Elucidating the molecular genetics of host and nonhost resistance in barley to stripe rust

Andrew Marc Dawson

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Abstract.

Plants have a remarkable ability to resist the majority of pathogenic microbes they encounter. As such, they are described as nonhosts. Nonhost resistance is often conceptualised as a qualitative separation from host resistance. Classification into these two states is generally facile, as they fail to fully describe the range of states that exist in the transition from host to nonhost. This poses a problem when studying pathosystems that cannot be classified into either of these categories due to their intermediate status relative to the two extremes. Therefore, the terms intermediate host and intermediate nonhost have been proposed to describe pathosystems in the evolutionary transition between host and nonhost status. At present, a significant amount of research exists into the molecular genetics of host and nonhost pathosystems but very little is known about intermediate systems. The work in this Ph. D. thesis focuses on the interaction of barley with *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust, as an intermediate host pathosystem.

The first research chapter describes the development of two microscopic phenotypic assays used to quantify *P. striformis* f. sp. *tritici* in barley leaves challenged with the pathogen. These assays are then used to screen a large panel of barley accessions to define the intermediate host status of barley relative to a host pathosystem. Subsequently, these assays play a key role in determining that the genetic architecture of resistance in barley is underpinned by three major effect resistance loci: *Rpst1*, *Rpst2*, and *Rpst3*. Using a combination of classical map-based genetics and contemporary genomics information I identify a candidate NLR gene underlying *Rpst2* resistance on chromosome 7HL. Furthermore, I show that distinct genes condition host and nonhost resistance in barley by mapping the host resistance gene, *rps2* to chromosome 2HL.

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Contributions to research.

Andrew Dawson (AD), John Ferguson (JF), Matthew Gardiner (MG), Phon Green (PG), Amelia Hubbard (AH), Matthew Moscou (MM), Inmaculada Hernández-Pinzón (IHP), Burkhard Steuernagel (BS).

Chapter 2- The development of quick, robust, quantitative phenotypic assays for describing the host-nonhost landscape to stripe rust.

Conception and the design of experiments: AD, MM.

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Data analyses: MM.

Chapter 3- An *R* gene complex protects barley against wheat stripe rust.

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Experimentation: AD, MG, PG, IHP, AH.

Data analyses: MM.

Chapter 4- Isolation and fine mapping of *Rpst2*- An intermediate host resistance gene in barley effective against wheat stripe rust.

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Data analyses: AD, MM.

Chapter 5- Fine mapping of *rps2*- A recessive host resistance gene in barley effective against barley stripe rust.

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Experimentation: AD, MG, PG, IHP, AH.

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1. General introduction.

The domestication of plants began around 10,500 years ago during an agricultural revolution that marked the cultural transition from hunting and gathering to settlement and non-nomadic lifestyles. In modern society, our dependence upon agricultural has not swayed and crop production is arguably the keystone that supports global food security. However, crop production and food security are being threatened by a multitude of problems that are converging to create the 'perfect storm'. Faced with these problems, it is vital that we develop our understanding of plant biology so that we may find ways to improve agricultural crop production. A key part of this endeavour will involve the deployment of durable disease resistance in crops effective against the multiplicity of pathogens they are exposed to.

1.1. Adaptive and innate immunity.

The immunity of an organism refers to its ability to resist particular diseases or infections (Ratcliffe, 1989). Both plants and animals are exposed to a plethora of microbial species, many of which could be potentially pathogenic, disease-causing agents. As a consequence, both have evolved complex systems for the detection, and subsequent destruction of, pathogenic microbes. In mammals, there are two forms of immunity: Innate and adaptive (Akira et al., 2006, Litman et al., 2010). Most research has focussed on adaptive immunity, where clonal propagation of somatically rearranged antigen binding receptors has the capacity to create an unlimited supply of pathogen perception systems (Litman et al., 2010). Adaptive immunity is specific and usually combats infection at a late stage (Akira et al., 2006). In contrast, innate immunity is mediated by the recognition of more general pathogen components by germline encoded immune receptors (Kawai and Akira, 2009). These receptors are known as pattern recognition receptors (PRRs) and the products they serve to recognise are known as pathogen associated molecular patterns (PAMPS) or microbe associated molecular patterns (MAMPS) (Akira et al., 2006, Boller and Felix, 2009). In mammalian immunity, there are three types of PRR: retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs), and Toll-like receptors (TLRs) (Kawai and Akira, 2009). TLRs are membrane bound receptors operating on the cell surface while RLRs and NLRs survey the intracellular compartments (Akira et al., 2006). Mammals tend to harbour a finite number of PRRs that are expressed constitutively, thus proffering the first line of defence against an invading pathogen. Interestingly, the general mechanisms underlying innate immunity are conserved across species ranging from humans and invertebrates through to plants

(Ausubel, 2005; and Akira et al. 2006). However, in plants, innate immunity is essential as they lack the adaptive immune system seen in mammals (Jones and Dangl, 2006).

1.2. The plant immune system.

1.2.1. Historical perspectives.

At the beginning of the 20th century, Sir Rowland Biffen discovered that disease resistance in wheat was inherited according to Mendelian laws of inheritance (Biffen, 1905). In his landmark publication, "Mendel's laws of inheritance and wheat breeding", Biffen crossed a stripe rust susceptible cultivar with a stripe rust resistant cultivar and found the F_1 progeny to be susceptible. Upon examining the F_2 progeny, the segregation ratio fit a 1:3 pattern of inheritance for resistant to susceptible plants, indicating that a recessive gene conditioned resistance. This was the first time Mendelian laws of inheritance were applied to a disease resistance trait and soon after similar results were reported in other crop species (Bushnell and Roelfs, 1984). The heritability of discrete traits separate from other plant traits was pivotal to our understanding of plant immunity and laid the foundation for many future discoveries.

Midway through the 20th century, Harold Flor made a seminal contribution to our understanding of the plant immune system through his pioneering works examining the interaction of flax (*Linum usitatissimum)* with flax rust (*Melampsora lini)*. From his observations, he concluded that the coevolution of plant and parasite had led to complimentary genic systems in the microbe and host plant. The gene-for-gene concept, as it was coined, surmised that for each gene conditioning resistance in the plant there was a specific gene conditioning pathogenicity in the parasite (Flor, 1956a). Flor's work explained very well how plants conditioned resistance against pathogens and soon after the concept was applied to other pathosystems. Moseman et al. (1965) published evidence that specificity between races of *Blumeria graminis* f. sp. *hordei* and barley followed the gene-for-gene model and Powers and Sando (1960) made similar observations while studying the interaction of *B. graminis* f. sp. *tritici* with wheat. Direct evidence supporting the gene-for-gene model emerged approximately 20 years later, when the first pathogen avirulence gene, *avrA*, was cloned from *Pseudomonas syringae* pv. *glycinea* (Staskawicz et al., 1984). Shortly after, the first plant disease resistance genes were cloned: maize *Hm1* (Johal and Briggs, 1992), tomato *Pto* (Martin et al., 1993), tobacco *N* gene (Whitham et al., 1994), tomato *Cf-9* (Jones et al., 1994), *Arabidopsis thaliana RPS2* (Bent et al., 1994, Mindrinos et al., 1994), *A. thaliana RPM1* (Grant et al., 1995), rice *Xa21* (Song et al., 1995), and tomato *Prf* (Salmeron et al., 1996).

1.2.2. PAMP triggered immunity (PTI).

Research over the next twenty years established that plant innate immunity is formed of two layers and can be viewed in the context of a zigzag model proposed by Jones and Dangl (2006). Each layer has its own distinctive properties but they are unified by the same overarching requirements: perception of pathogenic molecules and transduction of signal cascades. The first layer, PRR-triggered immunity (PTI), has many parallels with TLR mediated immunity in mammals, as it depends upon the recognition of conserved PAMPs by membrane bound PRRs (Boller and Felix, 2009). A well-characterised example is FLAGELLIN SENSING 2 (FLS2), a plant PRR that is activated upon detection of bacterial flagellin, analogous to TLR5 perception of bacterial flagellin in mammals (Zipfel et al., 2004). FLS2 directly binds to a highly conserved portion of the N terminus of the protein while TLR5 recognises a different epitope (Hayashi et al., 2001, Chinchilla et al., 2006). Upon perception, a series of downstream responses are activated such as the production of reactive oxygen species (Kadota et al., 2014), callose deposition, ion fluxes and transcriptional reprogramming via activation of mitogen activated protein kinases (MAPKs) (Nicaise et al., 2009). Ultimately, these responses are able to halt further pathogen colonisation and lead to a general immune response (Segonzac and Zipfel, 2011). However, pathogenic microbes can overcome PTI by actively suppressing PTI machinery. The pathogen achieves this by secreting an arsenal of effectors, into the host apoplast, or directly into the cell, which then modulate plant innate immunity and interfere with host targets (Stergiopoulos and de Wit, 2009). Effectors are typically characterised as being secreted, cysteine-rich proteins and often harbour conserved motifs of amino acids such as the RXLR and CRN motifs observed in oomycete effectors (Morgan and Kamoun, 2007, Stam et al., 2013). A well-characterised effector found in the *A. thaliana* bacterial pathogen *P. syringae* pv. tomato DC3000 (*Pto* DC3000), is AvrPtoB. AvrPtoB has intrinsic E3 ligase activity (Abramovitch et al., 2003) and is able to ubiquitinate FLS2 for degradation via the plant's own ubiquitin-proteasome pathway (Göehre et al., 2008). This reduces FLS2 protein concentration at the plasma membrane and dampens the resistance response mounted by the plant, thus promoting pathogen virulence. The use of effectors to suppress PTI and promote virulence is a shared mechanism among bacterial, fungal, and oomycete pathogens (Dodds and Rathjen, 2010). The collective term used to describe the process of suppressing plant immunity via effectors was coined effector triggered susceptibility (ETS) (Jones and Dangl, 2006).

1.2.3. Effector triggered immunity (ETI).

In order to combat ETS, plants have evolved a second layer of defence. The second layer, known as effector triggered immunity (ETI), involves the recognition of secreted pathogenic proteins by plant intracellular immune receptors, analogous to NLRs in mammalian innate immunity (Jacob et al., 2013). Plant NLRs are modular proteins containing nucleotide-binding site (NBS) and leucine rich repeat (LRR) domains (Dodds and Rathjen, 2010). In the plant genomes studied to date, NLRs are estimated to represent between 0.6-1.8% of all protein coding genes making them one of the most highly represented and diverse gene families (Meyers et al., 2003, Yang et al., 2006b, Yang et al., 2008, Guo et al., 2011, Jupe et al., 2012, Gu et al., 2015). However, the number of immune receptors encoded for by a plant genome does not explain the broad resistance observed in plants to the plethora of pathogens to which they are exposed (Niks, 1988). When implicated in resistance to a pathogen, NLRs are known as resistance (*R*) genes, or loci, and they act by recognising pathogen effectors, either directly or indirectly (Jones and Dangl, 2006). When a pathogen effector is recognised by an *R* gene the locus encoding the effector protein in the pathogen is termed an *Avirulence* (*Avr*) gene or locus. Disease resistance occurs when the R protein and Avr protein are present in the host and pathogen, respectively. Whereas, the absence, or inactivity, of either protein results in disease (Dangl and Jones, 2001). A hallmark of ETI is the hypersensitive response: a rapid localised cell death thought to occur due to build up of reactive oxygen species (ROS) that are toxic to plant cells (Coll et al., 2011). However, recognition of pathogen Avr proteins by plant immune receptors does not always manifest in a hypersensitive response (Goulden and Baulcombe, 1993, Jakobek and Lindgren, 1993, Rohe et al., 1995, Ori et al., 1997, Bendahmane et al., 1999, Bulgarelli et al., 2010). The hypersensitive response is analogous to programmed cell death (PCD) in mammalian immunity that is regulated by caspase proteases (Franchi et al., 2009). Disruption of distant caspase homologues in *Arabidopsis thaliana* (metacaspase 1 and 2; *AtMC1* and *AtMC2*) almost completely abolishes the hypersensitive response but does not lead to proliferation of pathogen growth (Coll et al., 2010). This uncoupling of the hypersensitive response, from disease resistance, suggests that it is not directly responsible for restricting pathogen growth. Alternatively, the hypersensitive response may be a consequence of elevated signalling events that occur after pathogen perception (Coll et al., 2011) or may play a role in defence response signalling in unchallenged cells (Torres et al., 2005). Understanding more about the hypersensitive response and defining exactly how pathogen growth is restricted during ETI, will address major conceptual hurdles that currently exist in the study of plant immunity.

1.2.4. Models for effector recognition.

The intimate interaction between a host plant and pathogen, whereby the products of *R* genes confer recognition of Avr proteins, was first proposed in Harold Flor's gene-for-gene hypothesis (Flor, 1956a). This work led others to speculate that the recognition mechanism would be initiated via direct binding of the R protein to the Avr protein in a ligand-receptor model (Keen, 1990). Indeed, a number of examples of direct recognition have been identified to support this notion. Dodds et al. (2006) were able to demonstrate direct recognition of AvrL567-A, AvrL567-B, and AvrL567-C proteins by the resistance proteins L5, L6 and L7, derived from the *L* locus in *Linum usitatissimum*. Interestingly, a high level of amino acid variation was observed at the *AvrL567* and *L* locus, indicative of diversifying selection. This observation is consistent with other loci known to be involved in direct interactions (Jia et al., 2000, Deslandes et al., 2003, Ueda et al., 2006). Therefore, direct recognition, via a receptor-ligand model, appears to promote a constant evolutionary arms race between the plant and pathogen where the need to escape host recognition drives evolution at the *Avr* locus. The direct recognition model for effector recognition is mechanistically intuitive, but may over simplify potentially complex interactions between plant host and pathogen. The small number of direct interactions that have been reported to date highlights this simplification. Several modifications have been proposed to the direct recognition model to try and describe the additional complexity that occurs during plant pathogen interactions.

- The guard model (Dangl and Jones, 2001) asserts that R proteins guard effector targets in the host and trigger a resistance response upon detection of perturbations to the host protein induced by the effector. *Pto* encodes a serine threonine protein kinase that was first cloned as a resistance gene in tomato effective against *P. syringae* pv. *tomato* (Martin et al., 1993). Pto interacts directly with the pathogen avirulence protein AvrPto but the resistance function of Pto was shown to be dependent upon an NLR, Prf (Ronald et al., 1992, Salmeron et al., 1996, Tang et al., 1996). The guard model was first proposed to reconcile the interactions observed between Pto, Prf, and AvrPto.
- The decoy model (van der Hoorn and Kamoun, 2008) is a slight modification to the guard hypothesis that accounts for conflicting evolutionary pressures that would arise if the original proposal were true. Here, a decoy protein evolves to mimic an effector target (guardee) thereby competing with the real effector target for binding with the pathogen effector. The decoy has no other role in plant signalling and evolves specifically for effector recognition. Therefore, selection pressures acting on the decoy are independent of other functions and relax some of the

evolutionary constraints of the guard model. Pto was suggested to be a decoy protein rather than a functional host protein. However, determining a decoy from a true effector target has proven difficult as some potential decoys, such as Pto, have active biological properties.

- The bait and switch model (Collier and Moffett, 2009) focuses on the mechanistic properties of effector recognition. It proposes that a decoy molecule is a 'bait' molecule that interacts with an NLR protein that is in an auto-inhibited state. In this state, the switch is primed but remains in an off position. During a second interaction the 'bait' interacts with the Avr protein from the pathogen resulting in a conformational change in the Avr protein. The NLR then interacts directly with the Avr protein triggering conformational changes in the R protein itself. Perturbation of the R protein activates the molecular switch and signalling for immune responses.
- The integrated decoy model (Cesari et al., 2014a) proposes that effector targets in the host plant have evolved to be directly guarded by NLRs via integration of the effector target into the NLR modular domain structure. Such a model is supported by resistance specificities that involve the concerted action of two NLRs: one functioning in recognition and the other in downstream signal transduction. In *A. thaliana*, RRS1 works in tandem with RPS4 to mediate race-specific resistance to two bacterial pathogens and one fungal pathogen (Narusaka et al., 2009). RRS1 harbours a WRKY transcription factor at its C terminal that is proposed to act as an integrated decoy for effectors targeting such transcription factors (Le Roux et al., 2015, Sarris et al., 2015). In rice (*Oryza sativa*), NLRs RGA4 and RGA5, have been shown to mediate resistance to two distinct rice blast (*Magnaporthe oryzae)* isolates. Direct recognition of the effectors AVR-Pia and AVR1-CO39 is mediated by effector binding at a RATX1 domain at the C terminal of the RGA5 protein (Okuyama et al., 2011, Cesari et al., 2014b). This happens independently of RGA4, which is thought to be involved in transduction of downstream signalling cascades after initial perception by RGA5. A similar RATX1 domain was shown to be the effector binding site of another NLR, Pik-1, also operating as part of an NLR pair against *M. oryzae* (Zhai et al., 2014).

While models play an important role in scientific conceptualisation it is important to remember that they often have limitations and do not always fully describe the wide range of observations that can be made in any particular biological system. It is likely that the models presented above explain the majority of recognition events currently seen in plant immunity but novel mechanisms and or combinations of existing models will likely be unearthed. For example, *rpg4* mediated recessive resistance to *P. graminis* f. sp. *tritici* in

barley is linked to the concerted action of an *Actin depolymerisation factor2* (*Adf2*), *Rpg5* (an NLR), and a second NLR encoding gene, *RGA1,* at the *rpg4* locus (Brueggeman et al., 2008, Brueggeman et al., 2009, Wang et al., 2013). Exactly how these factors interact to confer resistance remains unclear.

1.2.5. NLR structure and function.

To date, R proteins conferring ETI often confer qualitative resistance and are frequently inherited as dominant or semi-dominant traits (Hammond-Kosack and Jones, 1997). This has facilitated the discovery of the genes underlying R proteins via map-based cloning (Krattinger et al., 2009a). Based on these efforts, structure/function analyses of cloned R proteins have revealed that the majority encode NLR proteins (Ayliffe and Lagudah, 2004, Dodds and Rathjen, 2010). NLR proteins are modular and contain domains required for recognition and signal transduction (Jacob et al., 2013). The NBS domain is believed to play a role in signal transduction and is characterised by a conserved element containing three ATP/GTP binding motifs, known as the P loop (Qi and Innes, 2013). A linker sequence connects the NBS to the LRR domain. The LRR domain has widely been implicated in recognition specificity between an R protein and its cognate effector (Jia et al., 2000, Deslandes et al., 2003, Ueda et al., 2006). As a consequence the LRR domains of resistance genes often exhibit signatures of diversifying selection. This is thought to promote variation in recognition specificities to different pathogens but may be more evident in instances of direct recognition rather than indirect recognition (Jones and Dangl, 2006, van der Hoorn and Kamoun, 2008). NLRs often contain other modular domains particularly at the N termini (Jacob et al., 2013, Cesari et al., 2014a). In most plant genomes surveyed to date, NLRs harbour an N terminal domain with homology to the *Drosophila* Toll protein and mammalian interleukin-1-receptor (TIR; TNL) or those with the alternative coiled-coil (CC; CNL) domain, although alternative domains exist (Jacob et al., 2013). Interestingly, only CNLs have been causally linked to plant immunity in monocots, including the Triticeae tribe (Table 1) (Ayliffe and Lagudah, 2004, Krattinger et al., 2009a).

Table 1: An overview of cloned disease resistance genes to fungal pathogens in the Triticeae tribe.

