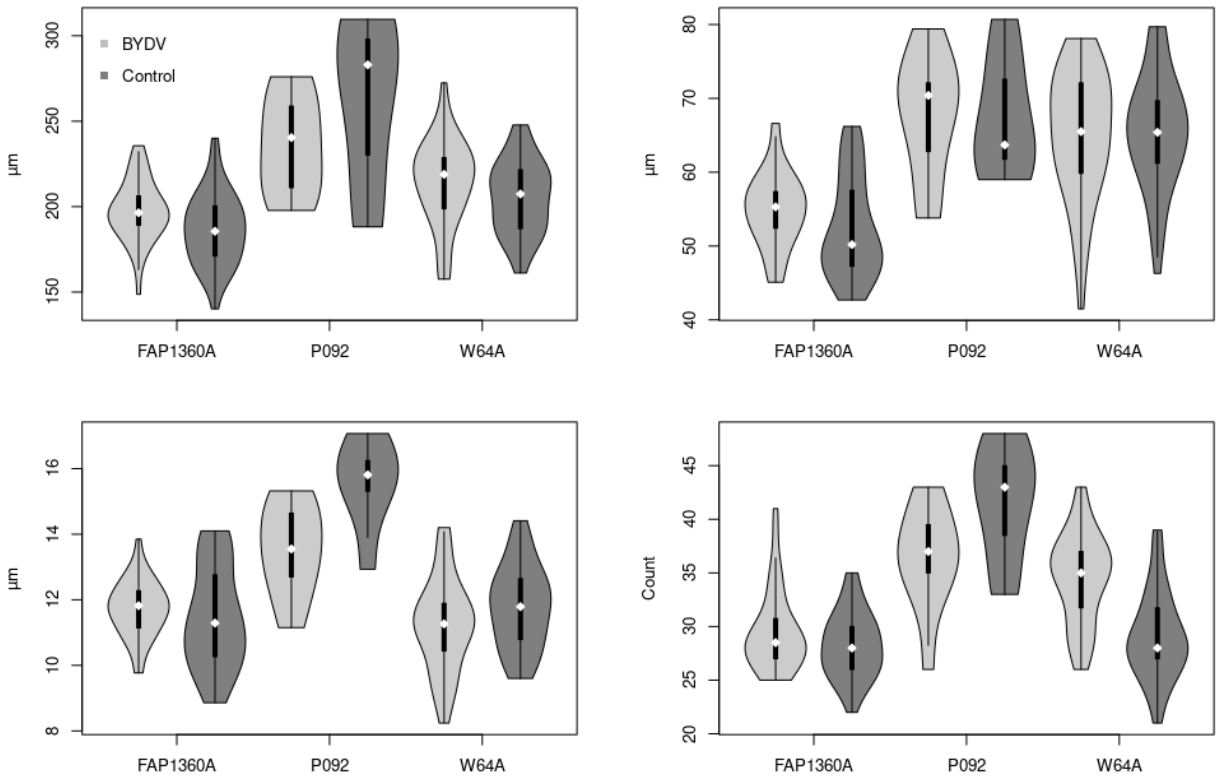


Figure 3.5: Morphological comparison of midrib vascular bundle parameters of healthy and BYDV-PAV infected maize inbreds. Top-left: vascular bundle diameter, top-right: xylem diameter, bottom-left: sieve element diameter, bottom-right: number of sieve elements. Please note the suppressed zero at the y-axis scale. The white diamond shape represents the median

We observed a significant inbred x treatment interaction effect for vascular bundle diameter, sieve element cell diameter, and number of sieve element cells of the midrib and number of sieve element cells of the marginal veins (Table 3.4 and Supplementary table 3.3). However, changes evoked by BYDV-PAV infection were usually of small scale. Parameters measured in BYDV-PAV infected plants differed from control plants less than $\pm$ 6 % with these exceptions: the diameter of the vascular bundle and sieve element cells of the midrib of P092 was strongly reduced in BYDV-PAV infected plants in comparison to control plants (-10.5 % and -13.5 %, respectively), as well as the number of sieve element cells in the midrib (-12.0 %) of P092. Conversely, the number of sieve element cells in the midrib (+18.7%) and marginal veins (+16.6 %) of inbred W64A was increased in BYDV-PAV infected plants compared to control plants. Additionally, BYDV-PAV infection strongly reduced plant height of inbred P092 (-18.5 %) and leaf width of inbred W64A (-9.1 %), compared to control plants.

Table 3.4: ANOVA of factors that influence morphological traits of the vascular bundle of the midrib of BYDV-PAV infected and control plants of maize inbreds FAP1360A, P092, and W64A. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

	Vascular bundle diameter	Sieve element diameter	Xylem diameter	Number of sieve elements
Inbred line	***	***	***	***
Treatment	.	.	ns	***
Replication	***	**	.	**
Inbred x treatment	**	**	ns	***

3.5 Discussion

Resistance to virus-vectoring insects can reduce primary and secondary spread of insect-transmitted plant viruses (Rodríguez-López et al. 2011). Plants have evolved different mechanisms to combat herbivores and pathogens, including physical barriers and toxic secondary metabolites (for review see Erb and Reymond 2019, Radchenko et al. 2022). Additionally, plants are able to sense herbivore or pathogen attack by specific receptors and activate molecular mechanisms that induce specific defense reactions (Castro et al. 2021, Couto and Zipfel 2016, Erb and Reymond 2019, Jiang et al. 2019, Radchenko et al. 2022). One of these defense reactions is the generation of ROS that act as local and systemic signaling molecules inducing defense responses (Castro et al. 2021, Couto and Zipfel 2016).

In the study we present here, no effect of aphid infestation or BYDV-PAV infection on ROS-levels was detected – neither in BYDV-PAV resistant nor BYDV-PAV susceptible or BYDV-PAV tolerant maize inbreds (Figure 3.4 and Table 3.3). This contradicts results of previous studies. Sytykiewicz (2015) found that *Z. mays* cultivars responded after *R. padi* infestation with an elevation in H_2O_2 content compared to the uninfested control, and this accumulation was linked with resistance degrees to the aphids' colonization. After 24 hours, H_2O_2 levels doubled in *R. padi* resistant cultivars but were only 1.5 times higher in susceptible cultivars (Sytykiewicz 2015). Recent studies suggest that ROS accumulation is also involved in plant reaction to YDV infection (Paulmann et al. 2018, Rong et al. 2018, Wang et al. 2018). Six weeks after infection, ROS levels of BYDV-PAV infected susceptible barley were significantly increased compared to BYDV-PAV resistant plants carrying the resistance gene *Ryd2* (Paulmann et al. 2018). Similarly, ROS levels were significantly increased in BYDV-GAV infected susceptible wheat compared to

uninfected plants or infected plants carrying the *Bdv2* resistance gene three, four, and five weeks after inoculation (Rong et al. 2018, Wang et al. 2018).

The reason for the contradiction between our study and others might be that investigation of ROS generation upon aphid infestation is experimentally challenging. The onset, peaks and duration of ROS accumulation caused by aphid infestation varies widely in different studies and not all have detected an increase of ROS in response to aphids (Goggin and Fischer 2022). Additionally, the magnitude of ROS induction varies depending on the aphid species, the aphid biotype, the plant cultivar, plant age, location of the aphid infestation on the plant, and aspects of the experimental design such as infestation levels and timing of measurement (Goggin and Fischer 2022).

Furthermore, the scattered and asynchronous nature of aphid feeding sites may limit the magnitude of ROS accumulation, increase random sample-to-sample variation, and make it difficult to pinpoint the timing of the responses (Goggin and Fischer 2022). In this study, we quantified ROS of whole leaves but organ and tissue level ROS measurements average the ROS levels of high numbers cells and neglect compartment-specific differences. Thus, these measurements might not give meaningful information about the changes at the single-cell and subcellular level, which can have important implications for signaling function (Waszczak et al. 2018). Thus, only experiments with a high spatial-temporal resolution may clarify if, where, and when ROS are produced during *R. padi* infestation and BYDV-PAV infection in maize.

Next to generation of ROS, other factors may influence feeding of *R. padi* on different maize inbreds. For example, volatile organic compounds (VOCs) and visual cues of host plants may attract or repel insects (Jiménez-Martínez et al. 2004). In our experiment, BYDV-PAV carrying *R. padi* significantly preferred BYDV-PAV resistant maize inbred FAP1360A over other BYDV-PAV resistant inbreds (D408 and Ky226), BYDV-PAV tolerant inbred P092, and BYDV-PAV susceptible inbred W64A (Figure 3.1), indicating that BYDV-PAV resistance in maize is not caused by reduced attraction of *R. padi to* BYDV-PAV resistant plants. This is in accordance with previous studies.