Name	Host	Protein	Extra/ novel domains	Chr.	Introgression Source	Resistance type	Pathogen	Disease	References
Lr1	Wheat	CNL	-	5DL	n/a	Race specific	*P. triticina*	Leaf rust	Cloutier et al. (2007)
Lr10	Wheat	CNL	-	1AS	n/a	Race specific	*P. triticina*	Leaf rust	Feuillet et al. (2003)
Lr21	Wheat	NLR	unknown 151 aa domain at N terminus	1DS	*A. tauschii*	Race specific	*P. triticina*	Leaf rust	Huang et al. (2003)
Lr34	Wheat	ABC transporter	-	7DS	n/a	Broad spectrum	Multiple	Multiple	Krattinger et al. (2009)
Mla[1]	Barley	CNL	-	1HS	n/a	Race specific	*B. graminis* f.sp. *hordei*	Barley powdery mildew	Zhou et al. (2001), Halterman et al. (2001), Halterman et al. (2003), Shen et al. (2003), Seeholzer et al. (2010)
mlo	Barley	unknown plant specific protein	seven transmembrane domains	4HL	n/a	Broad spectrum	*Bl. graminis* f.sp. *hordei*	Barley powdery mildew	Büschges et al. (1997)
Pm3[2]	Wheat	CNL	-	1AS	n/a	Race specific	*B. graminis* f.sp. *tritici*	Wheat powdery mildew	Yahiaoui et al. (2004), Yahiaoui et al. (2006), Yahiaoui et al. (2009), Bhullar et al. (2006), Bhullar et al. (2009)
Pm8	Wheat	CNL	-	1BS	*S. cereale* (1RS)	Race specific	*B. graminis* f.sp. *tritici*	Wheat powdery mildew	Hurni et al. (2013)
Pm21	Wheat	Serine threonine protein kinase	-	6AL	*H. villosa* (6VS)	Broad spectrum	*B. graminis* f.sp. *tritici*	Wheat powdery mildew	Cao et al. (2011)
Rpg1	Barley	Protein kinase-like	Two tandem protein kinase domains	7HS	n/a	Race specific	*P. graminis* f.sp. *tritici*	Wheat stem rust	Brueggeman et al. (2002)
Rpg5	Barley	NLR	C terminal serine/ threonine kinase domain	5HL	n/a	Race specific	*P. graminis* f.sp. *secalis*	Rye stem rust	Brueggeman et al. (2008)
Sr33	Wheat	CNL	-	1DS	*A. tauschii*	Race specific	*P. graminis* f.sp. *tritici*	Wheat stem rust	Periyannan et al. (2013)
Sr35	Wheat	CNL	-	3AL	*T. monococcum* ($3A^{m}L$)	Race specific	*P.graminis* f.sp. *tritici*	Wheat stem rust	Saintenac et al. (2013)
Yr10	Wheat	CNL	-	1BS	n/a	Race specific	*P.striiformis* f. sp. *tritici*	Wheat yellow rust	Liu et al. (2014)
Yr36	Wheat	Kinase-START	-	6BS	*T.turgidum ssp. dicoccoides*	Broad spectrum	*P. striiformis* f.sp. *tritici*	Wheat yellow rust	Fu et al. (2009)
Rdg2a	Barley	CNL	-	7HS	*n/a*	Race specific	*Pyrenophora graminea*	Barley leaf stripe	Bulgarelli et al. (2010)

[1]17 functional *Mla* alleles cloned.

[2]17 functional *Pm3* alleles cloned.

1.3. The molecular genetics of host resistance.

Plant resistance to potentially pathogenic microbes can be categorised into two states depending on the compatibility of a given pathogen species on a particular plant host. The compatibility of an interaction can influence the frequency, type, and durability of resistance observed in a pathosystem (Heath, 2000). As a generalisation, host resistance involves high levels of compatibility between a pathogen and host. Resistance is usually race specific and underpinned by recognition of effectors by NLRs (Dangl and Jones, 2001, Jones and Dangl, 2006, Dodds and Rathjen, 2010). A large portion of our current understanding of plant immunity is derived from the study of host systems, often in diploid, model organisms with small genomes. The following section aims to review molecular genetics of host immunity to agriculturally relevant fungal pathogens in monocots, specifically the Triticeae tribe of the grasses. This tribe contains many agriculturally important crop species such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*) and rye (*Secale cereal*). Efforts to understand the molecular mechanisms of host resistance in these species have focussed on identifying the causal genes, underlying resistance loci, using map-based cloning (Krattinger et al., 2009a). The first step in this process is to identify phenotypic variation between individuals that can be crossed and used to generate populations that segregate for a trait of interest. Then, high-density genetic maps are constructed to map the trait to a chromosomal locus, followed by physical mapping of the sequence information in the region. Historically, this was impractical for organisms such as wheat and barley due to the large genome sizes, approximately 5 and 18 Gbp, respectively, and high repetitive sequence content (>80%) (Feuillet et al., 2003). However, the advent of molecular markers, creation of bacterial artificial chromosome (BAC) libraries, and the use of syntenic approaches, via diploid progenitor species, have enabled the cloning of a substantial number of Triticeae resistance genes effective against fungal pathogens (Table 1).

1.3.1. Barley powdery mildew (*B. graminis* f. sp. *hordei*).

The interaction between barley and barley powdery mildew is the most well characterised pathosystem within the Triticeae tribe. In this system, resistance is conditioned by *Mildew loci* (*Ml*) and at least 11 *Ml* loci have been described throughout the barley genome (Wei et al., 1999). *Mildew locus a* (*Mla),* which resides on chromosome 1H, is known to harbour over 30 different race specificities that confer immune reactions differing in both severity and speed (Zhou et al., 2001, Seeholzer et al., 2010). Because of these characteristics, significant efforts have been made to untangle some of the complexity observed at the locus. The locus spans a 260 kbp region and contains three NLR encoding gene families;

RGH1, *RGH2*, and *RGH3* (Wei et al., 1999). Within each family, the members are highly conserved (60 to 98% amino acid similarity) but considerably lower similarity exists between NLR families. It has been proposed, that the NLR families arose from three single progenitor sequences that underwent duplication and inversion events (Wei et al., 2002). Subsequently, a series of transposon insertions increased the region size before a 40 kbp tandem duplication, of a gene rich region, expanded the locus to include 34 predicted genes (Wei et al., 2002). To date, only gene members of the *RGH1* family, *CNL* type *R* genes, have been shown to confer resistance to powdery mildew (Halterman et al., 2003, Zhou et al., 2001, Wei et al., 2002, Shen et al., 2003) and there are now 17 molecularly characterised interactions at the *Mla* locus (Table 1). Some, but not all, of the *Mla* specificities depend upon *Rar1* (*Required for Mla12 mediated resistance 1*) for immune function (Torp and Jørgensen, 1986, Halterman et al., 2003). *Rar1* appears to act downstream of initial recognition events and plays a role in H_2O_2 accumulation: a prerequisite of the hypersensitive response (Shirasu et al., 1999).

While the *Mla* locus appears to condition classical race-specific resistance mediated by CNLs, another mildew locus on chromosome 4H appears to depend on a novel mechanism. The *mildew locus o* (*mlo*) is a recessive resistance gene, which confers resistance to all known barley powdery mildew isolates. It is considered durable due to the longevity of resistance observed despite widespread use in European agriculture (Jørgensen, 1992). The gene underlying *mlo* resistance was identified as a seven transmembrane domain protein (Büschges et al., 1997). In its wildtype form, Mlo is thought to inhibit vesicle mediated SNARE protein dependent resistance to adapted forms of powdery mildew (Collins et al., 2003).

Two *mlo* alleles are used within agriculture, an induced mutant, *mlo-9*, and a natural *mlo* allele, *mlo-11*, derived from an Ethiopian landrace, which protects approximately half of all European spring barley cultivars against barley powdery mildew (McGrann et al., 2014). *mlo-11* confers a partial resistance phenotype when compared to the complete resistance observed in null alleles identified via mutagenesis during the cloning of *Mlo* (Büschges et al., 1997). No naturally occurring complete resistance *mlo* alleles were identified and it was unclear why *mlo-11* conferred a partial resistance phenotype. Interestingly, after cloning *mlo-11,* no differences could be observed in the coding sequence versus the wild type *Mlo* (Piffanelli et al., 2004). Consequently, a complex tandem repeat array, inserted upstream of the *Mlo* coding sequence, was shown to inhibit *Mlo* transcript and protein accumulation in *mlo-11* containing lines. The upstream regulation of the gene leads to the formation of aberrant transcripts that in turn promotes a partial resistance phenotype when compared to the null alleles generated via induced mutation approaches. This is

thought to be an evolutionary consideration that offsets the negative yield effects observed in null alleles and explains why no naturally occurring null alleles have been identified.

1.3.2. Wheat powdery mildew (*B. graminis* f. sp. *tritici*).

The interaction between wheat and wheat powdery mildew is highly analogous to the *Ml* specificities observed in barley. There are 43 *Pm* (*Powdery mildew*) loci with over 70 described alleles (Hurni et al., 2013). The major *Pm* locus in wheat, *Pm3,* residing on chromosome 1AS, was resolved using physical tiling paths developed in the A genome diploid model *T. monococcum* (A^mA^m genome) and the tetraploid *T. durum* (AABB genome) (Yahiaoui et al., 2004). The first allele to be cloned at the locus was a CNL type resistance gene called *Pm3b* that confers race specific resistance to mildew isolates harbouring the *AvrPm3B* gene. There are now 17 molecularly characterised CNL *Pm3* alleles that share more than 97% nucleotide similarity, congruent with the similarity seen between members of the *RGH1* family at the *Mla* locus in barley (Table 1, refs therein). Indeed, *Pm3* resides in the *Mla* syntenic region in wheat and was once proposed to be orthologous to *Mla* (Hartl et al., 1993). However, cloning of *Mla1* permitted high resolution mapping in wheat and it was found to localise 0.7 cM proximal to the *Pm3* locus (Zhou et al., 2001). This observation, coupled with low homology between cloned *Mla* and *Pm3* alleles, did not support the hypothesis that they were orthologous loci. However, another mildew locus, *Pm8*, that was introgressed into wheat from rye was recently cloned and shown to be orthologous to *Pm3* (Hurni et al., 2013). The gene is located on rye chromosome 1RS that shows remarkable yield benefits and a plethora of disease resistance specificities when translocated into wheat (Kim et al., 2004). This is consistent with other alien translocations in wheat that show remarkable disease resistance profiles (Wulff and Moscou, 2014). For example, the introgression of the short arm of chromosome 6V from *Haynaldia villosa* conferred broad-spectrum disease resistance against wheat powdery mildew (Cao et al., 2011). Remarkably, this resistance has endured for other 30 years despite significant disease pressure in the field. Unfortunately, the T6VS-6AL introgression line has a low frequency of pairing and suppressed recombination between the *H. villosa* and wheat chromosome. Therefore, a candidate gene for *Pm21* was identified using a microarray expression analysis on *H. villosa* RNA extracted from pathogen challenged and unchallenged samples. A serine threonine protein kinase (STPK) was up-regulated in response to challenge with the pathogen and was later shown to co-localise with the *Pm21* locus. Transcomplementation later confirmed that this was the gene conferring *Pm21* resistance (Cao et al., 2011). Interestingly, expression of the gene was found to be up-regulated in response to an initial H_2O_2 burst after the plant was challenged by the

pathogen. This suggests that *Pm21* acts upstream of a second H_2O_2 burst that ultimately leads to the hypersensitive response.

1.3.3. Wheat stem rust (*P. graminis* f. sp. *tritici*).

Wheat genes effective against wheat stem rust are known as *Sr* (*Stem rust*) genes. *Sr31* that resides in the rye 1BL.1RS translocation in wheat provided resistance to stem rust for over 40 years after its extensive use in public breeding programmes (Singh et al., 2011). Efforts to use a map-based cloning strategy for *Sr31* are nearing completion and it is now thought that the gene underlying this resistance is an *Mla* homolog (Hurni et al., 2013). *Sr31* was defeated in 1998 after a new race of stem rust (Ug99) emerged in Uganda that harboured virulence on *Sr31* containing wheat differentials (Pretorius et al., 2000). Alarmingly, estimates suggested that up to 90% of the worlds wheat supply was potentially at threat from the fungus (Singh, 2006). This led to heightened awareness of the threat posed by stem rust and renewed efforts to molecularly characterise *Sr* genes functional against Ug99. Two genes, *Sr33* and *Sr35*, were recently cloned from wild wheat relatives after showing functionality in wheat (Periyannan et al., 2013, Saintenac et al., 2013). *Sr33* was cloned from the D genome progenitor of hexaploid bread wheat, *Aegilops tauschii*, and was found to code for a CNL that was syntenic and orthologous to an *Mla RGH1* family member (Periyannan et al., 2013). This provided evidence of NLR divergence leading to recognition of taxonomically distinct pathogen species. The second gene, *Sr35*, was cloned from *T. monococcum*, a close relative of the A genome progenitor in wheat, *T. urartu*. Interestingly, *Sr35* is not present in *T. urartu*, which explains its absence from domesticated wheat.

1.3.4. Wheat and rye stem rust (*P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis*).

A stem rust isolate specifically adapted to the *Hordeum* genus is not known to exist but barley is susceptible to both wheat and rye stem rust. Wheat stem rust was a particular problem in the northern great plains of the U.S. until the deployment of a resistance gene, *Rpg1* (*Resistance to Puccinia graminis 1*), in 1942 (Brueggeman et al., 2002). *Rpg1* is considered to be durable and conferred resistance to a wide range of stem rust races for over 60 years (Nirmala et al., 2006). *Rpg1* was cloned after high-resolution mapping, comparative allele mining, and complementation in susceptible barley accessions (Brueggeman et al., 2002, Horvath et al., 2003). The gene encodes a protein with two kinase domains. Many proteins harbouring kinase domains have been implicated in plant immunity but the tandem arrangement of two kinase domains remains a novel protein structure. Recently, a dual kinase domain protein was proposed as a candidate gene for the *Un8* gene in barley providing resistance to *Ustilago nuda* (Zang et al., 2015). Additional

work is needed to validate this candidate and determine its functionality. However, in *Rpg1*, the second kinase domain is a functional serine threonine kinase but both domains are required to confer immunity in transgenic barley (Nirmala et al., 2006). While parallels have been sought with immune signalling mediated by the tomato receptor kinase *Pto* and to cytokine-receptor signalling in mammals, more information is needed to understand the mechanism by which *Rpg1* confers immunity (Brueggeman et al., 2002). Intriguingly, two additional stem rust resistance genes have been identified in barley that appear to have novel functional activities. The recessive resistance gene *rpg4* has been mapped to the long arm of chromosome 5H but the complexity of the locus has precluded the elucidation of the exact genes involved (Brueggeman et al., 2008). However, as described earlier, an *Actin depolymerisation factor* (*Adf2*), an NLR gene, and the cloned rye stem rust resistance gene *Rpg5*, are thought to underpin *rpg4*-mediated immunity (Brueggeman et al., 2008). While the contribution of *Rpg5* to *rpg4*-mediated immunity remains unclear, *Rpg5* confers resistance to rye stem rust in a more classical race specific manner and was shown to code for an NLR conjugated to a serine threonine protein kinase domain (Brueggeman et al., 2008). Although STPK domains are often found in plant immunity, the combination of STPK domain integrated into an NLR, in a single functional unit, is unique. A gene lacking the STPK domain was shown to be present in the susceptible cultivar Harrington providing evidence that the domain is required for function (Brueggeman et al., 2008). It is hypothesised, that *Rpg5* is an example of an integrated decoy but additional evidence will be required to prove this hypothesis (Cesari et al., 2014a).

1.3.5. Wheat leaf rust (*P. triticina*).

Three resistance genes conferring race specific resistance to wheat leaf rust (*Lr* genes) have been cloned in wheat (Table 1). *Lr1* is exceptional in that it was cloned directly from wheat without using a subgenomic insert library. This was due to the location of the gene at the telomeric end of the chromosome in a region of high recombination (Cloutier et al., 2007). Consistent with the majority of Triticeae *R* genes *Lr1* codes for a CNL. Similarly, *Lr10* and *Lr21* also share this common feature but the genomic location of both genes required more tactical approaches for molecular cloning (Feuillet et al., 2003, Huang et al., 2003). Both were mapped using hexaploid bread wheat but the *Lr10* and *Lr21* loci were physically mapped using *T. monococcum*/*T. durum* and *A. tauschii* subgenomic libraries, respectively.

Most of the CNL type resistance genes that have been cloned confer seedling stage resistance to wheat leaf rust via recognition of putative Avr proteins from the pathogen. However, the wheat leaf rust pathosystem has provided significant insight into adult plant

resistance through the cloning of *Lr34* (Krattinger et al., 2009b). The *Lr34* locus confers multiple pathogen resistance to leaf rust (*Lr34*), powdery mildew (*Pm38)*, and stripe rust (*Yr18)* via the action of a single adenosine triphosphate-binding cassette (ABC) transporter of the pleiotropic drug resistance family. Another ABC transporter, *PEN3/PDR8*, has been implicated in plant immunity to nonhost pathogens in *A. thaliana* (Stein et al., 2006). It is proposed that PEN3 plays a role in movement of toxic compounds to sites of pathogen penetration. A similar mode of action has been proposed for *Lr34* given its phylogenetic relationship to *PEN3* (Krattinger et al., 2009b).

1.3.6. Wheat stripe rust (*P. striiformis* f. sp. *tritici*).

The wheat-stripe rust pathosystem is relatively understudied considering the ubiquitous presence of stripe rust around the world and its impact on wheat production. Only recently, the first race-specific resistance gene, *Yr10*, was cloned and shown to code for a CNL (Liu et al., 2014). *Yr10* was identified using wheat DNA extractions that had been purified for low complexity sequence using a C_0t DNA preparation. Using these preps a RAPD (**R**andom **A**mplified **P**olymorphic **D**NA) marker was identified on resistant and susceptible bulks that perfectly co-segregated with the *Yr10* gene. Remarkably, it was later found that the probe was directly linked to the causal gene. Despite a novel cloning methodology for wheat resistance genes, the cloning of *Yr10* did not provide any new mechanistic insight into Triticeae plant immunity. Contrastingly, *Yr36* cloned by Fu and co-workers provided insight into broad spectrum, partial, adult plant resistance (APR) derived from the wild wheat, *T. turgidum ssp. dicoccoides* (Fu et al., 2009). *Yr36* was found to encode a kinase-START (StAR related lipid transfer) protein. StAR domains are known to be involved in lipid transfer, metabolism and sensing and upon binding with specific compounds undergo conformational changes in mammals (Alpy and Tomasetto, 2005). It was proposed that the active kinase domain might initiate downstream signalling after conformational change in the START domain of *Yr36* (Fu et al., 2009). Whether the START domain functions to recognise plant or pathogen derived cues remains to be elucidated.

1.4. The molecular genetics of nonhost resistance.

It has long been recognised that plants are not infected by the majority of microbes they encounter. As a consequence, pathogens often have restricted host ranges and can be highly specialised to given host genera or species. Therefore, all other plant species are considered nonhosts, by definition (Thordal-Christensen, 2003). The molecular mechanisms governing nonhost status are poorly understood relative to host resistance mechanisms. This is due, in part, to a lack of clear discernable phenotypes and a general inability to make wide crosses that make nonhost systems recalcitrant for classic genetic

analyses (Niks, 1987, Niks, 1988). However, nonhost resistance remains a key research area due to the perceived durability and broad-spectrum nature of this kind of resistance (Gill et al., 2015). The field holds significant potential for improvements in agricultural crop protection should the molecular components be engineered into economically important crop species (Jones et al., 2014).

Prior to the cloning of the first *R* and *Avr* genes in host pathosystems, Heath reported the 'Generalized Concept of Host-Parasite Specificity' that speculated on the molecular mechanisms that may determine specificity between plant hosts and pathogens (Heath, 1981). Similar to Day (1976) and Ellingboe (1976) before her, Heath conceptually distinguished host species specificity into two distinct layers. The first layer determined specificity at the species level and was coined 'plant species specificity'. At this level, compatibility was specific and resulted from the interaction between host plant and parasite over a given time to establish 'basic compatibility'. The failure to establish basic compatibility would lead to nonhost status. Concurrently, the second layer, 'cultivar specificity', acted in a gene-for-gene manner and was superimposed on basic compatibility. Despite a very simplistic composition, the model subscribed to the key observation that most plants are able to resist the majority of potentially pathogenic microorganisms. Thus, the 'Generalized Concept of Host-Parasite Specificity' could address the mechanism of nonhost resistance by using basic compatibility theory. Adaptations to this model were made in light of evidence that suggested that gene-for-gene interactions were governing nonhost resistance mechanisms at a much higher taxonomic level than suggested by Heath's model (Tosa, 1992). Furthermore, significant advances were made in understanding the molecular genetics of nonhost resistance and these discoveries significantly shaped future models. Nonhost resistance is now thought to involve a number of passive and active mechanisms that hinder pathogen ingress (Heath, 2000, Thordal-Christensen, 2003, Mysore and Ryu, 2004, Gill et al., 2015). Failure, by the pathogen, to overcome any of these obstacles results in a nonhost interaction and the pathogen is considered to be non-adapted.

1.4.1. Establishing basic compatibility: Cue recognition and spore differentiation into infection structures.

The intimate interaction between a pathogen and host plant involves reciprocal perception of signals that will determine resistance or susceptibility. The first challenge for the pathogen, after a spore makes contact with the leaf surface, is to detect physical or chemical cues from the plant that lead to spore differentiation and development of infection structures (Thordal-Christensen, 2003). Some pathogens (e.g. *Puccinia* spp.) use

infection structures to gain access to the host plant via stomata and it is suggested that leaf topography plays a role in this process (Hoch et al., 1987, Allen et al., 1991). Other pathogens (e.g. *Blumeria* spp.) directly penetrate the mesophyll leaf surface and detection of epicuticular leaf waxes have been shown to be an important cue (Tsuba et al., 2002). Tsuba et al. (2002) demonstrated that waxes from the host plant, barley, induced greater barley powdery mildew spore differentiation than waxes from nonhost plants. Similarly, the absence of epicuticular wax on the abaxial leaf surface of *Medicago truncatula* plants was shown to greatly reduce spore differentiation of direct penetrating host and nonhost pathogens (Uppalapati et al., 2012). A failure to differentiate appropriate infection structures means that basic compatibility cannot be established. In the absence of basic compatibility, resistance ensues leading to a nonhost interaction.

1.4.2. Overcoming pre-formed chemical and physical impediments to entry.

The ability to establish basic compatibility is linked to the coevolution of the plant and pathogen so that the pathogen can adapt to overcome barriers to pathogenicity. Preformed barriers that exist in the plant can impede pathogen ingress and therefore determine basic compatibility. Impediments can be chemical, enzymatic or structural (Thordal-Christensen, 2003). The most compelling evidence of a preformed defence barrier, that determines nonhost resistance, comes from oat where the production of oat specific chemical compounds, avenacins, mediates resistance to the non-adapted pathogen *Gaeumannomyces graminis* var. *tritici*. *G. graminis* var. *tritici* is the causal agent of take-all disease on wheat and barley. Oat is considered a nonhost but is susceptible to an oat attacking variant *G. graminis* var. *avenae*. Indirect evidence for the role of avenacins came from the observation that *G. graminis* var. *avenae* requires avenacinase, a saponin detoxifying enzyme, for virulence on oat (Bowyer et al., 1995). However, a mutant screen on the diploid oat species *A. strigosa* provided genetic evidence for the role of avenacins in nonhost resistance (Papadopoulou et al., 1999). Several saponin deficient mutants were identified with varying degrees of susceptibility to the non-adapted pathogen. Interestingly, the decreased level of avenacin production in the mutants correlated with increased susceptibility, suggesting that avenacin mediated resistance is dosage dependent.

1.4.3. Suppression of basal immune responses mounted by the plant.

If a pathogen has all the necessary components for establishing basic compatibility it must then overcome active or induced defence responses launched by the plant after perception of the pathogen. Perception by this inducible system is mediated via recognition of highly conserved pathogen elements or by perception of danger signals emanating from

pathogen entry sites by PRRs (PTI; discussed in '1.2.2. PAMP triggered immunity') (Thordal-Christensen, 2003). Many downstream responses induced by PTI will be targeted to areas in the immediate vicinity of the pathogen, unlike constitutive preformed barriers that will be generally less localised. To prevent pathogen ingress via the leaf epidermis plants mount a resistance response known as pre-penetration resistance. Three genes in *A. thaliana*, *PEN1*, *PEN2,* and *PEN3* have been shown to condition pre-penetration resistance to the non-adapted pathogens *B. graminis* f. sp. *hordei* and *Phakopsora pachyrhizi* (Asian Soybean Rust) (Collins et al., 2003, Lipka et al., 2005, Stein et al., 2006, Hoefle et al., 2009). *PEN1* encodes a membrane-associated syntaxin and is thought to function in vesicle trafficking to the plasma membrane in response to danger signals emanating from pathogen attempted entry sites. *Required for mlo-specific resistance 2* (*Ror2*) in barley is a functional homologue of *PEN1* and is thought to play a similar role in membrane trafficking. Contrastingly, *PEN2* and *PEN3* are efflux-associated proteins coding for a glycosal hydrolase (Lipka et al., 2005) and an ABC transporter (Stein et al., 2006), respectively. Perturbation of the *PEN* genes in *A. thaliana* compromises pre-penetration resistance and leads to increased fungal entry into epidermal cells (Kwon et al., 2008). *B. graminis* f. sp. *tritici* was not able to mount a full infection on *PEN* compromised plants even on the *pen1/pen2* double mutant (Lipka et al., 2005). This was thought to be due to the action a separate layer of immunity involving *R* gene mediated resistance and the hypersensitive response. However, large microcolonies were visible on a *pen2*/*pad4*/*sag101* triple mutant indicating that this mutant was immune compromised against *B. graminis* f. sp. *hordei* (Lipka et al., 2005).

1.4.4. Avoiding recognition by intracellular NLR immune receptors.

Plants have evolved an intracellular surveillance system underpinned by modular NLR proteins. These proteins act by, directly or indirectly, recognising effectors secreted into the host by a pathogen. The role of R/Avr interactions in determining nonhost resistance is poorly understood. It has been hypothesised that resistance may be mediated by multiple independent recognition events that can condition broad spectrum, durable resistance across a host plant species (Thordal-Christensen, 2003). This view is supported by the observation of hypersensitive-like responses in *A. thaliana* plants compromised in pre-penetration resistance when challenged with a nonhost pathogen (Lipka et al., 2005). However, caution must be observed when trying to deduce molecular mechanisms based on phenotypes alone as different mechanisms can have similar phenotypic outputs. However, direct evidence exists for the role of a TNL in determining nonhost status to *Albugo candida* f. sp. *capsella* in *A. thaliana* (Borhan et al., 2008). *WHITE RUST RESISTANCE 4* (*WRR4*) was identified after subtle phenotypic differences were observed

under laboratory conditions that permitted map based cloning of the gene. Several loss of function *wrr4* alleles were shown to support the growth of the non-adapted pathogen, thus validating its role in nonhost resistance.

1.5. The evolutionary context of nonhost resistance.

Towards the end of the 19th century, Jakob Eriksson observed that rusts growing on cereal crops were pathogenically specialised (Eriksson, 1898). As a generalisation, each rust was exclusively confined to a particular host genera e.g. stems of oats could propagate stem rust of oat but not stem rust of rye, wheat or barley. Eriksson examined 37 different grass species and found 30 rust isolates, which he could group into seven forms according to their specialisation on different host genera. However, despite this specificity, the rusts were morphologically indistinguishable from one another. At that time, taxonomic classification of different species was based on morphological differentiation. As such, the specialised rust forms did not satisfy criteria for taxonomic classification as different rust species despite being pathogenically differentiable. This prompted Eriksson to propose a new taxonomic separation based on host species specificity: the *formae speciales* (Bushnell and Roelfs, 1984). The *formae speciales* have since become important evolutionary relevant model systems for understanding more about host species specificity, particularly, the role of gene-for-gene interactions in determining host and nonhost resistance.

1.5.1. The role of gene-for-gene interactions in determining nonhost resistance.

Gene-for-gene interactions have long been implicated in host resistance to adapted pathogens but their role in nonhost resistance has been the subject of debate amongst plant scientists. According to Heath's 'Generalised Concept of Host-Parasite Specificity' gene-for-gene interactions governed specific interactions at the race-cultivar taxonomic level only (Heath, 1981). However, opportunities to test this hypothesis were limited due to a lack of model pathosystems for dissecting nonhost resistance at an appropriate taxonomic threshold. An opportunity arose when (Tosa et al., 1987) developed a method for testing the species specificity concept using *formae speciales* of *B. graminis*. *B. graminis* f. sp. *hordei, tritici, agropyri, secale,* and *avenae* are appropriate pathogens to the genera *Hordeum, Triticum, Agropyron, Secalis, and Avena,* respectively, and are restricted to their given host genera with only a few exceptions (Tosa et al., 1987). Resistance to non-adapted *B. graminis formae speciales* had long been recognised but questions remained over the specificity and type of resistance mechanism governing these interactions. Firstly, according to Heath's model, did the *formae speciales*-host genus specificity belong to plant species specificity or cultivar specificity? And secondly, was the resistance to

inappropriate *formae speciales* nonhost resistance or cultivar resistance? To address these questions Tosa and Shishiyama (1985) began to make detailed cytological observations of the reaction of wheat to the non-adapted pathogen *B. graminis* f. sp. *hordei*. He concluded that the defence responses in wheat were highly similar to the responses of the host plant barley, including the hypersensitive reaction of mesophyll cells associated with race-cultivar specific resistance. Inspired by this observation Tosa began to investigate in more detail whether gene-for-gene interactions played a role in *formae speciales*-genus resistance to *B. graminis*. Tosa utilised *B. graminis* f. sp. *tritici* (Tk-1) and *B. graminis* f. sp. *agropyri* (Ak-1) for the basis of his research. Key to this work was the identification of a common host of both Ak-1 and Tk-1. This allowed him to make a cross between two isolates and propagate the segregating F_1 progeny without biased selection of virulent spores on either of the natural hosts (Tosa et al., 1987). Using classical genetics, four gene-for-gene interactions governing the specificity of wheat to Ak-1 were identified. Furthermore, the chromosomal location of each gene was inferred by using monosomic (Tosa et al., 1987), ditelocentric (Tosa et al., 1988) and nulli-tetrasomic (Tosa and Sakai, 1990) wheat lines.

In the last decade of the 20th century, scientists researching host species specificity began to realise the potential for powerful molecular genetic analysis using *M. oryzae* (later classified as two distinct taxonomic groups *M. grisea* and *M. oryzae*) (Valent and Chumley, 1991). *M. oryzae*, as with *B. graminis*, was known to exist in many subgroups that were specialised on specific hosts. However, the designation of taxonomic definitions to these subgroups (i.e. *formae speciales*) was not straightforward and ambiguities still exist today. Despite this, the *M. oryzae*-Poaceae host pathosystem had a few key advantages versus *B. graminis*. Primarily, it was, and still remains, a damaging fungal pathogen of many economically important crop plants (Wilson and Talbot, 2009). Furthermore, a potent mix of molecular biology, cell biology, classical genetics, and cytological studies added to its rapid adoption as a model system for studying host species specificity (Valent and Chumley, 1991). Many classical genetic studies were conducted utilising interfertile crosses between specialised strains of the pathogen in much the same way that Tosa had using *B. graminis*. For example, a strain specialised on rice could be crossed to a strain specialised on wheat and the resulting F_1 progeny would segregate for avirulence genes when applied to either of the natural hosts. Many gene-for-gene interactions determining host species specificity were identified using this approach (Murakami et al., 2000, Murakami et al., 2003, Oh et al., 2002, Takabayashi et al., 2002, Tosa et al., 2006, Chuma et al., 2010). Unfortunately, in many instances, the fine-mapping and eventual cloning of the genes underpinning these interactions, in both the host plant and pathogen, were never

carried out. An early exception was *PWL2* (*Pathogenicity to Weeping Lovegrass 2*) that was cloned in 1995 (Sweigard et al., 1995). Integral to this cloning effort was the detection, under laboratory conditions, of spontaneous mutations to the *PWL2* gene that gave rise to pathogenicity on weeping lovegrass (*Eragrostis curvula*). *PWL2* functioned to prevent infection on weeping lovegrass in much the same way as known *Avr* genes for cultivar specificity. Indeed, laboratory mutants facilitated map-based cloning until a specific ORF (open reading frame) was attributed to *PWL2*. The gene was found to encode for a glycine rich, hydrophilic protein (145 amino acids) with a putative secretion signal (Sweigard et al., 1995). A homologous gene family member, *PWL1*, was cloned shortly after and was also observed to function in preventing infection on weeping lovegrass (Kang et al., 1995). The cloning of the *PWL* gene family members allowed researchers to begin to look at field isolates to ascertain the role of *PWL* alleles in pathogenicity. The observation of spontaneous mutants in laboratory conditions led to the hypothesis that similar mutations would accumulate in the field. In contrast, in excess of 96% of the *M. oryzae* isolates specialised on *Oryza spp.* contained *PWL* homologs. A single strain that did not contain a *PWL* homolog was highly pathogenic on weeping lovegrass (Sweigard et al., 1995). When a wider survey of *M. oryzae* isolates, from diverse hosts, was analysed no correlation could be found between *PWL* sequences and pathogenicity on weeping lovegrass (Kang et al., 1995). This may be attributed to the use of DNA blot hybridisation for the analysis and subsequent appearance of false positive data. It has been shown elsewhere that conversion from *PWL2* (avirulence) to *pwl2* (virulence) can be attributed to a single nucleotide polymorphism (SNP) (Sweigard et al., 1995), a phenomenon too discrete to be detected by hybridisation. This was exemplified during a similar investigation into the *M. oryzae* (weeping lovegrass isolate) *AVR1-CO39* gene that had been cloned to a 1.06 kbp fragment by Farman and Leong (1998). In this study, polymerase chain reaction (PCR) based approaches resolved false positives reported using a DNA blot hybridisation approach. A correlation could be observed between the absence or presence of *AVR1-CO39* in *M. oryzae* isolates pathogenic on rice or other hosts, respectively. Thus, nonhost resistance at the level of host genera could be attributed to avirulence genes that were previously thought to condition only cultivar specificity.

Recently the corresponding resistance gene to *AVR1-CO39*, *Pi-CO39*, has been cloned (Cesari et al., 2013). Two closely linked, tandemly arranged NLR genes (*RGA4* and *RGA5*) were found to be required and sufficient for resistance mediated by recognition of *AVR1-CO39*. Remarkably, *RGA4* and *RGA5* had also been previously implicated in mediating resistance to the adapted host pathogen on rice via recognition of *AVR-Pia* (Okuyama et al., 2011). The dual recognition of two avirulence genes with no homology is all the more

intriguing in the context of plant immunity. It provides direct evidence for multiple pathogen recognition specificity and may explain how a finite number of immune receptors in a plant genome can confer resistance to the multiplicity of pathogens to which they are exposed. However, this *R*/*Avr* pairing also provides evidence that specificity at cultivar and species level not only resides on similar mechanisms, but can also involve the same genetic components.

1.5.2. Contemporary models of nonhost resistance.

A continuing theme in plant immunity considers the significant overlaps observed between host and nonhost resistance mechanisms (Heath, 2000, Thordal-Christensen, 2003, Mysore and Ryu, 2004, Schulze-Lefert and Panstruga, 2011, Bettgenhaeuser et al., 2014, Gill et al., 2015). This is demonstrated by the observation of functionally homologous genes (*PEN3*/*Lr34*; (Stein et al., 2006, Krattinger et al., 2009b)), or similar pathways (membrane trafficking *PEN1*/*ROR2*; Collins et al. (2003)), and even dependence on similar cues for establishing basic compatibility (epicuticular wax, *IRG1/PALM1;* (Uppalapati et al., 2012)). This suggests that the qualitative separation of host and nonhost does not fully capture the diversity of resistance outcomes possible in plant-microbe interactions. This has prompted a move towards continua based models. Schulze-Lefert and Panstruga (2011) have incorporated existing knowledge on the molecular mechanism of plant immunity to propose an evolutionary model that describes the transition from host through to nonhost status. In the model, the relative contribution of NLRs and PRRs to resistance is inversely correlated based on the evolutionary separation from the host species. This view is also supported by Bettgenhaeuser et al. (2014) who proposed that the interaction of rust fungi with plant hosts can be viewed as a continuum whereby the possible outcomes range from complete immunity through to completion of the pathogen lifecycle. In this instance, they propose separation of the pathosystems that exist in the transition from host through to nonhost into four states: host, intermediate host, intermediate nonhost, and nonhost. To date, the majority of the molecular genetic research into nonhost resistance has focused on nonhost systems evolutionary distant from the host species. Therefore, it will be necessary to identify pathosystems that occur in the transition phase between host and nonhost to reveal the underlying mechanisms that underpin resistance in these systems.

1.6. An evolutionary relevant pathosystem for studying nonhost resistance.

1.6.1. Stripe rust (*P. striiformis* Westend.).

All rust fungi are parasitic, obligate biotrophic pathogens, requiring living hosts to complete their lifecycle (Hovmøller et al., 2011). The rust fungi belong to the family Pucciniaceae within the order Puccinales (Bushnell and Roelfs, 1984). *P. striiformis* is the causal agent of stripe rust, a common rust fungus that infects gramineous plants (Wellings, 2011). *P. striiformis* is formed of four monophyletic lineages accommodated within a taxonomic series called the *Puccinia* Series *Striiformis* (Liu and Hambleton, 2010). The monophyletic lineage, known as *Puccinia striiformis sensu stricto*, harbours rusts that infect members of the Triticeae including species from the genera *Triticum* and *Hordeum* (Liu and Hambleton, 2010). Pathogenic specialisation onto different host genera prompted the classification of *P. striiformis* into different *formae speciales* (Eriksson, 1898). *P. striiformis* f. sp. *tritici* infects wheat and has been well characterised because it causes consistent yield losses on a global scale (Wellings, 2011). *P. striiformis* f. sp. *hordei* the barley specialised form, has received less attention due to the lower acreage of barley relative to wheat. Interestingly, it has been suggested that all *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates are derived from the same lineage (Liu and Hambleton, 2010). At present, very little is known about the molecular genetic determinants that drive the host specialisation of these pathogens.

P. striiformis is a heteroecieous rust fungus with five distinct spore stages that manifest during asexual reproduction on the gramineous host and sexual reproduction on the alternate host (*Berberis spp.*) (Hovmøller et al., 2011). The identity of an alternate host eluded researchers for over a century until the discovery of *P. striiformis* on *Berberis* spp. (Jin et al., 2010). The alternate host facilitates the sexual stage of the *P. striiformis* lifecycle. This commences via infection of *Berberis* spp. with basidiospores that lead to the production of haploid (n) pycnia. The pycnia contain pycniospores (spermatia) and receptive hyphae. Fertilisation occurs between pycniospores and receptive hyphae of opposite mating types giving rise to dikaryotic (n + n) mycelia. Following plasmogamy, dikaryotic aecia begin producing aeciospores that disperse and infect the primary gramineous host. Upon infection of the gramineous host a dikaryotic mycelium is formed and continued infection of wheat is propagated through the dispersal of urediniospores. The pathogen can remain in the asexual phase and causes continuous infections via the production urediniospores and the cyclical infection of the gramineous host. Upon contact with a compatible gramineous host plant urediniospores germinate and enter the leaf via stomata without the production of an appressorium (Chen et al., 2014). Upon entry to the

cell the primary germ tube differentiates into a substomatal vesicle. This vesicle then produces 2-3 primary hyphae that then form haustorial mother cells. Haustorial mother cells act as the interface between pathogen and host and are thought to play a role in signalling and nutrient uptake (Hovmøller et al., 2011). During infection the pathogen will continually produce infectious hyphae that spread throughout the apoplast. The spreading nature of *P. striiformis* differentiates it from other focally accumulating rust pathogens e.g. *P. graminis* (Bushnell and Roelfs, 1984). During a compatible interaction the asexual stage will conclude with the formation of urediniospores that form along the longitudinal axis of the leaf. As additional urediniospores are produced, pressure is exerted on the leaf epidermis, which eventually ruptures revealing the characteristic yellow lesions on infected leaves (Hovmøller et al., 2011). Production of urediniospores may be replaced by teliospores when older plants begin to senesce. Teliospore production usually commences in autumn towards the end of the growing season of the gramineous host. Karyogamy occurs early in teliospore development giving rise to the diploid state. Meiosis begins shortly after karyogamy but is suspended with the onset of winter whereby the fungus enters a period of teliospore dormancy. In early spring a hyphal protrusion emerges from one or both cells of the teliospore and meiosis is re-initiated. Upon completion, meiosis gives rise to four haploid basidiopsores that subsequently infect the alternate host and complete the lifecycle (Chen et al., 2014).

1.6.2. Barley (*H. vulgare Linnaeus.*).

Barley has many traits that make it an appealing model organism. It is an inbreeding crop, a true diploid, and has a rich pedigree of genetic research that spans more than a century (Ullrich, 2011). Despite its relatively large, repetitive genome (5.1 Gbp), it has been proposed as a model for genomic research within the Triticeae tribe (Schulte et al., 2009, IBGSC, 2012) and to date, there have been >20 genes isolated via map based cloning approaches (Krattinger et al., 2009a, Ariyadasa et al., 2014). Recently, significant advances have been made with regards to the genomic resources available in barley and these hold significant promise to assist gene isolation studies. The first major step towards a draft genome sequence was made when the International Barley Genome Sequencing Consortium published a 4.98 Gbp BAC-based physical map anchored to a high-resolution genetic map (IBGSC, 2012). In this study, sequencing of 6,278 BAC clones and 304,523 BAC end sequences (BES) allowed 112,989 whole genome shotgun (WGS) contiguous sequences (contigs) to be anchored to the physical map. Additionally, an estimation of the gene space was made by aligning full-length barley cDNAs and over 1.5 billion RNAseq reads to the WGS assembly resulting in the identification of over 26,000 high confidence genes (IBGSC, 2012). Shortly after the publication of the anchored physical map, Mascher

et al. (2013) used low read depth sequencing of progeny from a recombinant inbred line (RIL) population (POPSEQ) to genetically bin approximately 1.2 Gbp of sequence information. Subsequently, the integration of these two dataset and the anchoring of additional sequence information via alternative genetic maps led to the publication of a barley genomic resource, spanning ~98% of the barley genome, genetically anchored by two million SNPs (Ariyadasa et al., 2014). This resource will provide an invaluable tool for future gene isolation studies, as it provides physical sequence information that can be used for marker development, candidate gene analysis, and gene modelling.