In a two-hour choice experiment, Jiménez-Martínez et al. (2004) demonstrated that non-viruliferous *R. padi* significantly preferred BYDV-PAV infected over healthy plants. However, the results did not differ between BYDV-PAV susceptible and BYDV-PAV tolerant wheat varieties (Jiménez-Martínez et al. 2004). In a short-term experiment (2 h), Kern et al. (2021) found no difference in the choice of non-viruliferous *R. padi* between BYDV-PAV infected and

healthy resistant and susceptible plants, respectively. At a longer observation period (24 h), *R. padi* preferred BYDV-PAV infected over healthy susceptible plants but there was no difference choice of resistant plants carrying the YDV resistance gene *Ryd2 (*Kern et al. 2021). This suggests that healthy YDV susceptible and resistant plants may not differ in aphid-attracting VOCs but other factors on or in the leaves may prevent aphids from establishing a stable feeding site and thus move to a more suitable host.

The investigation of vascular bundle features revealed that inbred FAP1360A had the lowest numbers of sieve element cells and the smallest diameters of the vascular bundle, xylem and sieve element cells, compared to inbreds P092 and W64A, respectively (Figure 3.5 and Supplementary figure 3.1). Therefore, we suspected that these inbreds may differ in their accessibility of the phloem. To test this, we performed an electric penetration graph (EPG) analysis.

EPG-analysis revealed that BYDV-carrying *R. padi* feeding on the five virus-free maize inbreds D408, FAP1360A, Ky226, P092, and W64A differed significantly in time spend in the pathway phase (CF) (Table 3.1), that is the phase spend in epidermis and mesophyll. However, pairwise comparisons of CF between aphids feeding on the five maize inbreds did not reveal that *R. padi* spend more time in CF in BYDV-PAV resistant plants compared to BYDV-PAV susceptible or tolerant plants (Table 3.1 and Supplementary table 3.1). In contrast, *R. padi* spend least time in CF when feeding on plants of the susceptible inbred W64A (6700 s), suggesting that aphid resistance factors in the mesophyll or the accessibility of the phloem are not causative for BYDV-resistance.

The duration of E1 differed significantly between *R. padi* feeding on the five different maize inbreds (Table 3.1). This phase was both longest (D408, 877 s) and shortest (Ky226, 101 s) for aphids feeding on two of the three BYDV-PAV resistant inbreds, indicating that the duration of E1 does not correlate with BYDV-PAV susceptibility of maize. The E1 phase is important for transmission of viruses that are transmitted in a persistently, circulative manner such as YDVs. In this phase, virus particles are injected into the phloem along with the aphid's saliva (Prado and Tjallingii 1994).

However, in a meta-study on host-plant aphid resistance, Leybourne and Aradottir (2022) found that phloem access is restricted in aphid-resistant plants. Aphids probing on resistant plants take longer time to reach the phloem, as indicated by a longer time taken until E1. This is common in all plant families studied and effective against aphids with broad- and narrow-

host ranges, respectively (Leybourne and Aradottir 2022). These factors might be activated only through aphid feeding, which has been demonstrated for plant reaction to virus-free and viruliferus aphids (Ahmad et al. 2011, Givovich and Niemeyer, 1991; Leybourne et al. 2019, Louis et al. 2015, Meihls et al. 2013).

Interestingly, *R. padi* preferred BYDV-resistant inbred FAP1360A when aphids were allowed to choose freely between leaves of host plants but 12.5 % of aphids failed to reach phloem ingestion phase (E2) in the EPG-experiment (Figure 3.3). However, the duration of E2 differed not between *R. padi* feeding on the different maize inbreds (Table 3.1), indicating no difference in plant acceptance by the aphids.

In contrast to our findings, studies in barley and its wild relatives suggest a link between YDV resistance and aphid feeding. *R. padi* feeding on *H. bulbosum* clone A17 with complete resistance to BYDV and CYDV showed reduced number and duration of E1 and E2 phases compared to aphids feeding on a BYDV-susceptible *H. bulbosum* clone (Schliephake et al. 2013). Additionally, only 15% of the aphids feeding on the BYDV-resistant *H. bulbosum* clone showed E1 and E2 waveforms during the twelve-hour observation period compared to 60 % aphids feeding on the BYDV-susceptible *H. bulbosum* clone. The authors conclude that reduced phloem feeding might result in resistance to BYDV (Schliephake et al. 2013). *H. bulbosum* clone A17 is the source of the YDV-resistance gene *Ryd4Hb* (Scholz et al. 2009).

Additionally, *R. padi* feeding on the partially BYDV-resistant *H. spontaneum* accession Hsp5 showed a reduced duration of sustained E2 in comparison to a BYDV-susceptible barley variety (Leybourne et al. 2019). This was linked to a reduced nutritional quality of the phloem sap (Leybourne et al. 2019).

Differences in *R. padi* feeding behavior were also observed in *R. padi* resistant and susceptible maize cultivars (Sytykiewicz et al. 2019). In comparison to aphids feeding on the susceptible cultivar, *R. padi* feeding on the resistant cultivar spend longer time with non-probing (Np), exhibited a prolonged time of phloem salivation (E1) as well as phloem sap ingestion (E2). Interestingly, the same *R. padi* resistant cultivar generated higher H_2O_2 levels in response to aphid infestation than the susceptible cultivar, when compared to uninfested control (Sytykiewicz 2015).

Aphids in EPG-experiments cannot leave an unfavorable plant. Consequently, they may probe longer and more frequently than free aphids would do on the same plant (Alvarez 2006). Differences between susceptible and resistant plants may thus tend to be under-estimated by

EPG analysis (Tjallingii 1988). However, both choice and EPG experiments suggest that BYDV-PAV resistance in maize is not mediated through vector resistance.

3.6 Conclusion

Plants possess a variety of mechanisms to defend themselves against herbivore and pathogen attack. Of particular importance are ROS that accumulate in the apoplast and other cell compartments during the plant immune response. However, the study we present here suggests that ROS accumulation in leaves is not different between BYDV-PAV resistant, susceptible, and tolerant maize inbreds after *R. padi* infestation and virus infection, respectively. BYDV-PAV resistance might be conferred indirectly through resistance against the virus vector, *R. padi*. Comparison of the morphology of BYDV-PAV resistant, susceptible, and tolerant maize inbreds indicated that *R. padi* feeding on BYDV-PAV resistant inbreds may have difficulties to reach the phloem. However, *R. padi* feeding behavior indicated no aphid resistance of BYDV-resistant maize inbred lines. Thus, we conclude that BYDV-PAV resistance in maize is a direct resistance against the virus and not resistance against its vector, *R. padi*. However, resistance mechanisms remain unclear and it might be necessary to monitor them at single-cell level.

3.7 Acknowledgements

We would like to thank Mr. Sascha Feldt for conducting microscopy of vascular bundles.

3.8 References

Ahmad S., Veyrat N., Gordon-Weeks R., Zhang Y., Martin J., Smart L., Glauser G., Erb M., Flors V., Frey M., Ton J. (2011) Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology*, 157, 317–327.

Alvarez A.E., Tjallingii W.F., Garzo E., Vleeshouwers V., Dicke M., Vosman B. (2006) Location of resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid Myzus persicae. *Entomologia Experimentalis et Applicata*, 121, 145–157.

Aradottir G.I., Crespo-Herrera L. (2021) Host plant resistance in wheat to barley yellow dwarf viruses and their aphid vectors: a review. *Current Opinion in Insect Science*, 45, 59–68.

Bates D., Maechler M., Bolker B., Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Brault V., Uzest M., Monsion B., Jacquot E., Blanc S. (2010) Aphids as transport devices for plant viruses. *Comptes Rendus Biologies*, 333, 524–538.

Brown JK, Wyatt SD, Hazelwood D (1984) Irrigated corn as a source of Barley yellow dwarf virus and vector in eastern Washington. *Phytopathology* 74, 46–49.

Castro B., Citterico M., Kimura S., Stevens D.M., Wrzaczek M., Coaker G. (2021) Stress-induced reactive oxygen species compartmentalization, perception and signalling. *Nature Plants*, 7, 403–412.

Choudhury S., Hu H., Larkin P., Meinke H., Shabala S., Ahmed I., Zhou M. (2018) Agronomical, biochemical and histological response of resistant and susceptible wheat and barley under BYDV stress. *PeerJ*, 6, e4833.

Choudhury S., Hu H., Meinke H., Shabala S., Westmore G., Larkin P., Zhou M. (2017) Barley yellow dwarf viruses: infection mechanisms and breeding strategies. *Euphytica*, 213, 213-168.

Couto D., Zipfel C. (2016) Regulation of pattern recognition receptor signaling in plants. *Nature Reviews Immunology*, 16, 537-552

Dixon A.F.G. (1971) The life-cycle and host preferences of the bird cherry-oat aphid, *Rhopalosiphum padi* L., and their bearing on the theories of host alternation in aphids. *Annals of Applied Biology*, 68, 2, 135-147.

Eigenbrode S.D., Bosque-Pérez N.A., Davis T.S. (2018) Insect-borne plant pathogens and their vectors: ecology, evolution, and complex interactions. *Annual Review of Entomology*, 63, 169–191.