1.6.3. Rationale.

Nonhost resistance is often conceptualised as a qualitative separation from host resistance. Classification into these two states is generally facile, as they fail to fully describe the range of states that exist in the transition from host to nonhost. This poses a problem when studying pathosystems that cannot be classified as either host or nonhost due to their intermediate status relative to these two extremes. Bettgenhaeuser et al. (2014) defined the transition from host to nonhost with four states: host, intermediate host, intermediate nonhost, and nonhost. Classification into these four states depends on the degree of infection relative to a representative set of accessions from a species. In particular, intermediate host classification will often involve a small number of accessions being colonised or allowing for the completion of a pathogen's life cycle. The work described in this thesis uses the interaction between barley and *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* as representative systems for studying host and intermediate host resistance, respectively. Using these systems it is possible to dissect the genetic architecture of resistance and make significant progress in elucidating molecular genetic mechanisms of host and nonhost resistance in barley.

1.7. Dissertation organisation.

The research presented in this thesis is organised into chapters. The first research chapter details the initial exploration of the barley-*P. striiformis* interaction and the development of two microscopic phenotypic assays. The second section describes the application of these assays to structured barley populations for describing the genetic architecture of nonhost resistance. The third and fourth sections present the results of fine mapping a nonhost resistance locus and a host resistance locus, respectively. Each chapter contains a chapter-specific introduction, results, and discussion.

1. This study investigates the efficacy of the Poaceae-stripe rust (*P. striiformis*) interaction for describing the host-nonhost landscape. It details the finding that

the macroscopic observation of chlorosis is associated with hyphal colonisation by *P. striiformis* f. sp. *tritici*. This prompts the adaptation of a protocol for visualizing fungal structures into phenotypic assays that estimate the percent of leaf colonised and percent of leaf exhibiting pustules (pCOL and pPUST, respectively). Use of these assays on intermediate host and host systems finds that the frequency of infection decreases with evolutionary divergence from the host species. Similarly, the microscopic assays demonstrate that the pathogen's ability to complete its life cycle decreases faster than its ability to colonize leaf tissue, with significantly reduced pustule formation in the intermediate host system compared to the host system, barley-*P. striiformis* f. sp. *hordei*.

2. This chapter details the application of the microscopic assays to the SusPtrit x Golden Promise doubled haploid barley mapping population and the finding that the genetic architecture of resistance is underpinned by three major loci. Colonisation resistance maps to two major loci, *Rpst1* and *Rpst2*, which coincide with the race-specific mildew resistance loci *Mla* and *Mlf,* respectively. *Rpst3*, prevents lifecycle completion of *P. striiformis* f. sp. *tritici* and maps near the non-race-specific mildew resistance locus *mlo*. A suite of barley mapping populations are interrogated for resistance to wheat stripe rust and it is possible to observe the presence of either one or more loci that are varying combinations of *Rpst1*, *Rpst2,* and *Rpst3*. This chapter demonstrates that intermediate host resistance is conditioned by major genes and I propose that a naturally occurring *R* gene stack protects barley against PST infection.
3. This chapter details the map-based isolation of a candidate NLR gene underlying the colonisation resistance locus, *Rpst2*. *Rpst2* is mapped in two distinct populations involving different resistant and susceptible accessions. Using existing genomic resources of barley, a candidate gene is identified and mapped within a high-resolution genetic map. RNAseq experiments facilitate the development of molecular markers that are then used to anchor the *Rpst2* locus to the physical map of barley defining approximately 267 kbp region harbouring the gene.
4. The research presented in this chapter details the efforts to use a map-based cloning approach to identify *rps2*, a recessive resistance gene conferring resistance to the host pathogen of barley, *P. striiformis* f. sp. *hordei.* The gene is mapped to a putative 330 kbp region after anchoring a high resolution genetic map to the physical map of barley. Using a candidate gene approach tailored towards NLRs it is not possible to identify candidate genes at the locus using existing genomic resources.

2. Materials and methods.

2.1. General.

2.1.1. Plant materials.

Barley accessions were obtained from the United States Department of Agriculture Germplasm Resource Information Network (Aberdeen, ID, USA), the James Hutton Institute (Dundee, UK), Okayama University (Okayama, Japan), the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (Gatersleben, Germany), the Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (Madrid, Spain), Oregon State University (Corvallis, OR, USA), Washington State University (Pullman, WA, USA), and Wageningen University and Research Centre (Wageningen, Netherlands). All plants underwent single seed descent before performing pathogen assays. Plant materials are detailed in Table A1, Table A2, and Table A11.

2.1.2. Pathogen materials and assays.

P. striiformis f. sp. *tritici* isolate 08/21 was collected in the United Kingdom in 2008 and maintained at the National Institute of Agricultural Botany (NIAB) on the susceptible wheat cultivar Solstice. *P. striiformis* f. sp. *hordei* isolate B01/2 was collected in the United Kingdom in 2001 and maintained at NIAB on the susceptible barley cultivar Cassata. *P. striiformis* urediniospores were stored at 6°C after collection. Inoculations were carried out by sowing seeds in 1 L pots containing peat-based compost in groups of four, using eight seeds per accession. Plants were grown at 18°C day and 11°C night using a 16 h light and 8 h dark cycle in a controlled environment chamber at NIAB, with lighting provided by metal halide bulbs (Philips MASTER HPI-T Plus 400W/645 E40). Barley seedlings were inoculated at 14 days after sowing, where first leaves were fully expanded and the second leaf was just beginning to emerge. Urediniospores of *P. striiformis* were suspended in talcum powder, at a 1:16 ratio of urediniospores to talcum powder based on weight. Compressed air was used to inoculate seedlings on a spinning platform. After inoculation, seedlings were placed in a sealed bag and stored at 6°C for 48 h to increase humidity for successful germination of urediniospores. Subsequently, plants were returned to the growth chamber for the optimal development of *P. striiformis*.

2.1.3. Microscopic phenotyping.

We adapted a protocol described by Ayliffe and co-workers that uses wheat germ agglutinin (WGA; a lectin that interacts with chitin oligomers) conjugated with the fluorescein isothiocyanate (FITC) fluorophore to visualize the intercellular growth and

pustule formation on infected leaves (Ayliffe et al., 2011, Ayliffe et al., 2013). Three leaves were harvested at 14 dpi and placed in 15 mL centrifuge tubes containing 1.0 M KOH with a droplet of surfactant (Silwet L-77, Loveland Industries Ltd.). Leaves were cleared by incubating in the KOH solution at 37°C for 12 to 16 h. Subsequently, the KOH solution was decanted and leaves were neutralised by washing three times in 50 mM Tris at pH 7.5. After decanting of the final wash solution, a 1.0 mL stain solution (20 μg/mL WGA-FITC (L4895-10MG; Sigma-Aldrich) in 50 mM Tris at pH 7.5) was applied to the leaves. Leaf tissue was incubated overnight, then washed with water, mounted, and observed under blue light excitation on a fluorescence microscope with a GFP filter. We developed two microscopy-based phenotypic assays to estimate the percent of leaf colonised (pCOL) and percent of leaf harbouring pustules (pPUST) of *P. striiformis.* This was achieved by evaluating disjoint fields of view (FOV) covering the surface area of the leaf by scanning a mounted leaf segment along either side of the longitudinal axis for barley leaves. Within each field of view (FOV), a convex hull was determined based on the colonisation of *P. striiformis* and estimated to be less than 15%, between 15 and 50% or greater than 50% of the FOV area and given scores of 0, 0.5, or 1, respectively. The final pCOL score was determined by averaging these scores based on the total number of FOVs evaluated and ranged from 0 to 100%. pPUST was evaluated in a similar manner, although convex hulls were defined by the clustering pattern of *P. striiformis* pustules. A 5x objective with a FOV of 2.72 mm x 2.04 mm was used.

2.1.4. Macroscopic phenotyping.

Macroscopic symptoms were evaluated on the first leaf of all seedlings at 14 days post-inoculation (dpi). For the interaction between barley and *P. striiformis* f. sp. *tritici* the observation of chlorosis (CHL) and infection (pustule formation; INF) phenotypes were scored on a nine-point scale from 0 to 4, with increments of 0.5. With all phenotypes, the scale reflects the percentage of the leaf surface area expressing the respective phenotype. A score of 0 was asymptomatic, i.e. no chlorosis, browning, or pustules, and a score of 4 indicated full expression of the respective phenotype (i.e., 100% of the surface area). For the interaction between barley and *P. striiformis* f. sp. *hordei* we phenotyped using the McNeal scale. The scale is based on the observed disease symptoms: 0 (immune; no visible symptoms), 1 (necrotic/chlorotic flecks without sporulation), 2 (necrotic/chlorotic stripes (NCS) without sporulation), 3 (trace sporulation with NCS), 4 (light sporulation with NCS), 5 (intermediate sporulation with NCS), 6 (moderate sporulation with NCS), 7 (abundant sporulation with NCS), 8 (abundant sporulation with chlorosis), and 9 (abundant pustule formation, without chlorosis) (McNeal et al., 1971).

2.1.5. DNA extraction.

DNA from all structured populations was extracted from leaf tissue following a CTAB-based protocol adapted for 96-well based format modified from (Stewart and Via, 1993) that provides PCR-grade genomic DNA (Nick Lauter, personal communication).

2.1.6. Genotyping.

Oligonucleotide assay (OPA) genotyping using the barley BOPA1 design that includes 1,536 SNP-based markers was performed at the University of California, Los Angeles Southern California Genotyping Consortium (Los Angeles, CA, USA) (Close et al., 2009). The concentration of gDNA samples submitted for OPA genotyping were estimated using the PicoGreen dsDNA quantification assay (Life Technologies; P11496) and normalised to 60 ng/µL. Additional markers were developed as either cleaved amplified polymorphic sequence (CAPS) or Sequenom MassARRAY markers to bridge gaps between unlinked chromosome arms and increase marker densities. For CAPS marker development, we identified type II restriction enzymes that digest at polymorphic positions using CAPS Designer (http://solgenomics.net/tools/caps_designer/caps_input.pl). CAPS marker PCR reactions were prepared by mixing 2 µL buffer (10x), 0.4 µL dNTPs, 0.4 µL forward primer, 0.4 µL reverse primer, 0.2 µL Taq polymerase, 2 µL gDNA at 10 ng/µL, and 14.6 µL H_2O. The PCR cycling started with an initial denaturation step at 94°C for five minutes and then proceeded through a cycle of 94°C for 20 seconds, annealing at 56°C for 30 seconds and primer extension at 72°C for one minute for a total of 35 cycles. The procedure ended with a final extension at 72°C for five minutes before being held at 16°C. Digestions were performed according to the manufacturer's instructions for individual enzymes. Electrophoresis was used to resolve restriction fragments using 2.0% TBE agarose gels stained with ethidium bromide. Gel images were taken using a Bio-Rad ChemDoc XRS+ imaging system and markers were scored manually. GBS CAPS markers are described in (Kota et al., 2008). All primers and restriction enzymes for CAPS markers are detailed in Table A3. For Sequenom marker development, SNP sequences were extracted in IUPAC format with 40 to 60 bp flanking sequence. This sequence was used as a template for primer design using MassARRAY software v3.1 for the multiplexing up to 32 SNP assays. Sequenom genotyping was carried out at the Iowa State University Genomic Technologies Facility (Ames, IA, USA). All SNPs template source information for Sequenom markers are detailed in Table A4. For KASP genotyping, SNPs were converted into Kompetitive Allele Specific PCR (KASP) markers using a similar approach as described in (Ramirez-Gonzalez et al., 2015). KASP primer mix was prepared by mixing 12 µL VIC primer (s1), 12 µL FAM primer (s2), 30 µL reverse primer (r), and 46 µL H_2O. KASP PCR reactions contained 2µL

gDNA (~10-20 ng), 2 µL KASP V4.0 2x master mix, and 0.055 µL primer mix. KASP PCR cycling used an initial incubation at 95°C for 15 minutes followed by touchdown PCR cycling: 94°C for 20 seconds followed by ten 25 second cycles of touchdown PCR starting at 65°C decreasing by 1°C each cycle. Samples then cycled 30 times at 94°C for 20 seconds and annealed at 57°C for 1 minute before being held at 4°C. All WGS contig source information, SNPs, KASP marker template, and primers are detailed in Table A5. KASP assays were performed at the John Innes Centre Genotyping Facility (Norwich, UK).

2.1.7. QTL and ANOVA analyses.

Interval mapping was performed with QTL Cartographer (v1.17j) using model 3, a step size of 2 cM, and a window size of 10 cM, whereas composite interval mapping was performed using model 6 with the selection of five background markers (Basten et al., 1994). Significant QTLs were extracted using the Eqtl module under the H_0:H_3 model using experiment-wide thresholds (EWT) that were calculated using 1,000 permutations with the reselection of background markers using a threshold of $\alpha < 0.05$ (Doerge and Churchill, 1996, Lauter et al., 2008). ANOVA analyses for testing the linkage of individual markers were performed with R/qtl.

2.1.8. Transcriptome sequencing and assembly.

Leaf tissue was harvested from first and second leaves 18 days after sowing. Samples were flash frozen in liquid nitrogen, and stored at -80°C. Samples were homogenised in liquid nitrogen-chilled pestle and mortars. RNA was extracted from samples using TRI-reagent (Sigma-Aldrich; T9424) according to the manufacturers protocol. DNA was removed by treating samples with RQ1 RNase free DNase (Promega; M6101). Samples were purified using RNeasy mini spin columns following the RNA Cleanup protocol (Qiagen; product No. 74104). The quality and integrity of the RNA samples were assessed using RNA Nano Chips (Agilent Technologies; product no. 5067-1511) on an Agilent 2100 Bioanalyzer. Abed Binder 12 and Russell RNA libraries were constructed using Illumina TruSeq RNA library preparation (Illumina; RS-122-2001). Final library insert sizes were predicted to be 411 and 339 bp for Abed Binder 12 and Russell, respectively. Barcoded libraries were sequenced using 100 bp paired-end reads on one lane of a Hiseq 2000/2500. This generated 32.0 and 59.3 million paired end reads for Abed Binder 12 and Russell, respectively. All library preparation and sequencing was performed at The Genome Analysis Centre (Norwich, UK). RNAseq data quality was assessed with FastQC and reads were removed using Trimmomatic (v0.32) with parameters set at ILLUMINACLIP:TruSeq3-PE.fa:2:30:10, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, and MINLEN:100. These parameters will remove all reads with adapter sequence,

ambiguous bases, or a substantial reduction in read quality. Transcriptome assembly was performed using Trinity (v2013-11-10) using default parameters. Raw reads have been submitted to NCBI Short Read Archive under the BioProject ID PRJNA292371 and SRA accession SRR2153288 (cv. Abed Binder 12) and SRR2153285 (cv. Russell).

2.1.9. Motif alignment and search tool (MAST) analyses.

MAST analysis was carried out using 20 previously described NLR motifs (Meyers et al., 2003, Jupe et al., 2012). Six frame translations were performed on all WGS contigs prior to MAST analysis. WGS contigs harbouring multiple motifs and showing evidence of characteristic NLR domain structure were selected as candidates.

2.2. Chapter 3 specific methods.

2.2.1. Statistical analysis.

Pearson rank correlation coefficients (ρ) were determined using the *cor* command in R (v3.1.0).

2.3. Chapter 4 specific methods.

2.3.1. Phylogenetic analyses.

A total of 129 barley accessions were genotyped using the barley OPA 1 (BOPA1) platform with 1,536 potential SNPs. Only SNPs with a minor allele frequency greater than 5% and less than 10% missing data were considered for use in phylogenetic analyses. A cladogram incorporating 1,258 SNPs was generated with hierarchical clustering using the *hclust* command in the R module *ape*, using default parameters and Euclidean distance estimates. An unrooted phylogenetic tree was generated for all parental accessions used in mapping populations using the same parameters. The phylogenetic tree was generated with neighbour joining using 1,235 polymorphic sites. Support over 90% is shown at branch points in the phylogeny based on 1000 bootstraps.

2.3.2. Marker trait associations.

Closely linked markers at the *Rpst1*, *Rpst2*, and *Rpst3* were screened on F_2 populations. Marker trait association was performed using R/qtl with the *fitqtl* command. A marker-trait permutation thresholds (MTT) was determined by performing 1,000 permutations on the phenotype and controlling at $\alpha = 0.05$ (95th percentile of LOD scores).

2.4. Chapter 5 specific methods.

2.4.1. Genetic map construction.

A genetic map was constructed using 589 markers including 535 barley OPA (Close et al., 2009), 26 CAPS markers, and 28 Sequenom markers. Genetic map construction was performed using JoinMap v4 with default parameters and an independence LOD threshold of 4.0 (Van Ooijen, 2011). Genetic distances were estimated using the Kosambi mapping function. Integrity of the genetic map was evaluated through comparison with the current OPA consensus genetic map of barley (Muñoz-Amatriaín et al., 2011) and with two-point linkage tests using R/qtl (v1.33-7).

2.4.2. Marker development for saturation at the *Rpst2* locus.

Initial marker development was guided by two approaches to identify sequences anchored to the *Rpst2* region. This included the identification of anchored unigenes based on marker colinearity with existing genetic maps (Potokina et al., 2008, Moscou et al., 2011, Muñoz-Amatriaín et al., 2011) and orthologous rice genes based on the barley genome zipper (IBGSC, 2012). A region on rice chromosome 6 was selected including 38 genes (Os06g43140 to Os06g43900). Best BLASTn hits returned from the cv. Morex WGS assembly were used as template for PCR primer design using Primer3 (libprimer3 release 2.3.6). All BLASTn queries were performed using blastall (v2.2.23). Abed Binder 12 and Russell gDNA was used as template for PCR amplification and Sanger sequencing. SNPs were identified by aligning sequence files using Seqman software (DNAstar Lasergene v11). SNPs were then used to develop markers using Cleaved Amplified Polymorphic Sequences or Sequenom MassARRAY iPLEX platform as described above.

Subsequent marker development involved either (1) the comparison of genomic contigs derived from cvs. Barke, Bowman, and Morex or (2) the comparison of Abed Binder 12 and Russell RNAseq aligned reads to WGS contigs anchored to the *Rpst2* region (Mascher et al., 2013, IBGSC, 2012). Geneious (v8.1.6) was used for read alignment using Geneious mapping function with default parameters and data visualisation (Kearse et al., 2012).

2.4.3. Recombination screen and phenotyping.

A recombination screen was carried out using seed bulked from F_3 plants selected from a single $F_{2:3}$ family that were heterozygous for *Rpst2*. Sequenom markers were converted into KASP markers and used as flanking markers to identify recombinant chromosomes. Two independent progeny tests were performed using individuals with recombinant chromosomes. A total of 16 individuals per family per replicate were scored for macroscopic observation of chlorosis and infection.

2.4.4. BAC library construction and screening.

First and second leaves were sampled from 18 day-old Abed Binder 12 seedlings and flash frozen in liquid nitrogen. DNA was extracted and partially digested with *Hind*III. Restriction fragments were cloned into pIndigoBAC-5 vector and transformed into DH10B E. coli cells. The BAC library was pooled in 5 x 96 well plates with each plate containing ~500- 600 independent primary clones with an average insert size of 130 kbp which represents an ~6X coverage of the barley genome. The library was PCR screened using two primer pairs specific to the 5' and 3' end of the *NLR-A* coding sequence (5' primers= A02/A08, 3' primers= A05/A11. Fig. 14A-B and Table A6). A single BAC clone, #4931-1 11E, was identified and determined to harbour a 155 kbp insert by *Not*I restriction digestion analysis and pulsed field gel electrophoresis (PFGE). All BAC library construction and screening was carried out by Bio S&T Inc. Quebec, Canada.

2.4.5. Long range PCR.

Long range PCR was carried out using primers amplifying the full-length *NLR-A* gene and a shorter fragment spanning Barke contigs 54347 and 2780081 (Table A6 and Fig. 14C-D). PCR reactions were performed using Pomega Gotaq Long PCR Master Mix (Catalogue No. M4021). Long PCR reactions were prepared by mixing 25 µL Gotaq Long PCR Master Mix (2x), 1 µL forward primer (10 µm), 1 µL reverse primer (10 µm), 1 µL gDNA (~100 ng/µL), and 22 µL H_2O. The PCR cycling started with an initial denaturation step at 95°C for two minutes followed by 30 cycles at 94°C for 30 seconds and a combined annealing and extension step at 65°C (six minutes= short fragment, 15 minutes= long fragment). The procedure ended with a final extension at 72°C for 10 minutes before being held at 16°C. PCR amplicons were resolved by gel electrophoresis using 0.8% TBE agarose gels visualised by ethidium bromide staining.

2.4.6. BAC fingerprinting and sequencing.

BAC clone #4931-1 11E was spread on LB plates containing 12.5 μg/ml chloramphenicol. Plasmid DNA was extracted from 10 independent clones using standard laboratory procedures. pDNA of each clone was digested with *Hind*III-HF enzyme overnight and resolved on a 0.95% TBE agarose gel at 4°C: 100V for 10 minutes then 65V for ~20 hours. Gel was stained using SYBER green and washed with TBE buffer before being visualised on a Bio-Rad ChemDoc XRS+ imaging system. All clones gave the same banding pattern. pDNA was then extracted from one clone using the Qiagen Large Construct Kit (cat No. 12462). pDNA preparation was checked by fingerprinting and the concentration was estimated visually by comparing to Lambda DNA standard on TBE gel agarose gel. The BAC clone was sequenced by The Genome Analysis Centre (TGAC), UK, with Pacific Biosciences long read sequencing using a SMRT cell with C4-P6 chemistry.

2.4.7. Bioinformatic annotation pipeline for bacterial artificial chromosome.

De novo assembly of PacBio sequenced bacterial artificial chromosome (BAC) was performed using the Hierarchical Genome Assembly Process 3.0 pipeline (Chin et al., 2013). The BAC was manually curated to identify the insert, removing backbone sequence (pIndigoBAC-5) and additional sequence integrated in flanking regions in the assembly process. The quality of the assembly was assessed using BLAT alignment of raw reads onto the final assembly. Annotation of repetitive elements was performed using RepeatMasker using the Monocotyledons repeat library. Annotation of genes was performed using (1) using high and low confidence gene models in barley (IBGSC, 2012) and (2) Bowtie and TopHat alignment of RNAseq data derived from Abed Binder 12 leaf tissue.

2.5. Chapter 6 specific methods.

2.5.1. Genetic map construction.

A genetic map of 120 markers was constructed using 107 Sequenom and 12 CAPS markers. Initially, 89 Sequenom markers were developed using the consensus map of Muñoz-Amatriaín et al. (2011), selecting markers at ~10 cM intervals across the genetic map. Marker saturation at the *rps2* locus was achieved by designing 12 CAPS and 18 Sequenom markers based on an integrated consensus map (Potokina et al., 2008, Close et al., 2009, Moscou et al., 2011, Muñoz-Amatriaín et al., 2011). JoinMap v4 was used using default parameters and an independence LOD threshold of 4.0 (Van Ooijen, 2011). Genetic distances were estimated using the Kosambi mapping function. Integrity of the genetic map was evaluated through comparison with the current OPA consensus genetic map of barley (Muñoz-Amatriaín et al., 2011) and with two-point linkage tests using R/qtl (v1.33-7).

2.5.2. Recombination screen and phenotyping.

A recombination screen was carried out using F_2 seed from the Abed Binder 12 x Russell population. CAPS markers C_1449 and C_6562 were used as *rps2* flanking markers to screen a total of 768 plants (1,536 gametes). Two independent progeny tests were performed using individuals with recombinant chromosomes. A total of sixteen individuals per family were scored using the McNeal scale.

2.5.3. Marker saturation at the *rps2* locus.

The International Barley Genome Sequencing Consortium sequenced five BAC clones that anchored to the *rsp2* region (IBGSC, 2012). Sequence derived from each of these clones was fragmented into 1,000 bp contigs to serve as template sequence for primer design using Primer3 software (libprimer3 release 2.3.6). SNPs were identified by PCR amplifying gDNA from Abed Binder 12 and Russell and Sanger sequencing PCR amplicons. Thirty-six SNPs were successfully converted into Sequenom markers for saturating the *rps2* locus.

3. The development of quick, robust, quantitative phenotypic assays for describing the host-nonhost landscape to stripe rust.

3.1. Introduction.

Plants have a remarkable ability to resist the majority of pathogenic microbes they encounter. It is now widely posited that the molecular networks underlying this resistance are multi-factorial and can depend upon active or passive defence mechanisms (Thordal-Christensen, 2003, Fan and Doerner, 2012). While the individual contribution of each mechanism is hard to quantify, their common objective is to provide barriers that impede the development of pathogens (Heath, 1980). Thordal-Christensen (2003) proposed a minimum of four barriers that included (1) germination and penetration of the leaf epidermis by a pathogen, (2) the ability to overcome pre-formed physical and/or chemical barriers, (3) the ability to avoid the inducible defence responses that govern pre-penetration resistance, and (4) the ability to avoid detection by membrane bound and intracellular defence surveillance system. A microbe that can circumvent or suppress these four barriers, and establish a compatible interaction, is known as an adapted pathogen. Contrastingly, a microbe that is impeded by any of the mechanisms described above is unable to establish a compatible interaction and is declared a nonhost pathogen (Zimmerli et al., 2004).

The identification of overlap between host and nonhost resistance prompted the development of models that integrate membrane and intracellular signalling pathways involved in plant immunity (Thordal-Christensen, 2003, Schulze-Lefert and Panstruga, 2011). Schulze-Lefert and Panstruga proposed an evolutionary model wherein the relative contribution of PRRs and NLRs in conditioning resistance would be inversely correlated based on the phylogenetic distance to the host species (Schulze-Lefert and Panstruga, 2011). While an intriguing proposal, the majority of research on the molecular mechanisms underlying nonhost resistance has been derived from nonhost systems that are phylogenetically distant to the host system (Fan and Doerner, 2012, Gill et al., 2015). Therefore, it will be necessary to identify biological systems that span the transition from host to nonhost. Bettgenhaeuser et al. (2014) defined the transition from host to nonhost with four states: host, intermediate host, intermediate nonhost, and nonhost. Classification into these four states depends on the degree of infection relative to a representative set of accessions from a species. In particular, intermediate classification will often involve a small number of accessions being colonised or allowing for the completion of a pathogen's life cycle (Bettgenhaeuser et al., 2014). Investigating systems on the boundary requires

the development of appropriate phenotypic assays, which are often distinct from those used in host systems.

Several microscopy-based approaches have been developed to interrogate host-nonhost pathosystems. Shafiei et al. (2007) found that early barriers conditioned nonhost resistance in *A. thaliana* to *P. triticina*. This was predominantly observed as a reduction in the ability for germ tubes to identify stoma and concomitant reduction in haustorial formation (Shafiei et al., 2007). Genetic dissection of guard cell death and substomatal vesicle formation found independent architectures, suggesting that several layers of microbial perception limit the development of *P. triticina* on *A. thaliana*. In contrast, resistance in *Brachypodium distachyon* to *P. graminis* f. sp. *tritici* manifested as a reduction in the formation of penetration pegs, substomatal vesicles, and primary hyphae, whereas appressoria formation was unaffected (Figueroa et al., 2013). Ayliffe et al. found a general requirement for microscopy-based approaches to visualise the development of infection structures in the interactions of *B. distachyon* and rice with several cereal rusts, although some symptoms on *B. distachyon* were macroscopically visible (Ayliffe et al., 2010, Ayliffe et al., 2011, Ayliffe et al., 2013). Niks and colleagues adopted a microscopy-based phenotypic assay to determine the number of pustules forming per unit area in the interaction of barley and *P. triticina* (Jafary et al., 2006, Jafary et al., 2008). This assay was critical, as the majority of the differential phenotypes between accessions were exhibited as variation in pustule formation rather than colonisation.

In this chapter, we describe the interaction of barley with *P. striiformis* f. sp. *tritici* as a representative pathosystem for describing intermediate host resistance. We take advantage of the stepwise infection process of *P. striiformis* that begins with intercellular colonisation of leaves and then transitions to pustule formation (Hovmøller et al., 2011). We develop a complimentary pair of phenotypic assays, pCOL and pPUST, to estimate the colonisation and pustule formation of *P. striiformis*, and apply them in the context of host and intermediate host systems to show that the frequency of infection decreases with evolutionary divergence from the host species. In parallel, we observe that the pathogen's ability to complete its life cycle decreases faster than its ability to colonise leaf tissue with lower incidence of pustules observed in the intermediate host system than in the host system.

3.2. Results.

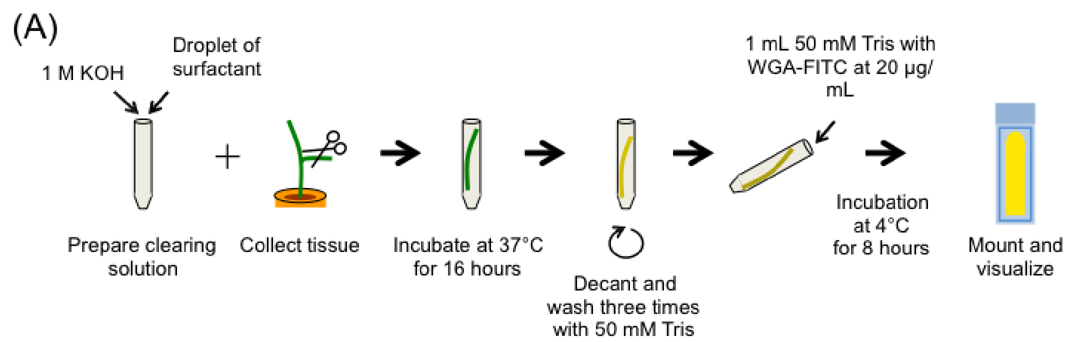
3.2.1. Barley is predominantly immune to *P. striiformis* f. sp. *tritici* and represents an intermediate host system.

The interaction of *P. striiformis* f. sp. *tritici* with barley has been proposed as an intermediate host system (Bettgenhaeuser et al., 2014). Previous research has demonstrated the occurrence of susceptibility to this pathogen in barley (Straib, 1937, Chen et al., 1995, Sui et al., 2010, Niks et al., 2015) but the frequency of susceptibility has never been systematically studied in a large collection of germplasm. Our initial approach was to screen a large collection of barley germplasm with *P. striiformis* f. sp. *tritici* to establish the frequency and degree of susceptibility. To do this, we inoculated and macroscopically phenotyped a panel of 237 barley accessions with *P. striiformis* f. sp. *tritici* isolate 08/21 (Fig. 1 and Table A1). We observed a few instances (3%; 7/237) of the completion of pathogen life cycle, namely, pustule formation (Fig. 1C). In most cases, leaves challenged with *P. striiformis* f. sp. *tritici* were immune, i.e., green and free of disease symptoms (77%; 184/237; Fig. 1A). However, a significant proportion (23%; 54/237) of genotypes exhibited a third phenotype that manifested as varying degrees of chlorosis (Fig. 1B). It was unclear whether chlorosis was a direct response to pathogen ingress or a general stress response. However, no chlorotic symptoms were evident on leaves in the absence of *P. striiformis* f. sp. *tritici*. In addition, the patterning of chlorosis in barley was not random and was often associated with stripe-like patterns on the leaf. Therefore, we hypothesised that this phenotype was associated specifically with *P. striiformis* f. sp. *tritici*.

3.2.2. Chlorosis is associated with hyphal colonisation of the leaf tissue.

To investigate whether chlorosis was a direct result of *P. striiformis* f. sp. *tritici* colonisation, we adapted a staining method in combination with fluorescence microscopy to visualize the presence of hyphal structures (Fig. 2A; (Ayliffe et al., 2011, Ayliffe et al., 2013). Initial observations demonstrated little or no hyphal growth in immune barley accessions. In contrast, barley accessions harbouring chlorosis appeared to have substantial *P. striiformis* f. sp. *tritici* hyphae (Fig. 2B). To quantify the association of this phenotype with *P. striiformis* f. sp. *tritici* infection, it was necessary to develop a phenotypic assay to quantify the area of the leaf infected by *P. striiformis* f. sp. *tritici* (pCOL; Fig. 2B). When we applied pCOL to barley, a strong association was observed between accessions displaying chlorotic symptoms and colonisation of *P. striiformis* f. sp. *tritici* (ρ = 0.84; Fig. 3). Exceptions did exist, including a few accessions displaying chlorotic symptoms but comparatively reduced pCOL (Fig. 3). While chlorosis does not

Figure 1: Macroscopic phenotypes observed on barley accessions challenged with *P. striiformis* f. sp. *tritici*. Accessions challenged with *P. striiformis* f. sp. *tritici* were generally categorised into three groups: (A) immune (no observable macroscopic symptoms; accession Abed Binder 12), (B) chlorotic (accessions displaying varying degrees of chlorosis; accession Foster), and (C) compatible (pustules observed, indicative of life cycle completion; accession Manchuria).



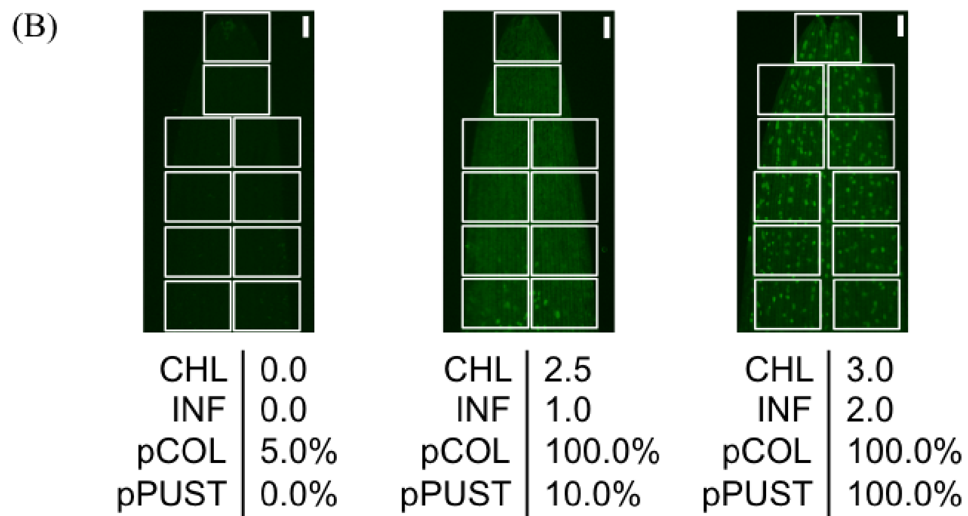


Figure 2: Quantitative microscopic phenotyping of *P. striiformis* colonisation and pustule formation using pCOL and pPUST on barley. (A) A modified WGA-FITC staining protocol based on the procedure proposed by Ayliffe et al (2011, 2013). (B) Representative samples that were macroscopically analysed for chlorosis (CHL) and pustule formation (INF) and microscopically analysed using pCOL and pPUST based on stereo micrographs of the first leaf. From left to right, accessions CIho 4196, Russell, and Manchuria. The scale bar is 1 mm and white boxes reflect the field of view used on barley.

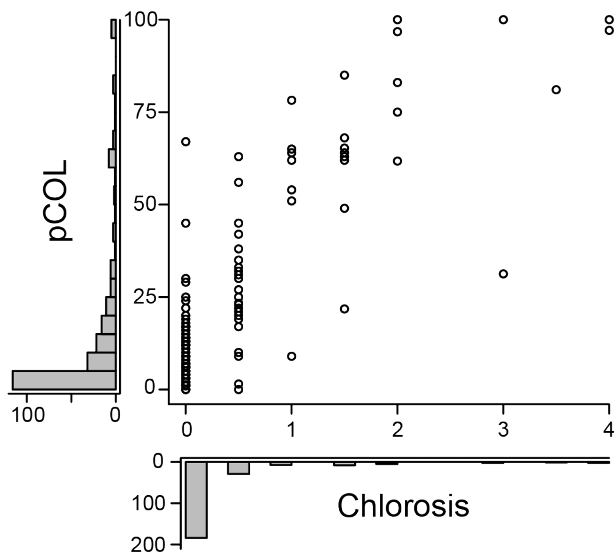


Figure 3: Macroscopic symptoms of chlorosis in barley are associated with leaf colonisation by *P. striiformis* f. sp. *tritici*. Plot comparing macroscopic chlorosis with microscopic pCOL phenotypes in barley challenged with *P. striiformis* f. sp. *tritici* isolate 08/21. Histograms showing the frequency of phenotypic observations are displayed on the corresponding axis.

fully predict pCOL, the correlation suggests that chlorosis is strongly associated with colonisation by *P. striiformis* f. sp. *tritici* in barley.

3.2.3. Barley is highly susceptible to *P. striiformis* f. sp. *hordei* and represents a host system.

To provide context for the differentiation between host and intermediate host systems it is essential to characterise each system in detail with identical phenotypic assays. On this premise, we assessed the applicability of pCOL on the barley-barley stripe rust (*P. striiformis* f. sp. *hordei*) system. To do this, we inoculated a collection of barley accessions with *P. striiformis* f. sp. *hordei* isolate B01/2 and phenotyped using the ten-point scale proposed by McNeal (McNeal et al., 1971) and pCOL (Fig. 4A). All accessions exhibited some degree of colonisation with the lowest observed pCOL at 29.1% and only four accessions exhibiting less than 50% pCOL. The majority of accessions (90%; 173/193) displayed greater than 75% pCOL. When phenotyped using the McNeal scale, accessions exhibited phenotypes ranging from 1 (necrotic/chlorotic flecks without pustule formation) to 8 (abundant pustule formation with chlorosis) in their phenotype and had a relatively equal distribution across the McNeal scale (Fig. 4A). Strikingly, the majority of accessions (64%; 123/193; McNeal scale ≥ 3) showed some degree of pustule formation. This observation highlights the differentiation between host and nonhost systems and the need to develop a microscopic assay to specifically quantify pustule formation. Thus, we established a phenotypic assay to determine the percent of the leaf harbouring pustules (pPUST; Fig. 2B). We observed a strong correlation between the McNeal and pPUST phenotypes ($\rho = 0.92$; Fig. 4B). This association was expected, as the McNeal scale was

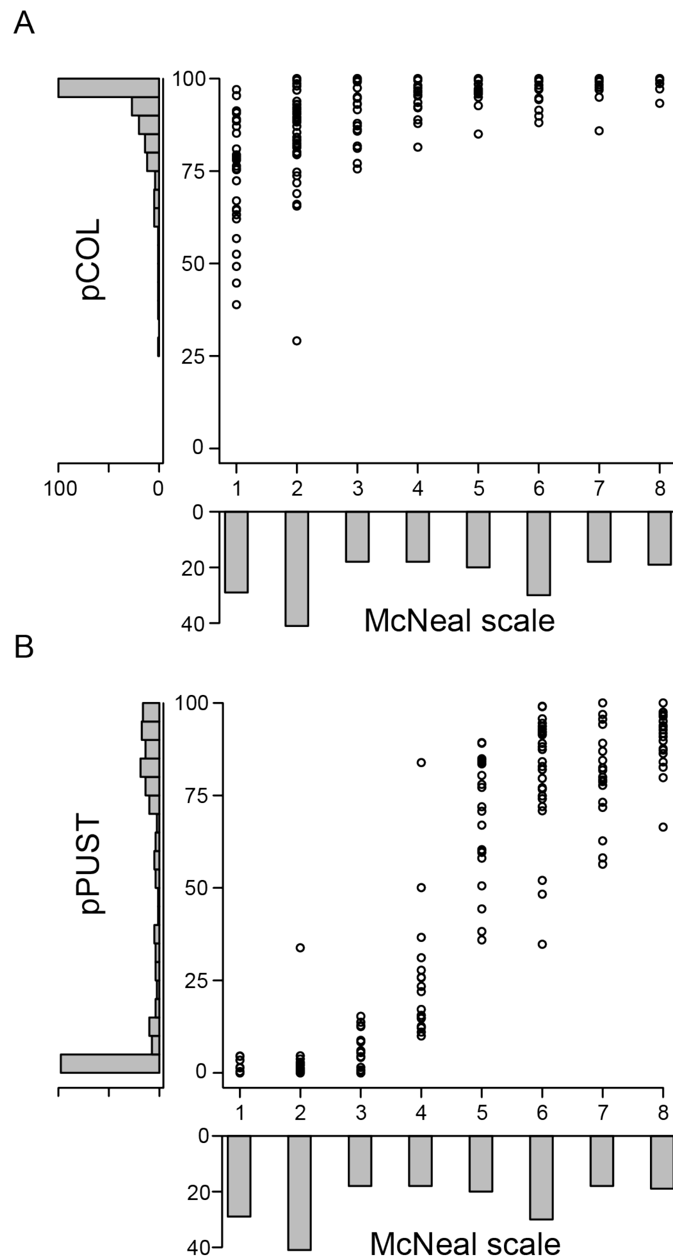


Figure 4: Comparison of macroscopic and microscopic phenotypes in the barley-*P. striiformis* f. sp. *hordei* interaction. Plots comparing the macroscopic McNeal scale with the microscopic phenotypes (A) pCOL and (B) pPUST in barley accessions challenged with *P. striiformis* f. sp. *hordei* isolate B01/2. Histograms showing the frequency of phenotypic observations are displayed on the corresponding axis.

developed for use on host systems, particularly for assessing the extent of pustule formation on infected leaves. However, a non-linear relationship was clearly evident between the McNeal and pPUST phenotypes. This suggested that the McNeal scale was not optimal for describing variation in leaf area with pustules, particularly when pustule density was between 25% and 75% of the leaf surface area. At these pustule densities pPUST is a more suitable phenotype as it identifies variation in a linear scale. Similarly, pCOL also captured additional variation in disease severity at the lower end of the McNeal scale (0 and 3). However, the McNeal scale may be describing additional variation as compared to pPUST when pustule density increased above 75% of the leaf surface area

(scores of 7 or above). The McNeal scale takes into account greater pustule density and stress responses (necrosis/chlorosis) at scores greater than or equal to 7, which is not taken into account with the pPUST microscopic phenotype. Taken together, these differing phenotypic scales uncover additional variation in the interaction of barley-*P. striiformis* f. sp. *hordei* and can be used in conjunction with existing macroscopic phenotypic assays to provide greater resolution of the phenotypic variation observed within a large collection of accessions.