Erb M., Reymond P. (2019) Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology*, 70, 527–557.

Esau K. (1957) Anatomic effects of barley yellow dwarf virus and maleic hydrazide on certain Gramineae. *Hilgardia*, 27, 15–69.

Gildow F.E., Gray S.M. (1993) The aphid salivary gland basal lammina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteoviruses. *Phytopathology*, 83, 1293–1302.

Givovich A., Niemeyer H.M. (1991) Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata*, 59, 79–85.

Goggin F.L., Fischer H.D. (2021) Reactive oxygen species in plant interactions with aphids. *Frontiers in Plant Science*, 12, 811105.

Grüntzig, M, Fuchs E (2000) Occurence of luteoviruses of cereals in Zea mays L. *Journal of Plant Diseases and Protection,* 107, 523–538.

Haack L., Courbon R., Riault G., Tanguy S., Le Vilain D., Henry M., Dedryver C.A. (1999) A plant and field study of BYDV-PAV and-MAV distribution on maize in France. *Journal of Plant Diseases and Protection*, 297–303.

Halbert S., Voegtlin D. (1995) Biology and taxonomy of vectors of barley yellow dwarf viruses. In *Barley yellow dwarf: 40 years of progress*, pp. 9–28. Eds C. J. D'Arcy and P. A. Burnett. St Paul: APS Press.

Henry M., Dedryver C.A. (1989) Fluctuations in cereal aphid populations on maize (*Zea mays*) in western France in relation to the epidemiology of barley yellow dwarf virus (BYDV). *Journal of Applied Entomology*, 107, 401–410.

Hernández J.A., Gullner G., Clemente-Moreno M.J., Künstler A., Juhász C., Díaz-Vivancos P., Király L. (2016) Oxidative stress and antioxidative responses in plant–virus interactions. *Physiological and Molecular Plant Pathology*, 94, 134–148.

Hewer A., Will T., van Bel A.J.E. (2010) Plant cues for aphid navigation in vascular tissues. *Journal of Experimental Biology*, 213, 4030–4042.

Horn F., Habekuß A., Stich B. (2013) Natural variation for BYDV resistance in maize. *Maydica*, 58.

Horn F., Habekuß A., Stich B. (2014) Genes involved in barley yellow dwarf virus resistance of maize. *Theoretical and Applied Genetics*, 127, 2575–2584.

Horn F., Habekuß A., Stich B. (2015) Linkage mapping of barley yellow dwarf virus resistance in connected populations of maize. *BMC Plant Biology*.

Ingwell L.L., Eigenbrode S.D., Bosque-Pérez N.A. (2012) Plant viruses alter insect behavior to enhance their spread. *Scientific Reports*, 2, 578.

Jambunathan, N. (2010) Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. *Methods in molecular biology*, 639, 292-8.

Jiang Y., Zhang C.-X., Chen R., He S.Y. (2019) Challenging battles of plants with phloem-feeding insects and prokaryotic pathogens. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 23390–23397.

Jiménez J., Moreno A., Fereres A. (2021) Transmission of phloem-limited viruses to the host plants by their aphid vectors. In *Progress in Botany Vol. 82*. pp. 357–382.

Jiménez-Martinez E.S., Bosque-Pérez N.A., Berger P.H., Zemetra R.S., Ding H., Eigenbrode S.D. (2004) Volatile cues influence the response of *Rhopalosiphum padi* (*Homoptera: Aphididae*) to barley yellow dwarf virus-infected transgenic and untransformed wheat. *Environmental Entomology*, 33, 1207–1216.

Jones J.D.G., Dangl J.L. (2006) The plant immune system. *Nature*, 444, 323–329.

Kern M., Meiners T., Schliephake E., Habekuss A., Ordon F., Will T. (2022) Infection of susceptible/tolerant barley genotypes with Barley yellow dwarf virus alters the host plant preference of *Rhopalosiphum padi* clones depending upon their ability to transmit BYDV. *Journal of Pest Science*, 95, 215–229.

Leybourne D.J., Aradottir G.I. (2022) Common resistance mechanisms are deployed by plants against sap-feeding herbivorous insects: insights from a meta-analysis and systematic review. *Scientific Reports*, 12, 17836.

Leybourne D.J., Valentine T.A., Robertson J.A.H., Pérez-Fernández E., Main A.M., Karley A.J., Bos J.I.B. (2019) Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *Journal of Experimental Botany*, 70, 4011–4026.

Loi N, Osler R, Snidaro M, Ardigo A, Lorenzoni C (1986) Tolerance to BYDV (Barley yellow dwarf virus) in inbred and hybrids of maize. *Maydica*, 31, 307–314.

Louis J., Basu S., Varsani S., Castano-Duque L., Jiang V., Williams W.P., Felton G.W., Luthe D.S. (2015) Ethylene contributes to *maize insect resistance1*-mediated maize defense against the phloem sap-sucking corn leaf aphid. *Plant Physiology*, 169, 313–324.

Meihls L.N., Handrick V., Glauser G., Barbier H., Kaur H., Haribal M.M., Lipka A.E., Gershenzon J., Buckler E.S., Erb M., Köllner T.G., Jander G. (2013) Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *The Plant Cell*, 25, 2341–2355.

Mittler R., Zandalinas S.I., Fichman Y., Breusegem F. (2022) Reactive oxygen species signalling in plant stress responses. *Nature Reviews. Molecular Cell Biology*, 23, 663–679.

Nancarrow N., Aftab M., Hollaway G., Rodoni B., Trębicki P. (2021) Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: a three-year field study in South-Eastern Australia. *Microorganisms*, 9.

Ng J.C.K., Perry K.L. (2004) Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, 5, 505–511.

Osler R, Loi N, Lorenzoni C, Snidaro M, Refatti E (1985) Barley yellow dwarf virus infections in maize (*Zea mays* L) inbreds and hybrids in Northern Italy. *Maydica*, 30, 285-229.

Oswald J.W., Houston B.E. (1953) The yellow-dwarf virus disease of cereal crops. *Phytopathology*, 43, 128–136.

Paulmann M.K., Kunert G., Zimmermann M.R., Theis N., Ludwig A., Meichsner D., Oelmüller R., Gershenzon J., Habekuss A., Ordon F., Furch A.C.U., Will T. (2018) Barley yellow dwarf virus infection leads to higher chemical defense signals and lower electrophysiological reactions in susceptible compared to tolerant barley genotypes. *Frontiers in Plant Science*, 9, 145.

Prado E., Tjallingii W.F. (1994) Aphid activities during sieve element punctures. *Entomologia Experimentalis et Applicata*, 72, 157–165.

R Core Team (2020) R: A language and environment for statistical computing. URL https://www.R-project.org/.

Radchenko E.E., Abdullaev R.A., Anisimova I.N. (2022) Genetic resources of cereal crops for aphid resistance. *Plants*, 11.

Rodríguez-López M.J., Garzo E., Bonani J.P., Fereres A., Fernández-Muñoz R., Moriones E. (2011) Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of tomato yellow leaf curl virus. *Phytopathology*, 101, 1191–1201.

Rong W., Wang X., Wang X., Massart S., Zhang Z. (2018) Molecular and ultrastructural mechanisms underlying yellow dwarf symptom formation in wheat after infection of barley yellow dwarf virus. *International Journal of Molecular Sciences*, 19.

Schliephake E., Habekuß A., Scholz M., Ordon F. (2013) Barley yellow dwarf virus transmission and feeding behaviour of *Rhopalosiphum padi* on *Hordeum bulbosum* clones. *Entomologia Experimentalis et Applicata*, 146, 347–356.

Scholz M., Ruge-Wehling B., Habekuss A., Schrader O., Pendinen G., Fischer K., Wehling P. (2009) *Ryd4 (Hb)*: a novel resistance gene introgressed from *Hordeum bulbosum* into barley and conferring complete and dominant resistance to the barley yellow dwarf virus. *Theoretical and Applied Genetics*, 119, 837–849.

Sharma S., Kooner R., Arora R. (2017) Insect pests and crop losses. In: *Breeding Insect Resistant Crops for Sustainable Agriculture*, 1st ed., pp. 45–66. Springer, Singapore.

Stoner WN (1977) Barley yellow dwarf virus infection in maize. *Phytopathology*, 67, 975–981.

Sytykiewicz H. (2015) Transcriptional responses of catalase genes in maize seedlings exposed to cereal aphids' herbivory. *Biochemical Systematics and Ecology*, 60, 131–142.

Sytykiewicz, H., Łukasik, I., Goławska, S., & Chrzanowski, G. (2019). Aphid-triggered changes in oxidative damage markers of nucleic acids, proteins, and lipids in maize (*Zea mays* L.) seedlings. *International journal of molecular sciences*, 20, 3742.

Tjallingii W.F (1988) Electrical recording of stylet penetration activities. In: *Aphids, their biology, natural enemies and control*. Eds.: Minks A.K., Harrewijn P., pp. 95-108. Elsevier, Amsterdam.