3.3. Discussion.

Pathosystems that exist in the transitory state between host and nonhost have been described as intermediate systems. Intermediate systems are proposed to involve a limited number of accessions being susceptible to a pathogen species, or limited numbers of isolates being able to infect a given plant species (Bettgenhaeuser et al., 2014). Few intermediate systems have been studied and we have a restricted understanding of the genetic architecture of resistance underlying such systems. This may be attributed to (1) a lack of robust, quantitative phenotypic assays applicable to these systems or (2) the inability to identify rare accessions that support colonisation or the full life cycle of a pathogen. In this chapter, we have developed two quantitative microscopic assays and applied them to barley-*P. striiformis* interactions to identify an intermediate host system.

3.3.1. pCOL and pPUST: Microscopic phenotypic assays for dissecting the lifecycle of *P. striiformis*.

Dissection of the architecture of resistance in intermediate systems requires robust phenotypes. Robustness is a broad term that describes the favourable combination of resolution, accuracy, precision, throughput, and biological context of the phenotypic assay. These five criteria for assessment will be influenced by the nature of the information that can be assessed in any given pathosystem. Therefore, phenotypic assays require calibration to the system being studied and will differ depending upon the infection stage that is being observed (e.g., spore differentiation, haustoria formation, colonisation, or pustule formation). For example, Jafary and co-workers used macroscopic observation of pustule formation to show the incidence of quantitative trait loci (QTL) that govern intermediate host status in barley-*Puccinia* pathosystems (Jafary et al., 2006, Jafary et al., 2008). Similarly, macroscopic observation of life cycle completion has been used to identify gene-for-gene interactions in Poaceae-*B. graminis* and Poaceae-*M. oryzae* nonhost pathosystems (Tosa et al., 1987, Tosa et al., 1988, Tosa and Sakai, 1990, Inukai et al., 2006, Nga et al., 2012). However, the phenotypes used in these systems are largely dependent upon the completion of the pathogens' life cycle, something that may not always manifest

in intermediate systems. Therefore, in intermediate systems, it is necessary to use microscopic phenotypes to assess the extent to which a pathogen infects a potential host. Indeed, microscopic evaluation has been used to demonstrate variation in spore differentiation and infection structure development of *Puccinia* rust fungi on *B. distachyon* (Figueroa et al., 2013). Similarly, Ayliffe *et al*. (2011) used a WGA-Alexa488 microscopy-based assay to successfully demonstrate that several cereal rusts including *P. graminis* f. sp. *tritici* were able to establish basic compatibility on rice. We adapted this protocol to develop two assays, pCOL and pPUST, for quantifying levels of colonisation and pustule formation, respectively. Application of these assays to two intermediate Poaceae-stripe rust pathosystems allowed us to visualize the progression of this spreading pathogen. This revealed that resistance was conditioned at two different stages: colonisation and life cycle completion (i.e., pustule formation).

The development of microscopic assays established a link between macroscopic observations of chlorosis and browning with the ingress of *P. striiformis* f. sp. *tritici* in challenged barley leaves. Chlorosis is a common phenotypic response observed during plant-pathogen interactions (Mishra et al., 2015). However, they are also prototypical phenotypes implicated in a plethora of abiotic stress responses (Drew and Sisworo, 1977, Sicher, 1997, Shaibur et al., 2008). As such, ambiguity can exist as to the exact underlying cause of such phenotypes and this may impede their use in classical genetic analyses. This study has provided strong evidence that the macroscopic observation of chlorosis in barley is linked to leaf colonisation by *P*. *striiformis* f. sp. *tritici*. Disambiguation of this response from other potential abiotic responses opens up the possibility of using this phenotype to dissect the genetic architecture of resistance to nonhost pathogens in intermediate systems in parallel with macroscopic phenotypic assays.

3.3.2. The barley-*P. striiformis* f. sp. *tritici* interaction can be used as a model for investigating intermediate host resistance.

Conceptualisation of nonhost resistance has moved away from binary approaches to continuous models. The terms intermediate host and intermediate nonhost have been introduced to describe systems that are in the transition between host and nonhost states (Bettgenhaeuser et al., 2014). Classification into these four states is dependent upon the analysis of representative sets of accessions from different plant species relative to host and nonhost pathogens. We applied pCOL and pPUST to representative samples of the barley-*P*. *striiformis* f. sp. *hordei* (host system) and barley-*P*. *striiformis* f. sp. *tritici* (intermediate host system) pathosystems. These systems represent a stepwise progression from host through to intermediate host status and clearly demonstrated a

general reduction in infection with increasing evolutionary distance from the host. The incidence of life cycle completion decayed quicker than incidence of pustule formation as seen by the absence of pustules in the intermediate systems and limited variation for colonisation in the host system. To further validate this hypothesis, it will be necessary to survey more pathosystems that span the evolutionary continuum between host and nonhost.

As a whole, this study has demonstrated the efficacy of the interaction of stripe rust with barley as model systems for studying resistance to nonhost pathogens in intermediate systems. The development of two robust, quantitative phenotypic assays facilitated the disambiguation of asymptomatic phenotypes observed in nonhost interactions. Using these systems we now hope to dissect the genetic architecture underlying resistance.

4. An *R* gene complex protects barley against wheat stripe rust.

4.1. Introduction.

Plants depend on an innate immune system to fend off would be pathogens (Jones and Dangl, 2006). This system involves the action of immune receptors, which serve to recognise pathogen-derived molecules and perceive danger signals (Boller and Felix, 2009). The intimate interaction between plant host and pathogen often results in a simple interaction whereby a single gene in the host codes for a protein that detects, directly or indirectly, a single pathogen protein (Jones and Dangl, 2006, van der Hoorn and Kamoun, 2008). In this instance, the trait has a qualitative contribution to the phenotype being studied and is inherited according to Mendelian principles (Flor, 1956a). Many single gene interactions have been described for host pathosystems (Jia et al., 2000, Deslandes et al., 2003, Dodds et al., 2006, Ueda et al., 2006). In contrast, during nonhost interactions, the phenotypes involved are often quantitative and the genetic factors underlying the interaction are often polygenic, functionally overlapping, and influenced by the environment (Niks, 1987, Niks, 1988). Deciphering the genetic basis of these complex traits is an important endeavour as nonhost resistance is durable and may benefit agriculture if the determinants can be engineered into crop species (Stuiver and Custers, 2001). To date, progress has been made in understanding the pre-penetration resistance conferred by *PEN* genes in *A. thaliana* during the nonhost interaction with *B. graminis* f. sp. *hordei* (Collins et al., 2003, Lipka et al., 2005, Stein et al., 2006). However, less is known about the genetic determinants of intermediate systems that span the transition from host to nonhost. Intermediate pathosystems are proposed to rely upon a mixture of simple Mendelian factors, and more complex quantitative elements, depending on the evolutionary separation form the host (Schulze-Lefert and Panstruga, 2011, Bettgenhaeuser et al., 2014).

The key to dissecting the genetic factors underlying resistance in host, intermediate, or nonhost systems is the identification of phenotypic variation. Nonhost pathosystems are defined by a complete lack of susceptibility to a given pathogen species and consequently lack variation (Niks, 1987, Niks, 1988, Heath, 2000). Therefore, defining the genetic factors in these systems requires induced variation approaches using mutagenesis (Collins et al., 2003, Ayliffe et al., 2010). Contrastingly, intermediate systems are defined by low frequencies of susceptibility, namely pustule formation, to a given pathogen species (Bettgenhaeuser et al., 2014). This presents an opportunity to study nonhost resistance using phenotypic variation that exists within intermediate host systems. In parallel with phenotypic variation, the advent of high throughput sequencing technologies has

prompted unparalleled access to genetic variation, allowing for the reconstitution of the genomic structure of almost any organism (Wu and Lin, 2006). Using these technologies, in conjunction with quantitative genetics, it is now possible to dissect the genetic architecture of QTLs to decipher their number, genomic position, effect size, interactions, and contribution to the phenotypic variance (Zeng et al., 1999). Furthermore, it is possible to map traits to the resolution of the causal genes in many crop species with large, repetitive genomes (Price, 2006).

Niks and co-workers have used the interaction between barley and several nonhost *Puccinia* species as evolutionary relevant models for understanding the genetic architecture of intermediate host resistance (Jafary et al., 2006, Jafary et al., 2008, Yeo et al., 2014, Niks et al., 2015). Key to these endeavours was the development of a highly susceptible barley accession known as SusPtrit (Atienza et al., 2004). SusPtrit was developed by identifying transgressive segregants in F_2 populations derived from crosses between landrace barley accessions. Subsequently, three DH populations were created using Vada, Golden Promise, and Cebada Capa crossed to SusPtrit (Jafary et al., 2006, Jafary et al., 2008, Yeo et al., 2014). The genetic architecture underlying resistance to several nonhost *Puccinia formae speciales* has been investigated using these populations. The resistance in all instances was reported to be complex and underpinned by multiple small effect loci located throughout the barley genome (Jafary et al., 2006, Jafary et al., 2008, Yeo et al., 2014, Niks et al., 2015). Occasionally, loci conferring resistance to different rust species co-localised. Two major effect loci were identified after screening the DH populations with eight distinct *Puccinia formae speciales.* The first locus, found in cv. Vada against *P. hordei* f. sp. *secalini,* mapped to chromosome 1H and the second, from Golden Promise effective against *P. hordei* f. sp. *murini,* mapped to chromosome 7H (Jafary et al., 2006, Yeo et al., 2014). Recently, Niks et al. (2015) reported on the genetic architecture of resistance to nonhost *P. striiformis formae speciales* using the Vada x SusPtrit DH population. Consistent with the observations made for other nonhost *Puccinia* rusts, they reported a polygenic architecture involving minor effect loci. Two loci, on chromosome 1H and 7H, conditioned resistance to both *P. striiformis* f. sp. *bromi* and *P. striiformis* f. sp. *tritici*. These observations contrasted with the relatively simple genetic architecture for resistance, to *P. striiformis* f. sp. *tritici*, published elsewhere. Pahalawatta and Chen (2005) reported two major effect loci, *RpstS1* and *rpstS2*, in barley cv. Steptoe that are effective against *P. striiformis* f. sp. *tritici*. While *rpstS2* was not mapped due to limited population size, *RpstS1* mapped to chromosome 4H. Sui et al. (2010) mapped a third major effect loci, *YrpstY1*, to the long arm of chromosome 7H. Interestingly, this locus has been shown to be functional against *P. striiformis* f. sp. *tritici* isolates in the U.S. (J.

Dubcovsky, personal communication). Resistance to *P. striiformis* f. sp. *pseudostriiformis*, a contemporary isolate of stripe rust found in Australia, is also underpinned by major effect loci on chromosomes 1H (Kamino et al., 2015), 5H, and 7H (Derevnina et al., 2015) although other minor effect loci also contribute to resistance. Taken together, these observations suggest that the genetic architecture of resistance to nonhost *formae speciales* in barley is mediated by the concerted action of major and minor effect loci. However, it is difficult to reconcile the findings of each of these studies due to the different phenotypes, size of mapping intervals, types of population used, and relatively small numbers of barley accessions interrogated in each study.

In this study, we use the interaction between barley and *P. striiformis* f. sp. *tritici* as a model for dissecting the genetic architecture of intermediate host resistance. We interrogate a representative panel of barley accessions for their reaction to the nonhost pathogen. Then, we utilise two microscopic assays, pCOL and pPUST, developed in the previous chapter, to identify three loci, *Rpst1*, *Rpst2*, and *Rpst3*, which determine host species specificity in barley at different stages of the pathogen lifecycle. *Rpst1* and *Rpst2* function in resistance at the level of colonisation by the pathogen and *Rpst3* functions at the level of pustule formation. We show that these loci function across a diverse range of barley accessions including wild, landrace, and elite accessions by assessing the contribution of each locus in 15 independent structured populations. We propose that they form part of a larger *R* gene complex that provides durable resistance.

4.2. Results.

4.2.1. There is a higher frequency of susceptibility to *P. striiformis* f. sp. *tritici* in wild versus 2-row elite barley accessions.

In the previous chapter, we inoculated a large panel of spring barley accessions, predominantly consisting of 2-row elite varieties, with *P. striiformis* f. sp. *tritici*. We wanted to investigate the genetic architecture of resistance in a more representative sample of the diversity found within barley. Therefore, we assembled a new panel of 129 accessions including wild (n=27), two-row landrace (n=4), six-row landrace (n=9), elite 2-row (n= 51), and elite 6-row (n=15) barley accessions (Table A2 and Fig. 5). To assess the genetic diversity of the panel we carried out a phylogenetic analysis using hierarchical clustering. To do this, we genotyped the panel, using the barley OPA1 (BOPA1) platform, which surveys 1,536 potential SNPs. A total of 1,258 SNPs with a minor allele frequency greater than 5% and less than 10% missing data were selected for inclusion in the cladogram. From this analysis we could observe four distinct clades (Fig. 5). Clade 1 (n=59) displayed clear differentiation of two-row elite spring barley accessions from all

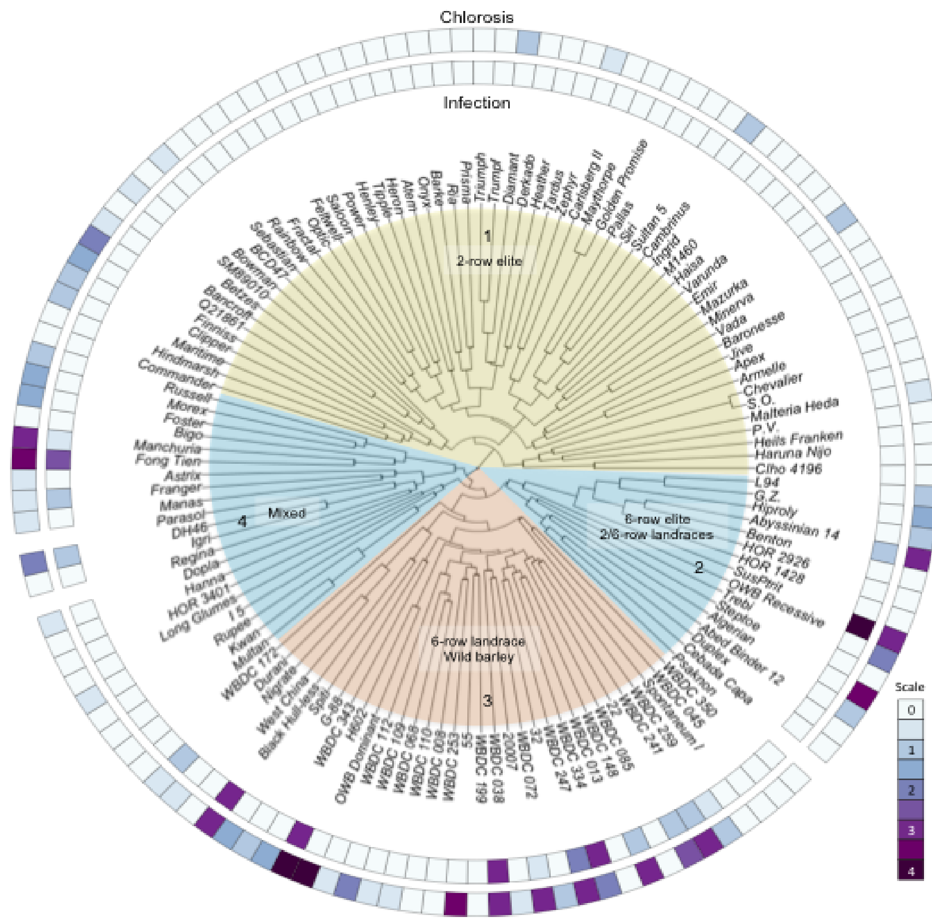


Figure 5: Unequal distribution of resistance to *P. striiformis* f. sp. *tritici* in diverse barley germplasm. A panel of 129 barley accessions were genotyped using Barley OPA1. A total of 1,258 SNPs having minor allele frequency greater than 5% and less than 10% missing data were used in generation of cladogram. Hierarchical clustering was performed using the *hclust* from the R module ape using default parameters with Euclidean distance estimates. Macroscopic phenotypes using 0 to 4 scales in 0.5 increments are displayed in surrounding rings. Outer and inner ring display chlorosis and infection, respectively.

other accessions. The second largest clade, clade 3 (n=33), harboured six-row landraces (n= 6) and wild (n=27) barley accessions. The two remaining clades, clade 2 and clade 4, did not harbour any wild accessions and were composed of admixtures of all other barley types. To assess the frequency of resistance in each clade we inoculated the panel with *P. striiformis* f. sp. *tritici* isolate 08/21 and phenotyped using the macroscopic observations of chlorosis and infection. We could observe that two-row elite barley accessions were highly resistant to *P. striiformis* f. sp. *tritici* with no accession in clade 1 exhibiting pustule formation (Fig. 5). Moreover, only 12 accessions (n=59; 20%) in clade 1 displayed the chlorosis phenotype and no score higher than 2. The observation of near complete resistance to *P. striiformis* f. sp. *tritici* in clade 1 contrasted sharply with the frequency of susceptibility observed in clade 3. Interestingly, this clade differentiated wild and six-row landrace accessions only. Of the 27 wild accessions in this clade, 18 (n=27; 67%) exhibited varying degrees of chlorosis and, of these accessions, nine displayed pustule formation (n=27; 33%).

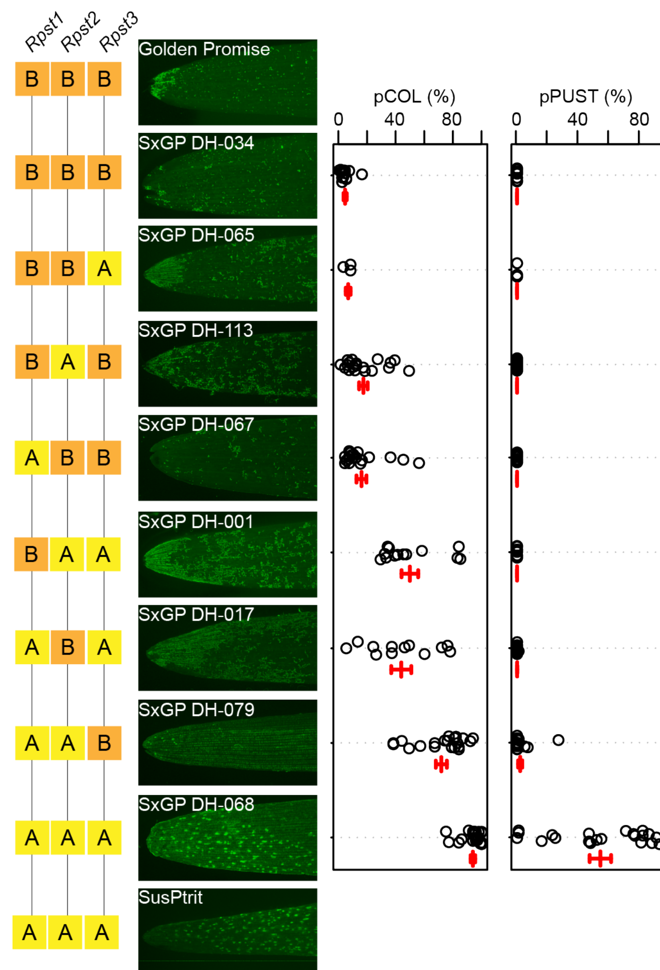


Figure 6: Three loci underpin resistance in Golden Promise to *P. striiformis* f. sp. *tritici*. Three-way phenotype x genotype plots for pCOL and pPUST show the contribution of *Rpst1*, *Rpst2*, and *Rpst3* to resistance in Golden Promise. Red error bars represent mean and standard deviation for each genotypic group. Micrograph images are of representative lines from the SusPtrit x Golden Promise DH visualised according to the pCOL staining procedure. 'A' and 'B' denote the SusPtrit and Golden Promise allele designation for each of the lines according to markers BOPA2_12_30817, SCRI_RS_155652, and BOPA1_4361−1867 for *Rpst1*, *Rpst2*, and *Rpst3*, respectively.

4.2.2. Three major effect loci confer resistance to *P. striiformis* f. sp. *tritici* in Golden Promise.

We observed that Golden Promise and SusPtrit were resistant and fully compatible, respectively, to *P. striiformis* f. sp. *tritici* isolate 08/21. Niks and co-workers previously used these accessions to dissect the genetic architecture of resistance to several nonhost *Puccinia* rust fungi by QTL mapping in a DH population (Yeo et al., 2014, Niks et al., 2015). Therefore, to investigate the genetic architecture of resistance to *P. striiformis* f. sp. *tritici* we inoculated the SusPtrit x Golden Promise DH population with race 08/21. We observed segregation of chlorosis and pustule formation in the population and microscopically quantified these traits using pCOL and pPUST. QTL analyses using composite interval mapping with the pCOL and pPUST phenotypic data detected three significant QTLs on chromosome 1HS, 4HL, and 7HL (Table 2). These loci mapped to, or near, the mildew

Table 2: Composite interval mapping of resistance to *P. striiformis* f. sp. *tritici* in several mapping populations.

Resistant Accession	Cross[1]	Type	Trait	Chromosome	cM	Peak Marker	LOD	EWT[2]	AEE[3]	PVE[4]
CIho 4196	Foster x CIho 4196	RIL	CHL	1H	8.93	owbGBS32429_p1	20.24	3.19	0.32	0.53
			CHL	6H	20.62	1_0136	3.82	3.19	0.12	0.07
			pCOL	1H	9.50	0711N16_R1_p1	14.99	3.17	0.10	0.38
			pCOL	1H	71.06	1_0357	4.69	3.17	0.05	0.09
			pCOL	7H	145.28	1_0888	4.37	3.17	0.05	0.09
Golden Promise	SusPtrit x Golden Promise	DH	CHL	1H	12.55	BOPA2_12_30817	19.64	3.26	0.76	0.28
			CHL	4H	89.12	BOPA1_4361-1867	6.73	3.26	0.39	0.07
			CHL	7H	161.20	SCRI_RS_155652	18.30	3.26	0.72	0.25
			INF	1H	14.21	BOPA2_12_30950	4.91	3.03	0.39	0.11
			INF	4H	89.95	SCRI_RS_121084	4.07	3.03	0.36	0.09
			INF	7H	164.52	SCRI_RS_206322	5.52	3.03	0.43	0.12
			pCOL	1H	12.55	BOPA2_12_30817	18.13	3.25	0.18	0.23
			pCOL	4H	89.12	BOPA1_4361-1867	12.54	3.25	0.14	0.14
			pCOL	7H	161.20	SCRI_RS_155652	20.20	3.25	0.19	0.26
			pPUST	1H	14.21	BOPA2_12_30950	4.51	3.10	0.08	0.10
			pPUST	4H	89.12	BOPA1_4361-1867	3.71	3.10	0.08	0.09
			pPUST	7H	164.52	SCRI_RS_206322	4.69	3.10	0.09	0.11
Haruna Nijo	Haruna Nijo x OUH602	DH	CHL	1H	13.34	1_0419	32.80	3.02	-1.23	0.69
			CHL	4H	91.75	2_1243	4.42	3.02	-0.37	0.06
			CHL	5H	109.89	1_1200	3.62	3.02	-0.33	0.05
			INF	1H	15.34	1_0419	7.52	2.92	-0.57	0.28
			INF	4H	91.49	1_0785	4.54	2.92	-0.39	0.13
			pCOL	1H	13.34	1_0419	32.88	3.12	-0.35	0.71
			pCOL	5H	97.21	2_1168	3.59	3.12	-0.09	0.05
			pCOL	7H	164.10	1_0454	4.05	3.12	-0.09	0.05
			pPUST	1H	15.34	1_0419	6.96	2.81	-0.14	0.27
			pPUST	2H	74.78	2_0528	2.81	2.81	0.07	0.08
			pPUST	4H	87.49	1_0785	3.66	2.81	-0.09	0.11

[1]Direction of cross is determined by Mother x Father, designation of alleles are A and B for Mother and Father, respectively.

[2]Allelic effect estimate, negative and positive values indicate resistance is contributed by the A and B alleles, respectively.

[3]Experiment-wide threshold.

[4]Percent of the phenotypic variation explained.

Table 3: Eighteen structured populations inoculated with *P. striiformis* f. sp. *tritici.*

Resistant Accession	Cross[1]	Type	N
Abed Binder 12	Manchuria x Abed Binder 12	F_2	96
	Manchuria x (Manchuria x Abed Binder 12 F1)	BC_1	90
Baronesse	Baronesse x Manchuria	F_2	93
	Manchuria x Baronesse	F_2	94
Betzes	Manchuria x Betzes	F_2	94
CIho 4196	Foster x CIho 4196	RIL	89
Duplex	Duplex x Manchuria	F_2	94
Emir	Manchuria x Emir	F_2	94
Golden Promise	SusPtrit x Golden Promise	DH	122
Grannenlose Zweizeilige	Grannenlose Zweizeilige x Manchuria	F_2	92
Haruna Nijo	Haruna Nijo x OUH602	DH	94
Heils Franken	Manchuria x Heils Franken	F_2	93
	Manchuria x (Manchuria x Heils Franken F1)	BC_1	93
HOR 1428	Manchuria x HOR 1428	F_2	94
HOR 2926	Manchuria x HOR 2926	F_2	92
I 5	I 5 x Manchuria	F_2	94
WBDC 008	WBDC 008 x Manchuria	F_2	92
WBDC 085	WBDC 085 x Manchuria	F_2	94

[1]Direction of cross is determined by Mother x Father, designation of alleles are A and B for Mother and Father, respectively.

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resistance loci *Mla* (1H), *mlo* (4H), and *Mlf* (7H) (Schönfeld et al., 1996, Büschges et al., 1997, Wei et al., 1999). We provisionally designated the 1H, 4H, and 7H QTLs *Resistance to Puccinia striiformis* f. sp*. tritici 1* (*Rpst1*), *Rpst3*, and *Rpst2*, respectively. *Rpst1*, *Rpst2,* and *Rpst3* collectively explained 63% and 30% of the phenotypic variation with respect to colonisation resistance (pCOL) and pustule formation (pPUST), respectively (Table 2). Interestingly, *Rpst3* explained less variation in the context of colonisation relative to *Rpst1* and *Rpst2* but was consistent with respect to preventing pustule formation. Three-way phenotype by genotype plots using pCOL and pPUST data confirmed the effect size estimates and additive effects of the three loci (Fig. 6). *Rpst1* and *Rpst2* provide complete resistance at the level of pustule formation regardless of their permutation with any of the other loci. *Rpst3* also inhibits pustule formation but permits very low levels of lifecycle completion in the absence of *Rpst1* and *Rpst2*. Individually, *Rpst1*, *Rpst2*, and *Rpst3* prevent pustule formation in isolation. The absence of all three loci results in a fully compatible interaction similar to the susceptible parent, SusPtrit. Comparably, the presence of all three loci results in an incompatible interaction similar to the resistant parent Golden

Promise (Fig. 6). The absence of *Rpst3* does not attenuate the resistance response conferred by *Rpst1* and *Rpst2*. However, variation in colonisation resistance is observed for each the three loci when present singularly. Independently, *Rpst1* and *Rpst2* permitted moderate levels of colonisation but the addition of *Rpst3* reduces colonisation by approximately 50%. *Rpst3* has a small effect on colonisation when functioning in isolation. *Rpst1*, *Rpst2*, and *Rpst3* follow a Mendelian pattern of inheritance when isolated independently in F_2 populations suggesting they are major *R* genes (data not shown). Taken together, these results demonstrate the genetic architecture underlying nonhost resistance to *P. striiformis* f. sp. *tritici* in Golden Promise is relatively simple and involves the concerted action of three genes: *Rpst1* and *Rpst2* conditioning colonisation resistance, and *Rpst3* conditioning resistance at the level of pustule formation. Furthermore, these results demonstrate that pCOL and pPUST can be used to dissect the *P. striiformis* f. sp. *tritici* life cycle, in a stepwise manner, to reveal the concerted action of complimentary resistance mechanisms functioning at different stages of pathogen infection.

4.2.3. *Rpst1*, *Rpst2*, and *Rpst3* function across the diversity of barley and are principal components of resistance to *P. striiformis* f. sp. *tritici*.

To assess the frequency of *Rpst1*, *Rpst2*, and *Rpst3* in barley, we inoculated the Foster x CIho 4196 recombinant inbred line (RIL) population and the Haruna Nijo x OUH602 DH population with *P. striiformis* f. sp. *tritici*. Composite interval mapping revealed that the major component of resistance in each population was *Rpst1*, explaining 38% and 71% of the phenotypic variation for colonisation resistance in the RIL and DH population, respectively (Table 2). In the Haruna Nijo DH population, evidence for *Rpst2* and *Rpst3* was observed in addition to weak QTLs on chromosome 2H and 5H (Table 2). *Rpst3* was detected at equivalent levels to the SusPtrit x Golden Promise DH population. The additional QTLs were minor in their effect size and only the 1H and 4H QTLs representing *Rpst1* and *Rpst3*, respectively, were identified across replicated datasets and phenotypes. The Foster x CIho 4196 population segregated for colonisation, but did not segregate for pustule formation. As a consequence, it is unlikely that *Rpst3* would be detectable. However, in addition to the major effect of *Rpst1* we observed minor effects for QTLs on 1H, 6H, and 7H (*Rpst2*). *Rpst1* was the only robust QTL identified in replicated datasets and consistent between the different phenotypes used. The observation of *Rpst1*, *Rpst2*, and *Rpst3* in CIho 4196 and Haruna Nijo prompted us to determine the extent to which these loci conditioned host status to *P. striiformis* f. sp. *tritici* in a more representative sample of barley accessions. Therefore, we initiated a crossing scheme to generatepopulations segregating for resistance to *P. striiformis* f. sp. *tritici* in order to understand the application of *Rpst1*, *Rpst2*, and *Rpst3* in a wider context.

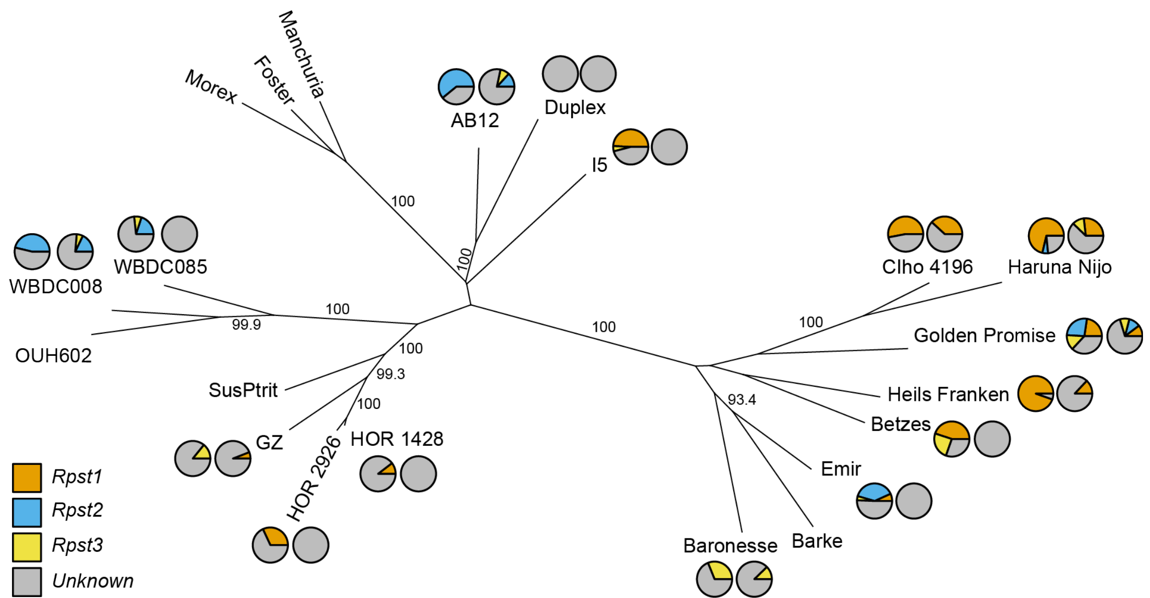


Figure 7: *Rpst1*, *Rpst2*, and *Rpst3* are present in diverse barley germplasm. The phylogenetic tree was generated with neighbour joining using 1,235 polymorphic sites according to parameters in Fig. 1. Support over 90% is shown at branch points in the phylogeny based on 1,000 bootstraps. Pie charts represent percent variation explained (PVE) for significant marker trait associations. Two pie charts are shown for each population analysed using two microscopic phenotypes, with the left chart representing pCOL and right chart representing pPUST.

Resistant accessions used as parental lines in crosses were selected using the phylogenetic relationship and phenotypic information collected for the barley diversity panel to achieve representative sampling of diversity. A total of 15 structured populations (F_2 and BC_1) were created and in each case the susceptible parent was Manchuria, a 6-row landrace (Table 3). Polymorphic markers linked to *Rpst1*, *Rpst2*, and *Rpst3* were identified for each population using the OPA genotyping data (Table 4). OPA markers were converted to KASP markers and assayed on a minimum of 90 individuals for each population. Populations were phenotyped and marker trait associations used to ascertain linkage with the *Rpst1*, *Rpst2*, and *Rpst3* loci in each population (Fig. 7 and Table 4). The original DH and RIL populations were also included in this analysis. Strikingly, evidence for all three genes was observed across the phylogenetic tree, shown as the proportion of phenotypic variation explained (PVE) for significant linkages (Fig. 7). Among all populations surveyed, only one 6-row landrace accession, Duplex, harboured a distinct resistance specificity. *Rpst1* was observed in 67% (10 out of 15) of the populations analysed. In four instances *Rpst1* was detected independent from *Rpst2* and *Rpst3* with effect sizes ranging from 10-95% PVE with respect to colonisation resistance (Fig. 7 and Table 4). *Rpst2* was detected at a lower frequency, contributing to resistance in 40% (6 out of 15) of the populations. *Rpst2* was not detected independently from *Rpst1* or *Rpst3* in any of the populations analysed. *Rpst3* was detected in 67% (10 out of 15) of the populations. *Rpst3* functioned independently from *Rpst1* and *Rpst2* in Baronesse with effect sizes of 31% and 12% for colonisation and pustule formation resistance, respectively. In the presence of *Rpst1*

Table 4: Marker-trait association at the *Rpst1*, *Rpst2*, and *Rpst3* loci in diverse barley F_2 populations inoculated with *P. striiformis* f. sp. *tritici.*

Resistant Accession	Cross[1]	Type	Trait	Chromosome	Locus	Marker	LOD	MTT[2]	AEE[3]	DEE[4]	DEE / AEE	PVE[5]
Abed Binder 12	Manchuria x Abed Binder 12	F_2	CHL	7H	Rpst2	U32_3345_P1	8.35	1.36	0.61	-0.77	-1.27	33.01
			INF	7H	Rpst2	U32_3345_P1	2.29	1.13	0.24	-0.24	-1.00	10.40
			pCOL	7H	Rpst2	U32_3345_P1	20.09	1.35	0.26	-0.20	-0.78	60.94
			pPUST	4H	Rpst3	3_0718_60_R	2.12	1.35	-0.06	-0.04	0.55	8.50
			pPUST	7H	Rpst2	U32_3345_P1	3.21	1.17	0.07	-0.05	-0.82	13.21
	Manchuria x (Manchuria x Abed Binder 12)	BC_1	CHL	4H	Rpst3	3_0718_60_R	2.28	0.98	NA	0.58	NA	2.93
			CHL	7H	Rpst2	U32_3345_P1	26.32	0.85	NA	2.72	NA	67.16
			INF	7H	Rpst2	U32_3345_P1	6.46	0.90	NA	1.47	NA	26.55
			pCOL	4H	Rpst3	3_0718_60_R	1.60	0.81	NA	0.12	NA	2.02
			pCOL	7H	Rpst2	U32_3345_P1	26.48	0.84	NA	0.69	NA	69.19
			pPUST	4H	Rpst3	3_0718_60_R	0.92	0.81	NA	0.10	NA	3.41
			pPUST	7H	Rpst2	U32_3345_P1	5.78	0.84	NA	0.27	NA	24.30
Baronesse	Baronesse x Manchuria	F_2	CHL	4H	Rpst3	2_0732_120_F	7.39	1.29	-0.85	-0.34	0.40	30.64
			INF	4H	Rpst3	2_0732_120_F	2.11	1.22	-0.06	-0.06	1.00	9.93
			pCOL	4H	Rpst3	2_0732_120_F	7.51	1.34	-0.12	-0.05	0.44	31.07
			pPUST	4H	Rpst3	2_0732_120_F	2.65	1.08	-0.02	-0.02	1.19	12.29
	Manchuria x Baronesse	F_2	INF	4H	Rpst3	2_0732_120_F	12.84	1.43	0.86	-0.81	-0.94	46.68
			pPUST	4H	Rpst3	2_0732_120_F	12.35	1.28	0.19	-0.17	-0.94	45.38
Betzes	Manchuria x Betzes	F_2	CHL	1H	Rpst1	HV5_963924_P1	14.19	1.35	0.95	-0.52	-0.54	40.68
			CHL	4H	Rpst3	1_0751_120_F	7.39	1.35	0.69	-0.35	-0.51	17.64
			pCOL	1H	Rpst1	HV5_963924_P1	17.74	1.43	0.29	-0.13	-0.44	44.95
			pCOL	4H	Rpst3	1_0751_120_F	9.71	1.39	0.20	-0.14	-0.69	19.73
			INF	1H	Rpst1	HV5_963924_P1	2.63	1.26	0.20	-0.29	-1.46	11.09
			INF	4H	Rpst3	1_0751_120_F	2.36	1.24	0.26	-0.18	-0.68	9.88
Emir	Manchuria x Emir	F_2	CHL	1H	Rpst1	HV5_963924_P1	3.19	1.32	0.28	-0.05	-0.16	8.13
			CHL	4H	Rpst3	GBS0288	1.81	1.41	0.22	-0.03	-0.13	4.46
			CHL	7H	Rpst2	U32_2966_P1	10.25	1.34	0.53	-0.50	-0.95	31.41

			pCOL	1H	Rpst1	HV5_963924_P1	2.88	1.54	0.07	0.02	0.34	6.80
			pCOL	4H	Rpst3	GBS0288	1.66	1.44	0.06	-0.02	-0.34	3.80
			pCOL	7H	Rpst2	U32_2966_P1	12.68	1.37	0.18	-0.16	-0.88	38.73
Grannenlose Zweizeilige	Grannenlose Zweizeilige x Manchuria	F_2	CHL	4H	Rpst3	2_0384_120_F	1.85	1.30	-0.44	0.26	-0.60	8.85
			pCOL	4H	Rpst3	2_0384_120_F	2.88	1.40	-0.13	0.12	-0.96	13.41
			INF	1H	Rpst1	206D11_T7_p1_281_F	2.30	1.16	0.19	-0.13	-0.66	10.89
			pPUST	1H	Rpst1	206D11_T7_p1_281_F	1.22	1.18	0.02	-0.01	-0.45	5.93
Heils Franken	Manchuria x Heils Franken	F_2	CHL	1H	Rpst1	206D11_T7_p1_281_F	61.27	1.45	1.49	-1.47	-0.99	95.19
			pCOL	1H	Rpst1	206D11_T7_p1_281_F	59.08	1.34	0.44	-0.41	-0.92	94.64
			INF	1H	Rpst1	206D11_T7_p1_281_F	2.82	1.35	0.30	-0.24	-0.81	13.05
			pPUST	1H	Rpst1	206D11_T7_p1_281_F	2.82	1.21	0.06	-0.05	-0.93	13.01
	Manchuria x (Manchuria x Heils Franken)	BC_1	CHL	1H	Rpst1	206D11_T7_p1_281_F	6.73	0.78	NA	-1.50	NA	28.88
			pCOL	1H	Rpst1	206D11_T7_p1_281_F	7.89	0.86	NA	-0.29	NA	32.36
			INF	1H	Rpst1	206D11_T7_p1_281_F	3.67	0.83	NA	-0.85	NA	16.97
			pPUST	1H	Rpst1	206D11_T7_p1_281_F	3.03	0.75	NA	-0.16	NA	13.92
HOR 1428	Manchuria x HOR 1428	F_2	CHL	1H	Rpst1	HV5_963924_P1	2.64	1.26	0.50	-0.30	-0.59	12.14
			pCOL	1H	Rpst1	HV5_963924_P1	2.24	1.35	0.14	-0.05	-0.35	10.41
HOR 2926	Manchuria x HOR 2926	F_2	CHL	1H	Rpst1	HV5_963924_P1	12.25	1.28	0.78	-0.55	-0.71	42.50
			pCOL	1H	Rpst1	HV5_963924_P1	7.69	1.39	0.19	-0.13	-0.65	31.95
I 5	I 5 x Manchuria	F_2	CHL	1H	Rpst1	206D11_T7_p1_281_F	11.71	1.27	-1.02	-0.54	0.53	43.67
			pCOL	1H	Rpst1	206D11_T7_p1_281_F	14.45	1.35	-0.30	-0.15	0.50	49.20
			pCOL	4H	Rpst3	1_0510_120_F	1.96	1.37	-0.09	-0.01	0.10	4.82
WBDC 008	WBDC 008 x Manchuria	F_2	CHL	7H	Rpst2	SCRI_RS_185445_60_F	13.49	1.32	-1.61	0.21	-0.13	49.09
			pCOL	7H	Rpst2	SCRI_RS_185445_60_F	12.41	1.29	-0.32	0.05	-0.14	46.26
			INF	4H	Rpst3	1_1398_77_F	2.05	1.27	-0.27	-0.16	0.61	7.92
			INF	7H	Rpst2	SCRI_RS_185445_60_F	3.92	1.28	-0.37	-0.36	0.95	15.87
			pPUST	4H	Rpst3	1_1398_77_F	1.50	1.13	-0.06	-0.03	0.46	5.75
			pPUST	7H	Rpst2	SCRI_RS_185445_60_F	4.34	1.46	-0.10	-0.09	0.90	17.89
WBDC 085	WBDC 085 x Manchuria	F_2	CHL	4H	Rpst3	1_1398_77_F	1.60	1.38	-0.35	0.54	-1.53	6.27
			CHL	7H	Rpst2	OZ_43640_p1_174_F	3.85	1.35	-0.81	0.30	-0.37	15.99

pCOL	4H	Rpst3	1_1398_77_F	1.83	1.44	-0.10	0.07	-0.66	6.84
pCOL	7H	Rpst2	OZ_43640_p1_174_F	4.92	1.43	-0.19	0.08	-0.43	19.93

[1]Direction of cross is determined by Mother x Father, designation of alleles are A and B for Mother and Father, respectively.