Tjallingii W.F., Hogen Esch T. (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology*, 18, 317–328.

van den Eynde R., van Leeuwen T., Haesaert G. (2020) Identifying drivers of spatio-temporal dynamics in barley yellow dwarf virus epidemiology as a critical factor in disease control. *Pest Management Science*, 76, 2548–2556.

Walls J., Rajotte E., Rosa C. (2019) The past, present, and future of barley yellow dwarf management. *Agriculture*, 9, 23.

Wang X., Rong W., Liu Y., Wang X., Zhang Z. (2018) Investigation of the mechanism of adult-stage resistance to barley yellow dwarf virus associated with a wheat-*Thinopyrum intermedium* translocation. *The Crop Journal*, 6, 394–405.

Waszczak C., Carmody M., Kangasjärvi J. (2018) Reactive oxygen species in plant signaling. *Annual Review of Plant Biology*, 69, 209–236.

Wickham H. (2007) Reshaping data with the reshape Package. *Journal of Statistical Software*, 21, 1–20.

Wickham H., François R., Henry L., Müller K. (2020) dplyr: A Grammar of Data Manipulation. R package version 1.0.2. URL https://CRAN.R-project.org/package=dplyr.

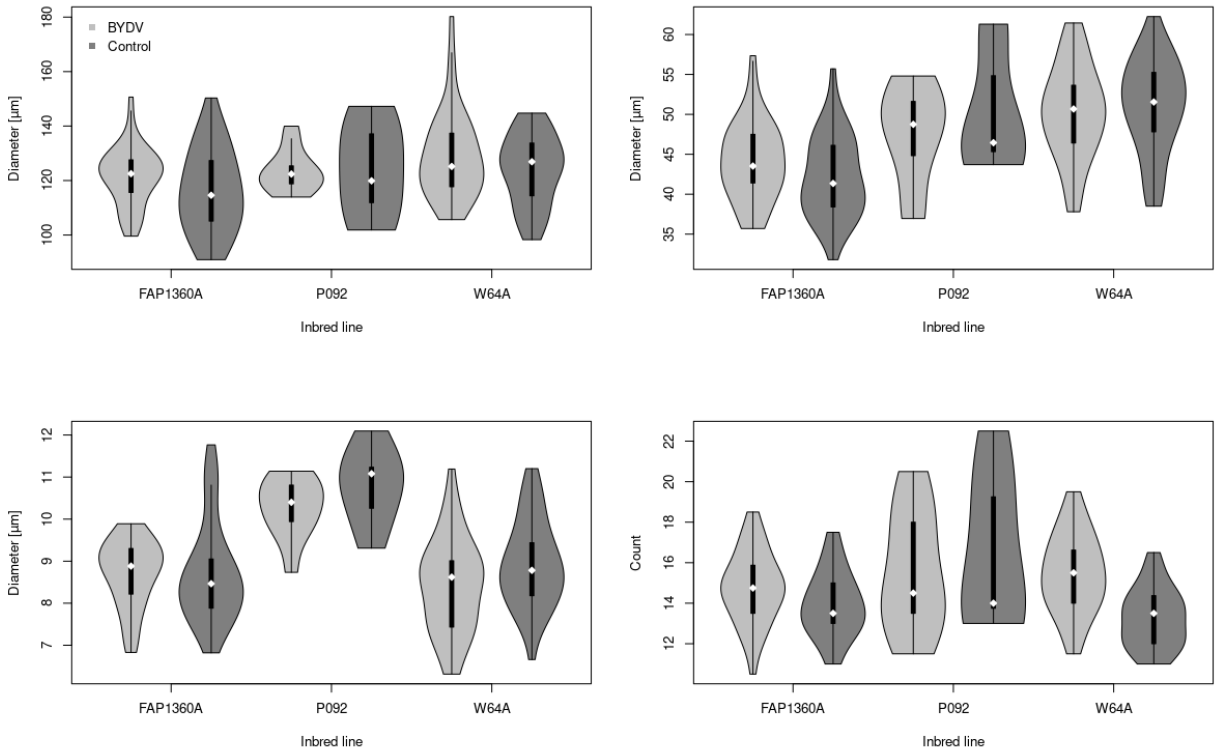
3.9 Supplementary material

Supplementary table 3.1: Dunn post-hoc test of CF and E1 of 8 h aphid feeding. CF: transition of the insect stylets through epidermis and mesophyll cells including difficulties in stylet penetrations; E1 (phloem salivation phase). Significant different pairs are marked with an asterisk (*)

Comparison	CF	E1
D408 – FAP1360A	1.0000	1.0000
D408 – Ky226	0.1560	0.0077*
D408 – P092	1.0000	1.0000
D408 – W64A	0.0182*	1.0000
FAP1360A – Ky226	1.0000	0.0924
FAP1360A – P092	1.0000	0.6884
FAP1360A – W64A	0.3341	1.0000
Ky226 – P092	0.1062	0.0015*
Ky226 – W64A	1.0000	0.0561
P092 – W64A	0.0085*	1.0000

Supplementary table 3.2: Dunn post-hoc test of significant hour-wise comparisons. Significant different pairs are marked with an asterisk (*)

	4 h		5 h	
Comparison	CF	E1	E2	E1
D408 – FAP1360A	1.0000	0.7243	1.0000	0.2554
D408 – Ky226	0.0078*	0.3865	0.0737	0.0566
D408 – P092	1.0000	1.0000	1.0000	1.0000
D408 – W64A	0.3780	1.0000	0.0153*	1.0000
FAP1360A – Ky226	0.0105*	1.0000	0.0640	1.0000
FAP1360A – P092	1.0000	0.0792	1.0000	0.1089
FAP1360A – W64A	0.4730	0.2037	0.0125*	1.0000
Ky226 – P092	0.0005*	0.0563	0.0503	0.0246*
Ky226 – W64A	0.4738	0.1224	1.0000	0.3604
P092 – W64A	0.0452	1.0000	0.0077*	1.0000



Supplementary figure 3.1: Morphological comparison of minor vein vascular bundle parameters of healthy and BYDV-PAV infected maize inbreds. Top-left: vascular bundle diameter, top-right: xylem diameter, bottom-left: sieve element diameter, bottom-right: number of sieve elements. Please Note the suppressed zero at the y-axis scale. The white diamond shape represents the median

Supplementary table 3.3: ANOVA of factors influencing morphological traits of minor veins of BYDV-infected and control plants of maize inbred lines FAP1360A, P092, and W64A. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

	Vascular bundle diameter	Sieve element diameter	Xylem diameter	Number of sieve elements
Inbred line	***	***	***	**
Treatment	*	.	ns	***
Replication	***	.	*	ns
Inbred x treatment	ns	ns	ns	**

4 Manuscript 3: Does Zm00001eb428020 Confer Resistance Against BYDV-PAV in Maize?

4.1 Abstract

Barley Yellow Dwarf (BYD) is one of the most devastating virus diseases of cereals, causing severe yield losses. BYDV-PAV is the most prevalent virus species of BYD and transmitted by the bird-cherry-oat aphid, *Rhopalosiphum padi.* Recently, a candidate gene conferring resistance against BYDV-PAV in maize, Zm00001eb428020*,* was proposed. The aim of this study was to confirm Zm00001eb428020 as the causative gene of BYDV-PAV resistance in maize. Therefore, inbred A188 was transformed with alleles of a BYDV-PAV resistant, tolerant, and susceptible inbreds FAP1360A, P092, and W64A. Virus titer of transformed plants did neither significantly differ between BYDV-PAV susceptible wildtype A188 and transformed plants, nor between plants that were transformed with different alleles of Zm00001eb428020. Thus, it is very unlikely that Zm00001eb428020 is the causative gene for BYDV-PAV resistance in maize.

4.2 Introduction

Plants are exposed to wide variety of fungal, bacterial, and viral pathogens. In contrast to vertebrates, their immunity does not rely on mobile immune cells and adaptive somatic variation (Jones and Dangl 2006). Instead, resistance mechanisms on a single-cell basis and disease resistance genes (*R*-genes) play a key role in the plant's immune responses to pathogen attack (Jones and Dangl, 2006, Andersen et al. 2018).

R-genes are usually dominant genes that provide full or partial resistance to one or more pathogens, but recessive *R*-genes also exist (Kourelis and van der Hoorn 2018). The first plant *R*-gene, *Hm1* of maize (*Zea mays*), was cloned in 1992 (Johal and Briggs 1992). To date, more than 300 R-genes are identified (Kourelis and van der Hoorn 2018). More than 60 % of them encode intracellular nucleotide binding/leucine-rich repeat immune receptor proteins (NLRs) that detect intracellular pathogen-derived molecules directly or indirectly, inducing effector-triggered immunity (ETI) (Kourelis and van der Hoorn 2018). The other main class of cloned *R*-

genes (19 %) encode receptor-like proteins and receptor-like kinases (RLPs/RLKs), which can recognize pathogen-derived components on the cell surface directly or indirectly and induce pathogen-associated molecular pattern-triggered immunity (PTI) (Kourelis and van der Hoorn 2018). Other *R*-gene based resistance mechanisms include, for example, the loss of susceptibility by directly disarming the pathogen by actively interrupting a key pathogenicity process, and the loss of susceptibility by mutation in a host component, leading to the inability of the pathogen to manipulate the host (Kourelis and van der Hoorn 2018). However, details of the resistance mechanisms remain unknown for most *R*-genes (Kourelis and van der Hoorn 2018).

Plant virus infections are responsible for about half of plant diseases worldwide (Jones and Naidu 2019). Unlike fungi or bacteria, viruses are harder to target by pesticides due to their intracellular parasitic nature (Akhter et al. 2021). Thus, the conventional method of viral disease control is to use the inherent resistance of plants through breeding (Akhter et al. 2021).

Barley Yellow Dwarf (BYD) is one of the most devastating virus diseases of cereals causing yield losses of up to 80 % (Choudhury et al. 2017, Nancarrow et al. 2021, van den Eynde et al. 2020, Walls et al. 2019). BYD is caused by at least ten different phloem-limited single stranded positive sense RNA viruses called barley yellow dwarf viruses (BYDV) and cereal yellow dwarf viruses (CYDV) that are transmitted by at least 25 different aphid species worldwide (Choudhury et al. 2017, Miller and Lozier 2022, Walls et al. 2019). BYDV-PAV is the most prevalent virus species of BYD and transmitted by the bird-cherry-oat aphid, *Rhopalosiphum padi* (Choudhury et al. 2017, van den Eynde et al. 2020, Walls et al. 2019).

Recently, a quantitative trait locus (QTL) for BYDV-PAV resistance in maize has been identified and a candidate gene, Zm00001eb428020, was suggested (Horn et al. 2014, Manuscript 1). Zm00001eb428020 encodes a 151 aa protein that is annotated to be located at the nuclear speckles and involved in response to oxidative stress and response to cadmium ion. The best BLAST hit in *Arabidopsis thaliana* is *oxidative stress 3* (*OXS3*, AT5G56550) (Horn et al. 2014), a gene involved in tolerance to oxidative stress and heavy metals like cadmium (Blanvillain et al. 2009). *AtOXS3* localizes in nuclear speckles associated with chromatin and might act as a chromatin remodeling factor and thus influences gene expression during stress response (Blanvillain et al. 2009). AT5G56550 is also a potential interaction partner of *ATAF2* (At5g08790), a NAC-domain transcription factor targeted for degradation by Tobacco mosaic

virus (Wang and Culver 2012). *OXS3* likely enhances virus resistance by the production of H_2O_2 (Wang and Culver 2012). The generation of reactive oxygen species (ROS) is a general pathogen resistance mechanism, triggering both local and systematic defense responses (for review see Hernández et al. 2016, Mittler et al 2022). In contrast, some viruses are dependent on ROS for robust viral RNA replication (Hyodo et al. 2017, Wang et al. 2021). In both cases, ROS levels are increased in infected plants.

Nuclear speckles (NS) are RNA–protein granules enriched in pre-mRNA splicing factors, located in the interchromatin regions of the nucleoplasm of eukaryotic cells (Spector and Lamond 2011). Many human viruses use host NS for processes that are vital for viral propagation, such as transcription, splicing and export (for review see Faber et al. 2022). NS were also found to be involved in plant susceptibility to oomycete pathogens (Li et al. 2018) and geminivirus infection (Rosas-Diaz et al. 2022).

The aim of this study is to validate the candidate gene Zm00001eb428020 as the causative agent for BYDV-PAV resistance in maize by a transgenic approach. Therefore, alleles of Zm00001eb428020 from a BYDV-susceptible, a BYDV-tolerant, and a BYDV-resistant maize inbred line were transferred into the BYDV-susceptible maize inbred line A188.

4.3 Methods

Aphid rearing

Aphids of species *R. padi* clone R07 were reared on BYDV-infected barley cultivar “Rubina” at room temperature and 16 h artificial lightening mimicking daylight conditions.

Vector construction

DNA from maize inbreds FAP1360A (BYDV-PAV resistant), P092 (BYDV-PAV tolerant), and W64A (BYDV-PAV susceptible) was extracted using the DNaesy Plant Mini Kit (Qiagen) according to the manufacturer’s instructions with optional steps.

From each inbred, full-length gene Zm00001eb428020 including promoter region was amplified via PCR (Supplementary tables 4.5 and 4.6). PCR products were cleaned using the PCR and Gel Clean-up kit (Macherey-Nagel), sequenced with the Sanger method and used for cloning. Zm00001eb428020-alleles were cloned separately into vector pUBI7intL (kindly provided by Dr. Dirk Becker from Crop Genetic Systems, Hamburg, Germany) using restriction

enzymes BamHI and EcoRI and DNA ligase T1 (ThermoFisher) according to the manufacturer's instructions.

pUBI7intL-Zm00001eb428020 were transformed into *E. coli* DH5α cells using the heat shock method. Transformed *E. coli* DH5α were cultivated over night at 37 °C on LB medium prepared with spectinomycin for selection. Ten *E. coli* DH5α colonies per construct were picked using sterile pipette tips and a colony PCR was performed to check if *E. coli* DH5α contained the full sequence of Zm00001eb428020 (Supplementary tables 4.5 and 4.6). Pipette tips with remaining cells were transferred into 15 ml liquid LB medium containing 1 µl spectinomycin and cultivated over night at 37 °C. Plasmids of three Zm00001eb428020 positive clones of each construct were extracted using the GeneJET Plasmid Mini Prep kit (ThermoFisher Scientific) following the manufacturer's instructions. Plasmids were checked for mutations of Zm00001eb428020 via Sanger-sequencing (see Supplementary table 4.11 for primer sequences). Plasmids of one clone per construct that showed no mutations in comparison to the original Zm00001eb428020 alleles of the maize inbreds were chosen for further steps.

Transformation of plants

Zm00001eb428020 alleles of the maize inbreds FAP1360A (BYDV-PAV resistant), P092 (BYDV-PAV tolerant), and W64A (BYDV-PAV susceptible) that were cloned into vector p7intL were transformed into maize inbred A188 (BYDV-PAV susceptible) via *Agrobacterium tumefaciens* method at Crop Genetic Systems (Hamburg, Germany) resulting in nine to twelve individual T0 plants per allele. Four plants transformed with the allele of W64A and one plant transformed with the allele of P092 were selfed and the other plants were back-crossed with A188 to generate seeds for T1 generation.

Selection of transgenic plants

DNA of transgenic T1 plants was extracted using the method described previously (Manuscript 1) but washing of the pellet with ethanol was conducted twice. The presence of the transgene was confirmed via PCR specific for *bar* resistance gene (Supplementary tables 4.7 and 4.8) and 35S promoter (Supplementary tables 4.9 and 4.10), respectively. Additionally, PCR on Zm00001eb428020 (Supplementary tables 4.5 and 4.6) was conducted to confirm presence of full-length transformed Zm00001eb428020 alleles. It was not possible to amplify

Zm00001eb428020 equivalent in A188. Plants that were positive for Zm00001eb42802 and bar or 35S or both were selfed.

Plants of the T2 generation were subjected to phenotyping (see next paragraph). DNA of T2 plants was extracted using the Nucleo Spin kit (Macherey-Nagel) and the presence of the transgene was confirmed via PCR specific for the *bar* resistance gene and 35S promoter, respectively (Supplementary tables 4.7 to 4.10).

Phenotyping

We conducted two independent experiments to analyze BYDV-PAV titer in transformed plants. In experiment 1, eight plants per T2 line were grown in a climate chamber (16 h light, 24 °C / 8 h darkness, 22 °C). In experiment 2, ten plants per T2 line were grown in a greenhouse (16 h light, 20 °C / 8 h darkness, 16 °C). In each experiment, maize inbred A188 (wildtype) was included four times and inbreds FAP1360A, P092, and W64A were included each twice for comparison.

In both experiments, BYDV-PAV carrying apterous *R. padi* were spread evenly between plants with at least ten aphids per plant. Aphids were killed with insecticide “Confidor” (Bayer CropScience) one week after inoculation. Six weeks after inoculation, the 6^{th} leaf of each plant was harvested individually and virus titer was quantified using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method with in-house BYDV-PAV antibodies as described by Horn et al. (2013).

Statistics

Presence of the transgene in T2 plants was confirmed (see above) and plants with negative or ambiguous results were excluded from further analysis.

The following linear model was applied on data of individual experiments:

$$Y_i = \mu + g_i + e_i$$

where Yi is the observed virus titer, μ the general mean, g_i the genotype, and e_i the error.

For the combined analysis of data from both experiments, the following model was applied:

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

where Y_{ij} is the observed virus titer, μ the general mean, g_i the genotype, r_j the experiment, and e_i the error.

Significance of factors of the model was calculated using ANOVA. Pairwise comparisons were conducted using the function "pairwise" of R-package emmeans with tukey adjustment method for correction for multiple testing. Analyses were conducted using R version 3.6.3 (R Core Team 2020, https://www.R-project.org/) with package "emmeans" version 1.5.1 (Lenth, 2020), and RStudio version 1.3.1073 (RStudio Team 2020, http://www.rstudio.com/).

4.4 Results

In experiment 1, average virus titer was highest in BYDV-tolerant and BYDV-susceptible maize inbreds P092 (1.05) and W64A (0.87), respectively (Supplementary table 4.1). In contrast, BYDV-resistant inbred FAP1360A had the lowest average level (0.29). Virus titer of A188 wildtype and A188 transformed with different alleles of Zm00001eb428020 were intermediate, ranging from 0.54 (A188-FAP1360A) to 0.67 (A188-P092). Despite virus titer of A188-FAP1360A was lower than virus titer of A188 wildtype and A188 transformed with the alleles of P092 and W64A, these differences were not statistically significant (α = 0.05) (Supplementary table 4.2). However, BYDV-PAV titer of FAP1360A was significantly lower than of P092 and W64A, respectively (Figure 4.1 and Supplementary table 4.2).

Virus titers in experiment 2 were higher than in experiment 1 (Figure 4.1). Averages of virus titer values ranged between 1.60 (W64A) and 1.92 (A188-W64A) (Supplementary table 4.3). Accordingly, infection rates (IR) were very high, ranging from 0.94 (P092 and A188 wildtype) to 1.00 (W64A) (Supplementary table 4.3). The only exception was BYDV-PAV resistant maize inbred FAP1360A with average virus titer levels of 0.06 and an IR of 0.00 (Supplementary table 4.3). There was no statistically significant difference (α = 0.05) in virus titer levels between A188 wildtype and transformed A188 plants – neither those plants that carried alleles of the BYDV-PAV tolerant and BYDV-PAV susceptible maize inbreds, nor those plants that carried Zm00001eb428020 alleles of the BYDV-PAV resistant inbred FAP1360A (Supplementary table 4.4).

In the combined analysis of both experiments shows a significant ($p < 0.001$) effect of the experiment and the genotype on virus titer (Table 4.1). Virus titers of maize inbred FAP1360A were significantly lower ($p < 0.001$) than in all other genotypes (Table 4.1).

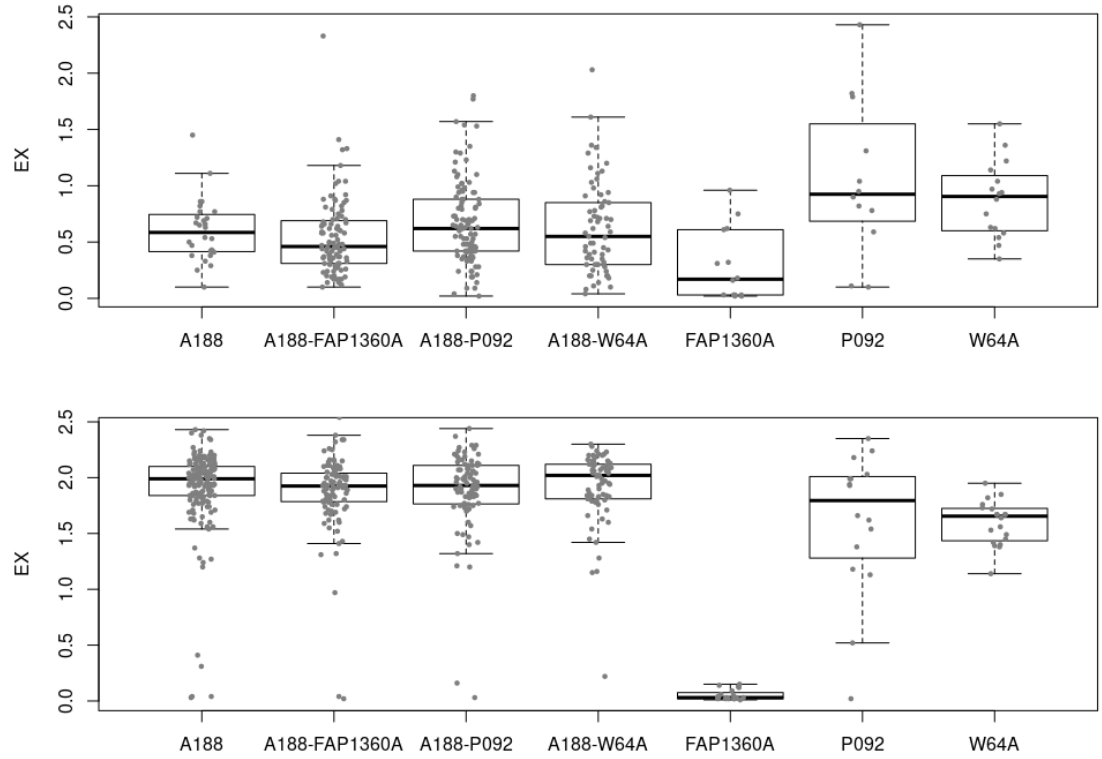


Figure 4.1: Virus titer measured in experiments 1 (upper panel) and 2 (lower panel). Maize inbred line A188 was transformed with alelles of the BYDV-PAV resistant (A188-FAP1360A), the BYDV-PAV tolerant (A188-P092), and the BYDV-PAV susceptible (A188-W64A) inbred. Untransformed A188 and maize inbreds FAP1360A, P092, and W46A were used for comparison. Grey dots represent measurements of individual plants.

Table 4.1: Pairwise comparisons of virus titers per genotype in combined analysis of both experiments. Maize inbred A188 (BYDV-PAV susceptible) was transformed with alleles of gene Zm00001eb428020 of the BYDV-PAV resistant (FAP1360A), the BYDV-PAV tolerant (P092), and the BYDV-PAV susceptible (W64A) inbred. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1. ns: not significant

	A188	A188-FAP1360A	A188-P092	A188-W64A	FAP1360A	P092
A188-FAP1360A	ns	-	-	-	-	-
A188-P092	ns	ns	-	-	-	-
A188-W64A	ns	ns	ns	-	-	-
FAP1360A	***	***	***	***	-	-
P092	ns	ns	ns	ns	***	-
W64A	ns	ns	ns	ns	***	ns

4.5 Discussion

We could not detect a difference between the virus titres of wildtype A188 and plants that were transformed with alleles of Zm00001eb428020 from BYDV-PAV resistant (FAP1360A), BYDV-PAV tolerant (P092), and BYDV-PAV susceptible (W64A) maize inbreds, respectively.

One reason might be that the resistant Zm00001eb428020 allele is recessive and the effect of wildtype Zm00001eb428020 in A188 masks the effect of the resistant allele. In fact, about half of the *R*-genes for virus-resistance in crops are recessive (Kang et al. 2005). The main group of identified recessive *R*-genes for virus-resistance are eukaryotic translation initiation factors (eIF) 4E and eIF4G (Kang et al 2005). Additional potential recessive *R*-genes include other translation factors like polyA-binding protein (PABP), NAC-domain transcription factor and DEAD-box RNA helicase; a knockout mutation in *DNA-binding protein phosphatase 1* (DBP1) gene; a nuclear-encoded chloroplast phosphoglycerate kinase cPGK2; mutated host factors that interact with viral proteins directly; genes that are involved in the unfolded protein response (UPR); and mutations in genes involved in phytohormone metabolism (for review see Hashimoto et al. 2016, Sanfaçon, 2015). However, our previous experiments suggested that the BYDV-PAV resistance gene in maize is not recessive (Manuscript 1), thus masking of effects of the resistant allele can be excluded.

Integration of foreign DNA into the host genome via *Agrobacterium* transformation happens randomly (Kim et al. 2007, Shilo et al. 2017). Transgenic DNA that is integrated into transcriptionally inactive regions might not be expressed. We examined transgenic plants that originated from nine to twelve individual T0 plants per transformed allele. This minimizes the likelihood that positional effects of single insertion events have a significant influence on gene expression. Additionally, there is a selection bias towards insertion events in transcriptionally active genomic regions if metabolic processes are used as selection markers (Kim et al. 2007, Shilo et al. 2017). This was the case for the T0 plants in this study. Thus, it is very likely that transformed DNA was integrated in transcriptionally active regions.

Zm00001eb428020 alleles were transformed including their natural promoter region. However, regulatory elements that influence gene expression are often located kilobases to megabases apart from their target genes and sometimes on different chromosomes (Schmitz et al. 2022). Thus, transformed Zm00001eb428020 alleles might not be expressed in A188 because regulatory elements are lacking. However, expression of Zm00001eb428020 did not change when allele donor inbreds FAP1360A, P092, and W64A were challenged with BYDV-PAV (Manuscript 1). Thus, regulation of expression of Zm00001eb428020 may not be of importance during BYDV infection.

4.6 Conclusion

Zm00001eb428020 is a candidate gene for conferring resistance to BYDV-PAV in maize. However, the results from our experiments revealed that the Zm00001eb428020 allele from the BYDV-PAV resistant maize inbred FAP1360A does not confer BYDV-PAV resistance in transformed A188 plants. Positional effects of transgene insertion sites or lacking regulatory sequences of Zm00001eb428020 in transformed plant may be excluded. Thus, it is very likely that Zm00001eb428020 is not the causative gene for BYDV-PAV resistance in maize.

4.7 Acknowledgements

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4.8 References

Akhter M.S., Nakahara K.S., Masuta C. (2021) Resistance induction based on the understanding of molecular interactions between plant viruses and host plants. *Virology journal*, 18, 176.

Andersen E.J., Ali S., Byamukama E., Yen Y., Nepal M.P. (2018) Disease resistance mechanisms in plants. *Genes*, 9.

Blanvillain R., Kim J.H., Wu S., Lima A., Ow D.W. (2009) OXIDATIVE STRESS 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress. *The Plant Journal*, 57, 654–665.

Choudhury S., Hu H., Meinke H., Shabala S., Westmore G., Larkin P., Zhou M. (2017) Barley yellow dwarf viruses: infection mechanisms and breeding strategies. *Euphytica*, 213, 213-168.

Faber G.P., Nadav-Eliyahu S., Shav-Tal Y. (2022) Nuclear speckles - a driving force in gene expression. *Journal of Cell Science*, 135.

Hashimoto M., Neriya Y., Yamaji Y., Namba S. (2016) Recessive resistance to plant viruses: potential resistance genes beyond translation initiation factors. *Front. Microbiol.*, 7.

Hernández J.A., Gullner G., Clemente-Moreno M.J., Künstler A., Juhász C., Díaz-Vivancos P., Király L. (2016) Oxidative stress and antioxidative responses in plant–virus interactions. *Physiological and Molecular Plant Pathology*, 94, 134–148.

Horn F., Habekuß A., Stich B. (2014) Genes involved in barley yellow dwarf virus resistance of maize. *Theoretical and applied genetics*, 127, 2575–2584.

Hyodo K., Hashimoto K., Kuchitsu K., Suzuki N., Okuno T. (2017) Harnessing host ROS-generating machinery for the robust genome replication of a plant RNA virus. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E1282-E1290.

Johal G.S., Briggs S.P. (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. *Science*, 258, 985-987.

Jones J.D.G., Dangl J.L. (2006) The plant immune system. *Nature*, 444, 323–329.

Jones R.A.C., Naidu R.A. (2019) Global dimensions of plant virus diseases: current status and future perspectives. *Annual Review of Virology*, 6, 387–409.

Kang B.-C., Yeam I., Jahn M.M. (2005) Genetics of plant virus resistance. *Annual Review of Phytopathology*, 43, 581–621.

Kim S.-I., Veena, Gelvin S.B. (2007) Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the *Arabidopsis* genome generated under non-selective conditions. *The Plant Journal*, 51, 779–791.

Kourelis J., van der Hoorn R.A.L. (2018) Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *The Plant Cell*, 30, 285–299.

Lenth R. (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means: R package version 1.5.1. URL https://CRAN.R-project.org/package=emmeans.

Li H., Wang H., Jing M., Zhu J., Guo B., Wang Y., Lin Y., Chen H., Kong L., Ma Z., Wang Y., Ye W., Dong S., Tyler B., Wang Y. (2018) A *Phytophthora* effector recruits a host cytoplasmic transacetylase into nuclear speckles to enhance plant susceptibility. *eLife*, 7.

Nancarrow N., Aftab M., Hollaway G., Rodoni B., Trębicki P. (2021) Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: A three-year field study in South-Eastern Australia. *Microorganisms*, 9.

Miller W.A., Lozier Z. (2022) Yellow dwarf viruses of cereals: taxonomy and molecular mechanisms. *Annual Review of Phytopathology*, 60, 121–141.

Mittler R., Zandalinas S.I., Fichman Y., Breusegem F. (2022) Reactive oxygen species signalling in plant stress responses. *Nature Reviews. Molecular Cell Biology*, 23, 663–679.

R Core Team (2020) R: A language and environment for statistical computing. URL https://www.R-project.org/.

Rosas-Diaz T., Cana-Quijada P., Wu M., Du H., Fernandez-Barbero G., Macho A.P., Solano R., Castillo A.G., Wang X.-W., Lozano-Duran R., Bejarano E.R. (2022) The transcriptional regulator JAZ8 interacts with the C2 protein from geminiviruses and limits the viral infection in *Arabidopsis*.

Sanfaçon H. (2015) Plant translation factors and virus resistance. *Viruses*, 7, 3392–3419.

Schmitz R.J., Grotewold E., Stam M. (2022) *Cis*-regulatory sequences in plants: their importance, discovery, and future challenges. *The Plant Cell*, 34, 718–741.

Shilo S., Tripathi P., Melamed-Bessudo C., Tzfadia O., Muth T.R., Levy A.A. (2017) T-DNA-genome junctions form early after infection and are influenced by the chromatin state of the host genome. *PLoS genetics*, 13, e1006875.

Spector D.L., Lamond A.I. (2011) Nuclear Speckles. *Cold Spring Harbour Perspectives in Biology*, 3.

van den Eynde R., van Leeuwen T., Haesaert G. (2020) Identifying drivers of spatio-temporal dynamics in barley yellow dwarf virus epidemiology as a critical factor in disease control. *Pest Management Science*, 76, 2548–2556.

Walls J., Rajotte E., Rosa C. (2019) The past, present, and future of barley yellow dwarf management. *Agriculture*, 9, 23.

Wang X., Culver J.N. (2012) DNA binding specificity of ATAF2, a NAC domain transcription factor targeted for degradation by tobacco mosaic virus. *BMC Plant Biology*, 12.

Wang X., Jiang Z., Yue N., Jin X., Zhang X., Li Z., Zhang Y., Wang X.-B., Han C., Yu J., Li D. (2021) Barley stripe mosaic virus γb protein disrupts chloroplast antioxidant defenses to optimize viral replication. *The EMBO Journal*, 40.

4.9 Supplementary Material

Supplementary table 4.1: Descriptive statistics on virus titer in experiment 1. IR: proportion of plants with virus titer above 0.05 (infection rate)

Genotype	mean	median	var	sd	sem	IR
A188	0.60	0.59	0.0768	0.2772	0.0524	0.61
A188-FAP1360A	0.54	0.46	0.1154	0.3398	0.0347	0.45
A188-P092	0.67	0.62	0.13949	0.3735	0.0370	0.61
A188-W64A	0.62	0.55	0.1640	0.4050	0.0502	0.52
FAP1360A	0.29	0.17	0.1014	0.3184	0.0851	0.29
P092	1.05	0.93	0.47895	0.6921	0.1998	0.83
W64A	0.87	0.91	0.1135	0.3370	0.0842	0.88

Supplementary table 4.2: Pairwise comparisons of virus titers per genotype in experiment 1. Maize inbred A188 (BYDV-PAV susceptible) was transformed with alleles of gene Zm00001eb428020 of the BYDV-PAV resistant (FAP1360A), the BYDV-PAV tolerant (P092), and the BYDV-PAV susceptible (W64A) inbred. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1. ns: not significant

Genotype	A188	A188-FAP1360A	A188-P092	A188-W64A	FAP1360A	P092
A188-FAP1360A	ns	-	-	-	-	-
A188-P092	ns	ns	-	-	-	-
A188-W64A	ns	ns	ns	-	-	-
FAP1360A	ns	ns	**	*	-	-
P092	**	***	*	**	***	-
W64A	ns	*	ns	ns	***	ns

Supplementary table 4.3: Descriptive statistics on virus titer in experiment 2. IR: proportion of plants with virus titer above 0.05 (infection rate)

Genotype	mean	median	var	sd	sem	IR
A188	1.88	2.00	0.26797	0.5177	0.0888	0.94
A188-FAP1360A	1.87	1.93	0.1260	0.35498	0.0355	0.98
A188-P092	1.88	1.93	0.1422	0.3772	0.0412	0.98
A188-W64A	1.92	2.02	0.1062	0.32588	0.0390	0.99
FAP1360A	0.06	0.03	0.0021	0.0462	0.0106	0.00
P092	1.61	1.80	0.4092	0.6397	0.1599	0.94
W64A	1.60	1.66	0.0384	0.1958	0.0438	1.00

Supplementary table 4.4: Pairwise comparisons of virus titers per genotype in experiment 2. Maize inbred A188 (BYDV-PAV susceptible) was transformed with alleles of gene Zm00001eb428020 of the BYDV-PAV resistant (FAP1360A), the BYDV-PAV tolerant (P092), and the BYDV-PAV susceptible (W64A) inbred. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1. ns: not significant

	A188	A188-FAP1360A	A188-P092	A188-W64A	FAP1360A	P092
A188-FAP1360A	ns	-	-	-	-	-
A188-P092	ns	ns	-	-	-	-
A188-W64A	ns	ns	ns	-	-	-
FAP1360A	***	***	***	***	-	-
P092	ns	.	.	*	***	-
W64A	.	*	*	*	***	ns

Supplementary table 4.5: Reagents used for amplification of Zm00001eb428020. In case of colony PCR, E. coli DH5α cells were given directly into reaction tubes instead of purified DNA

Reagent	Volume per sample
DNA	variable
5x GC Buffer	5.0 µl
dNTP (2 mM each)	2.0 µl
ZmOXS3_1 (10 mM)	1.0 µl
ZmOXS3_2 (10 mM)	1.0 µl
DMSO	1,0 µl
Phusion polymerase	0.2 µl
H_2O	Ad 25.0 µl

Supplementary table 4.6: Temperature program used for amplification of Zm00001eb428020

Step	Temperature	Duration	
1	95 °C	5 min	
2	95 °C	30 s	Repeat 40 times
3	69 °C	30 s	
4	72 °C	1 min	
5	72 °C	5 min	
6	4 °C	hold	

Supplementary table 4.7: Reagents used for verification of transgenes by *bar* gene-specific PCR

Reagent	Volume per sample
DNA	3.0 µl
10x Dream Taq Buffer	2.0 µl
dNTP (2 mM each)	2.0 µl
bar_3F (10 mM)	1.0 µl
bar_3R (10 mM)	1.0 µl
Dream Taq polymerase	0,1 µl
H_2O	10.9 µl

Supplementary table 4.8: Temperature program used for verification of transgenes by *bar* gene-specific PCR

Step	Temperature	Duration	
1	95 °C	5 min	
2	95 °C	30 s	Repeat 35 times
3	60 °C	30 s	
4	72 °C	1 min	
5	72 °C	5 min	
6	4 °C	hold	

Supplementary table 4.9: Reagents used for verification of transgenes by 35S promoter-specific PCR

Reagent	Volume per sample
DNA	3.0 µl
10x Dream Taq Buffer	2.0 µl
dNTP (2 mM each)	2.0 µl
35S_1F (10 mM)	1.0 µl
35S_1R (10 mM)	1.0 µl
Dream Taq polymerase	0,1 µl
H_2O	10.9 µl

Supplementary table 4.10: Temperature program used for verification of transgenes by 35S promoter-specific PCR

Step	Temperature	Duration	
1	95 °C	5 min	
2	95 °C	30 s	Repeat 35 times
3	60 °C	30 s	
4	72 °C	1 min	
5	72 °C	5 min	
6	4 °C	hold	

Supplementary table 4.11: Sequences of primers that were used for PCR

Name	Sequence	Purpose
ZmOXS3_1	gactGGATCCACTCACAAGCAACCCTCAAAATCT	Amplification and sequencing of Zm00001eb428020
ZmOXS3_2	agatcGAATTCGGCAGCACATTTATCAAGCGTGT	
ZmOXS3_3	CTCCGTGACGACCGCCAATAAG	Sequencing of Zm00001eb428020
ZmOXS3_4	AGATAAAGGAGCCGTCAGTCAGC	
ZmOXS3_5	CCAGCTACTCGTCATTCTTATC	
bar_3F	GAGTCCACCGTGTACGTCTC	Confirmation of presence of the *bar* gene
bar_3R	CTGAAGTCCAGCTGCCAGAA	
35S_1F	GTCTCAGAAGACCAGAGGGC	Confirmation of presence of the 35S promoter
35S_1R	GTGCGTCATCCCTTACGTCA	

5 Summary

Barley yellow dwarf (BYD) is one of the economically most important virus diseases of cereals. BYD is caused by at least ten different virus species that are vector-transmitted by at least 25 aphid species. The most prevalent BYD causing virus is BYDV-PAV. The main vector of BYDV-PAV is the bird-cherry oat aphid, *Rhopalosiphum padi*. Climate change is expected to promote abundance of *R. padi* and incidence of BYDV-PAV. Possibilities to control BYD are limited and growing BYD resistant crops is the most practical approach. However, only a few resistance genes are known for barley and wheat, respectively. Due to low efficiency and/or linkage with adverse traits, most of these genes are not employed in breeding programs.

Maize plays a central role in the infection cycle, serving as a reservoir for vector and virus in summer. Thus, growing BYD resistant maize will reduce BYD pressure not only in maize but also in small-grain cereals like wheat and barley. BYDV-PAV resistance in maize shows high genotypic variance and high heritability, making it a promising target for breeding. Recently, a quantitative trait locus (QTL) for BYDV-PAV resistance in maize has been identified at the distal end of chromosome 10 and a candidate gene, GRMZM2G018027 (Zm00001eb428020), conferring resistance was suggested.

The objectives of this thesis are i) to identify and validate the causative gene for BYDV-PAV resistance in maize and ii) to gain insights into the resistance mechanism.

To characterize the mode of action of the BYDV-PAV resistance gene, three BYDV-PAV resistant (D408, FAP1360A, and Ky226), one susceptible (W64A) and one tolerant (P092) inbred were used.

Experiments on choice and feeding behavior of viruliferous *R. padi* on virus-free plants of the five inbreds were conducted to evaluate if the resistance targets the vector or the virus. Aphids preferred plants of BYDV-PAV resistant inbred over plants of the other inbreds, suggesting that BYDV-PAV resistant plants do not repel viruliferous aphid vectors. Compared to inbreds P092 and W64A, inbred FAP1360A displayed the lowest numbers of sieve element cells and the smallest diameters of the vascular bundle, xylem and sieve element cells, in healthy and BYDV-PAV infected plants, respectively. This suggests that aphids may have difficulties reaching or feeding on the phloem of FAP1360A plants. However, monitoring of

aphid feeding behavior via electric penetration graph (EPG) analysis revealed that BYDV-PAV resistance has no impact on phloem accessibility or acceptance.

The generation of reactive oxygen species (ROS) is a conserved process during plant defense against herbivores and pathogens. Opposed to this, an increased ROS production upon *R. padi* infestation and BYDV-PAV infection in the tested inbreds FAP1360A, P092, and W64A was not confirmed. However, due to the generally complex spatio-temporal patterns of ROS formation, it is difficult to clarify if, where, and when ROS are produced during *R. padi* infestation and BYDV-PAV infection in maize.

Analyzing the effect of BYDV-PAV infection on the vascular bundle of FAP1360A, P092, and W64A, we observed a significant inbred x treatment interaction effect for vascular bundle diameter, sieve element cell diameter, and number of sieve element cells of the midrib and number of sieve element cells of the marginal veins. However, differences between healthy and infected plants did not exceed +/-19 %.

Two connected biparental mapping populations were used for finemapping of the BYDV-PAV resistance gene. These populations originate from crosses between BYDV-PAV tolerant P092 and BYDV-resistant inbreds FAP1360A and Ky226, respectively. The previously published ~8 Mbp QTL interval could be confirmed and reduced to ~0.3 Mbp, comprising nine genes. Two of these nine genes, Zm00001eb428010 and Zm00001eb428020, were expressed. However, expression of these genes did not differ between aphid infested, BYDV-PAV infected and untreated control plants in any of the maize inbreds that were tested.

An association study was conducted to further narrow down the QTL interval. This analysis confirmed Zm00001eb428010 and Zm00001eb428020 as candidate genes for resistance to BYDV-PAV in maize. Analysis of transgenic maize plants revealed that plants carrying Zm00001eb428020 alleles of FAP1360A, P092, or W64A did not differ from each other or from BYDV-PAV susceptible wildtype A188 regarding BYDV-PAV virus titer. Thus, BYDV-PAV resistance in maize is very likely linked to Zm00001eb428010. Proteins encoded by Zm00001eb428010 are predicted to belong to the DNA2/NAM7-like helicase family. Proteins of this family are known to target plant and animal viruses for nonsense-mediated mRNA decay. The analysis of gene sequences revealed that Zm00001eb428010 is affected by three structural variations (SVs), compared to the reference sequence of B73. One SV is located in the 5'-UTR and two SVs are located in intronic regions. These SVs are shared between all three BYDV-PAV resistant inbreds but are not present in P092 or W64A. Additionally, a SNP with

predicted protein-altering effect was identified in Zm00001eb428010 of all three BYDV-PAV resistant inbreds but not P092 and W64A.

Taken together, biparental mapping, association analysis, gene expression, predicted gene product function, and analysis of gene sequence strongly suggest that Zm00001eb428010 is the causative gene conferring BYDV-PAV resistance in maize. Interestingly, resistance genes to multiple other viruses were mapped to the same genomic region on chromosome 10, suggesting that Zm00001eb428010 might be involved in a general virus resistance mechanism in maize. Thus, Zm00001eb428010 may also be used to control other virus diseases in maize. The genetic information that is provided in this work may be used to develop marker for marker-assisted selection in breeding.

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7 Curriculum Vitae

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8 Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.
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Düsseldorf, im September 2023

Maria Schmidt