[2]Marker-trait permutation threshold.

[3]Allelic effect estimate, negative and positive values indicate resistance is contributed by the A and B alleles, respectively.

[4]Dominance effect estimate.

[5]Percent of the phenotypic variation explained.

and/or *Rpst2*, *Rpst3* consistently had smaller effect sizes due to the masking effect of the other genes. It is entirely feasible that *Rpst3* was functional in more populations but we were unable to detect it due to the absence of pustule formation conferred by the presence of *Rpst1* and or *Rpst2*. Three two-row elite cultivars, Haruna Nijo, Golden Promise, and Emir, provided evidence for all three genes functioning together in the same accession. Taken together, these results suggest that three major loci govern resistance to *P. striiformis* f. sp. *tritici* in wild, landrace, and elite barley accessions. The prevalence of the loci and their major contribution to resistance in all but one accession suggest that they are principal components of resistance in barley.

4.3. Discussion.

The *formae speciales* divide of *Puccinia* rust species provides an opportunity to study nonhost resistance in evolutionary relevant pathosystems that occur in the transition between host and nonhost status. At present, there is a dearth of reliable, reproducible, and robust systems for studying intermediate host resistance. In this study, we have demonstrated that the genetic architecture underlying host status in the interaction between barley and *P. striiformis* f. sp. *tritici* is mediated by three loci, *Rpst1*, *Rpst2*, and *Rpst3*. Using microscopic phenotypes that dissect the pathogen lifecycle in a stepwise manner we show that *Rpst1* and *Rpst2* function in resistance at the level of colonisation and that *Rpst3* functions at the level of pustule formation. All three genes determine host status across the spectrum of barley diversity including wild and domesticated (landrace and elite) accessions. We propose that these genes are principal components of a natural, durable, *R* gene complex that protects barley against a nonhost pathogen.

Rpst2 and *Rpst3*, identified in this study, colocalise with two previously reported loci determining host species specificity to *P. striiformis* f. sp. *tritici* in barley. Pahalawatta and Chen (2005) mapped *RpstS1* to a 4.5 cM region on chromosome 4H using resistance gene analog polymorphism (RGAP) markers and it appears to colocalise with *Rpst3*. The same group identified an additional major effect locus but were not able to genetically map it due to its recessive mode of inheritance. Sui et al. (2010) identified a third major locus, *YrpstY1*, on the long arm of chromosome 7H using nine simple sequence repeat (SSR) markers. *YrpstY1* was mapped to a 40 cM region and colocalises with *Rpst2*. The observation of major effect loci conditioning nonhost resistance is consistent with those of Tosa and co-workers who first demonstrated that major effect genes played a role in the specificity of wheat to nonhost *B. graminis formae speciales* (Tosa and Sakai, 1990, Tosa et al., 1988, Tosa et al., 1987). This work set a precedent for re-evaluating the contribution of major effect genes to nonhost resistance, which were previously considered to determine

specificity at the race-cultivar taxonomic threshold only (Heath, 2000). The identification of three major loci determining host species specificity in barley to *P. striiformis* f. sp. *tritici* in this study provides additional evidence for the role of major *R* genes in determining resistance to nonhost pathogens.

4.3.1. An *R* gene complex protects barley against *P. striiformis* f. sp. *tritici*.

Barley is grown in temperate regions of the world and occupies an agricultural niche similar to wheat (Ullrich, 2011). Thus, barley, inevitably, is exposed to wheat-adapted pathogens at significant disease pressures. Despite this, barley remains highly resistant to *P. striiformis* f. sp. *tritici* yet is readily infected by the closely related, barley-adapted form, *P. striiformis* f. sp. *hordei* (Straib, 1937, Chen et al., 1995, Pahalawatta and Chen, 2005, Sui et al., 2010). A critical example of the durability of resistance is on going in Australia. *P. striiformis* f. sp. *tritici* was first detected in 1979 and to date has been unable to complete a host jump onto barley (Wellings, 2007). This prompts us to ask: what determines the specificity of this interaction and what are the costs of specialisation to the pathogen? And, why has barley, even in the absence of the host pathogen, maintained such a high frequency of durable resistance to *P. striiformis* f. sp. *tritici* despite, presumably, significant disease pressure in the field? Johnson (1981) first defined durability as disease resistance that remains effective despite widespread use within agriculture. Discussions on the best strategies and proposed mechanisms of durable disease resistance often revolve around the effectiveness of minor (or quantitative/partial) versus major (qualitative/complete) resistance (Poland et al., 2009). This distinction is inherently vague as the effect size of different loci can be subjective and dependent upon the phenotypic assays being used and genetic systems being studied. Niks and co-workers assert that intermediate host resistance in barley to several *Puccinia* species, including *P. striiformis* f. sp. *tritici*, is complex and involves the concerted action of multiple minor effect loci (Jafary et al., 2008, Jafary et al., 2006, Yeo et al., 2014, Niks et al., 2015, Niks and Marcel, 2009). This conclusion is principally based on QTL analyses in three DH populations inoculated with *P. hordei* f. sp. *murini*, *P. hordei* f. sp. *secalini*, *P. triticina*, *P. striiformis* f. sp. *tritici*, and *P. persistens*. In each instance, the DH populations segregate for multiple QTLs. In the present study, we provide evidence that QTLs involved in intermediate host resistance are additive and often mask the observable effect of other resistance loci. We also show that the phenotypic assay being used for QTL analyses will influence the effect size of a locus depending on how well suited the assay is for describing the biological context of the system. For example, *Rpst3* could be considered a minor effect locus when using QTL analyses on the Abed Binder 12 x Russell F_2 population when phenotyped with pPUST (9% PVE). This is because *Rpst2* masks the effect of *Rpst3*. In contrast, *Rpst3* has a much larger

effect size (45% PVE) in the Baronesse x Manchuria F_2 population in the absence of *Rpst1* and *Rpst2*. pPUST is a more representative assay for assessing the effect size of a locus that inhibits pustule formation. Indeed, in Niks' own work, two major effect loci have been detected on chromosome 1H and 7H against *P. hordei* f. sp. *secalini* and *P. hordei* f. sp. *murini*, respectively (Jafary et al., 2006, Yeo et al., 2014). Intriguingly these loci cosegregate with other "minor" effect loci detected against other *Puccinia formae speciales*. However, the major effects are observed in populations segregating for two QTLs whereas the minor effect loci often occur in the presence of ≥4 QTLs. Based on these observations, and the work presented in this chapter we transition away from a debate centred on major versus minor effect loci. Instead, we propose that intermediate host resistance in barley is conferred by a broad complex of *R* genes that function at different stages of the pathogen lifecycle to confer durable disease resistance. The prevalence of the loci detected in this study across a diverse panel of barley lines suggests that they are principal components of this resistance. However, we fully expect to identify additional loci in cultivars such as Duplex, which exhibits complete resistance to *P. striiformis* f. sp. *tritici* but does not harbour *Rpst1*, *Rpst2*, or *Rpst3*. This scenario would be highly analogous to the proposed stacking (or pyramiding) of *R* genes within agricultural cultivars to obtain durable disease resistance (McDonald and Linde, 2002, Mundt, 2014). Such stacks are proposed to be durable due to the costs associated with fitness decreases to the pathogen if it were to mutate multiple avirulence alleles simultaneously. Taken together we hypothesise, that durability of resistance in barley to *P. striiformis* f. sp. *tritici* is due to a broad complex of *R* genes. The cost of specialisation to the pathogen is extremely high and explains why no host jump has occurred in the absence of the host pathogen in Australia. This view is inline with the model for the evolution of the *formae speciales* proposed by Tosa, whereby the accumulation of *R* genes in a host leads to specialisation of the pathogen (Tosa, 1992).

4.3.2. The barley OPA does not fully describe variation in wild and landrace accessions due to ascertainment bias.

Several publications have reported on the rich genetic diversity found within wild and landrace accessions using traditional morphological marker based approaches (Brown et al., 1978, Nevo et al., 1986a, Nevo et al., 1986b, Jana and Pietrzak, 1988, Nevo et al., 1979, Liu et al., 2002), molecular markers (Baum et al., 1997, Fu and Horbach, 2012), and more contemporary approaches using high throughput genotyping and RNAseq analysis (Moragues et al., 2010, Dai et al., 2014, Russell et al., 2014). However, in the present study we do not observe significant variation in diversity between wild/landrace accessions and elite cultivars using genotypic information derived from the barley OPA. Moragues et al. (2010) made a similar observation after genotyping 169 barley landraces from Syria and

Jordan and 171 European barley cultivars using the barley OPA. The same group observed that the SNPs incorporated into the OPA assay were originally mined from expressed sequence tag (EST), cDNA libraries, or from PCR amplicons derived from a small panel of parental lines, often elite varieties used in existing DH populations. As the original assay was designed based on such a small population, only a subset of SNPs were discovered and rare alleles would not be included. Thus, when the assay is applied to larger populations, or more diverse materials, an ascertainment bias is introduced (Moragues et al., 2010). For our present work, the barley OPA provided sufficient resolution to enable us to differentiate wild and landrace cultivars from elite 2-row cultivars. However, a rigorous assessment of barley accessions according to their domestication status and row number will be needed to assess the context of *P. striiformis* f. sp. *tritici* resistance across the diversity of barley. Therefore, genotyping by sequencing, capture (complexity reduction) based approaches or RNAseq analysis tailored specifically to wild barley would be a viable option for representing the diversity in wild and landrace accessions.

4.3.3. Man-guided evolution of the *formae speciales.*

At a micro-evolutionary scale, we know human practice has a substantial impact on pathogen populations as observed through the constant emergence of new races virulent on deployed resistance genes (Johnson, 1961). In contrast, we have very little knowledge of the impact humans have, on the long-term evolution of plant pathogens during the process of domestication and improvement of the crops they infect. That said, it has been hypothesised that the *formae speciales* divide arose as a consequence of man's domestication and breeding of plants to support agriculture (Johnson, 1961, Tosa, 1992). These activities first started around 10,000 to 13,000 years ago (Pringle, 1998). Therefore, the *formae speciales* represent evolutionary relevant models for understanding more about human impact on the long-term evolution of plant pathogens. In the present work, it is striking that the incidence of susceptibility to *P. striiformis* f. sp. *tritici* is notably different with higher frequencies of susceptibility observed in wild versus domesticated accessions (33% versus 3%, respectively for pustule formation; INF). Furthermore, interrogation of two wild barley F_2 populations revealed the presence of *Rpst2* and *Rpst3* functioning in wild barley, suggesting that similar resistance alleles confer resistance in wild and domesticated plants. We hypothesise that the heightened frequency of resistance observed in domesticated barley is a consequence of a population bottleneck that occurred during domestication. Subsequently, the reduction in population size led to an increase in resistance allele frequency either due to passive mechanisms (genetic drift) or by active mechanisms (selection). It is difficult to speculate on the exact mechanism driving the increase in allele frequency. However, this led to higher allele frequencies that

promoted specialisation of the pathogen to the host. Tosa (1992) first proposed that the *formae speciales* arose due to the accumulation of major *R* genes in a specific host genus. In parallel, a proportion of the pathogen population then specialised to this host by losing corresponding avirulence determinants. This repeated process occurred on numerous host genera leading to the genesis of specialised forms adapted to specific host genera. Such a model would also reconcile the findings of Straib (1937) who observed that the *formae speciales* specialisation on domesticated plants quickly eroded when applied to wild relatives. In order to validate these ideas, it will be necessary to survey the genetic architecture of resistance in larger panels of wild, landrace, and domesticated species to ascertain allele frequencies in each evolutionary stepwise progression.

4.3.4. Multiple pathogen recognition specificity.

While humans have both innate and adaptive immune systems, plant immunity is based solely on innate immunity (Jones and Dangl, 2006). This means that the immune response of a plant is completely dependent on the finite number of immune receptors encoded by its genome. After sequencing several plant genomes, we know that the number of genes encoding immune receptors does not necessarily correlate with the number of pathogens that infect plant species (Meyers et al., 2003, Yang et al., 2006b, Yang et al., 2008, Gu et al., 2015, Guo et al., 2011, Jupe et al., 2012). Thus, a central question in plant immunity is: How can a plant be a nonhost to so many pathogens but encode a finite number of immune receptors? We have found that resistance to wheat stripe rust in barley colocalises with a significant number of previously reported loci conferring resistance to host and nonhost pathogens. This includes multiple intermediate host resistance specificities effective against nonhost *Puccinia formae speciales* (Pahalawatta and Chen, 2005, Jafary et al., 2008, Jafary et al., 2006, Sui et al., 2010, Derevnina et al., 2015, Kamino et al., 2015, Niks et al., 2015, Yeo et al., 2014) as well as nonhost resistance to *M. oryzae* (Inukai et al., 2006). In the latter example, a major effect locus, *Rmo1*, colocalised with *Rpst1* and QTLs were detected that colocalise with *Rpst2* and *Rpst3*. In the case of host resistance, *Rpst1*, *Rpst2*, and *Rpst3* colocalise with *B. graminis* f. sp. *hordei* resistance loci *Mla*, *Mlf*, and *mlo*, respectively (Wei et al., 1999, Schönfeld et al., 1996, Büschges et al., 1997). At present, the genetic resolution and different genetic backgrounds used in these studies precludes causal association between any of these loci. However, the recognition of multiple pathogens at each chromosomal region remains intriguing. The repeated finding of resistance specificities emanating from similar regions on chromosome 1H, 4H, and 7H suggests that similar mechanisms may underlie host and nonhost resistance to evolutionary distinct pathogens. Additional fine mapping and characterisation of these loci will be required to elucidate whether the same genes, different alleles, or closely linked genes from distinct gene families underlie these overlapping resistance specificities.

5. Isolation and fine mapping of *Rpst2*: An intermediate host resistance gene in barley effective against wheat stripe rust.

5.1. Introduction.

Nonhost resistance is often described as the complete resistance of an entire plant species to a specific pathogen (Heath, 2000, Mysore and Ryu, 2004, Nürnberger and Lipka, 2005). In the majority of cases, this definition will hold true, as generally, most plants remain healthy, despite the ubiquity of potentially pathogenic microbes in the environment. However, it is clear that some plant pathogen interactions do not prescribe to the qualitative separation of host and nonhost. Instead, they appear to exist in a transitional phase between the two states, where radial coevolution with microbial species leads to the erosion, or reinforcement, of host status to pathogenic microbes (Niks and Marcel, 2009, Schulze-Lefert and Panstruga, 2011). This 'coevolution' can be considered a short-term interaction relative to the evolutionary time of plant speciation. Under long-term timescales, the preponderance of evidence supports host-shift speciation rather than cospeciation in the evolution of plant and microbial species (de Vienne et al., 2013). In contrast, our understanding of the short-term dynamics of host specialisation remains poorly understood.

Host specialisation is often observed in the interaction of mildew and rust fungi with grasses, particularly the *formae speciales* divide of cereal rusts (Eriksson, 1894, Bushnell and Roelfs, 1984, Niks and Marcel, 2009). Eriksson (1894) first proposed the *formae speciales* to differentiate forms of cereal rusts that were pathogenically specialised to given host genera but were otherwise morphologically indistinguishable. However, it was found that the *formae speciales* were not exclusively restricted to their host genera (Straib, 1937) and the application to plant species outside of the host genera can result in varying degrees of compatibility: ranging from haustoria formation and hyphae colonisation continuing through to lifecycle completion and pustule formation (Bettgenhaeuser et al., 2014). Despite the observation of non-exclusivity, the *formae speciales* division has been maintained. Bettgenhaeuser et al. (2014) proposed that interactions involving inappropriate *formae speciales* and nonhost plant genera are intermediate host systems that exist in the evolutionary transition between host and nonhost.

To date, a number of studies have reported on the genetic architecture of intermediate host systems with the majority reporting evidence for the role of major loci underlying resistance to nonhost *formae speciales* (Tosa et al., 1988, Tosa and Sakai, 1990, Pahalawatta and Chen, 2005, Jafary et al., 2006, Jafary et al., 2008, Sui et al., 2010). So far,

no major locus conditioning intermediate host resistance has been cloned within the Triticeae tribe. However, numerous major loci have been cloned for host pathosystems with the majority coding for intracellular NLR proteins (Krattinger et al., 2009a). Whether the same observations will be made for major loci in intermediate systems is unclear. However, the proposed contribution of NLRs to nonhost immunity is now widely accepted despite the relatively few well-characterised examples (Thordal-Christensen, 2003, Mysore and Ryu, 2004, Schulze-Lefert and Panstruga, 2011). *RGA4* and *RGA5*, two tandemly arranged NLRs, have been shown to condition *Pi-CO39(t)* mediated resistance to a nonhost *M. oryzae* isolate in rice (Cesari et al., 2013). Similarly, *WRR4* conditions nonhost resistance to *A. candida*, the causal agent of white blister rust, in *A. thaliana* (Borhan et al., 2008). These observations support the molecular evolutionary model proposed by Schulze-Lefert and Panstruga (2011) that implicates NLRs in nonhost resistance. In the model, the authors assert that the contribution of NLR triggered immunity will decrease as a function of evolutionary divergence time from the host. Given the presumed evolutionary infancy of the *formae speciales* divide, one may hypothesise that major loci governing nonhost resistance in intermediate host systems may be underpinned by NLRs analogous to host systems. However, very little evidence exists to support this notion due to a lack of well-resourced, model pathosystems, with robust phenotypes, that permit the elucidation of the underlying molecular mechanisms of resistance.

In the previous chapter, we used barley as a model system for elucidating the genetic architecture determining specificity in its interaction with the nonhost pathogen, *P. striiformis* f. sp. *tritici*. We were able to dissect the pathogen lifecycle in a stepwise manner using two microscopic phenotypic assays for quantifying hyphal colonisation (pCOL) and pustule formation (pPUST) in leaves challenged with the pathogen. Linkage mapping using these phenotypic assays revealed that *P. striiformis* f. sp. *tritici* resistance in barley is conditioned by three major loci; *Rpst1* and *Rpst2* conferring colonisation resistance, and *Rpst3* conditioning resistance at the level of pustule formation. In this study, we test whether there is an overlap between resistance to the host pathogen, *P. striiformis* f. sp. *hordei* and the intermediate host pathogen, *P. striiformis* f. sp. *tritici*. We use the barley accession Abed Binder 12 that contains the *P. striiformis* f. sp. *hordei* resistance gene *rps2* (Nover and Scholz, 1969), which also harbours the intermediate host resistance gene *Rpst2*. After mapping *P. striiformis* f. sp. *tritici* resistance to chromosome 7H, we determine that host and intermediate host resistance are uncoupled and confirm that *Rpst2* is the major determinant of resistance to *P. striiformis* f. sp. *tritici* in Abed Binder 12. Subsequently, we isolate and fine map *Rpst2* to a 0.1 cM region and anchor the region to a single fingerprinted contig (FPC) in barley. We also use transcriptome assemblies in

combination with existing barley genomic resources to identify a candidate NLR gene that maps to the *Rpst2* locus. Future work on the cloning of *Rpst2* will establish the genetic basis for resistance and its contribution to nonhost resistance.

5.2. Results.

5.2.1. Nonhost resistance in Abed Binder 12 maps to chromosome 7HL and is genetically uncoupled from host resistance gene *rps2*.

Our initial hypothesis was that resistance to host pathogens would overlap with resistance to intermediate host pathogens. To test this hypothesis, we focused our attention on the unmapped *P. striiformis* f. sp. *hordei* resistance gene *rps2* that is present in the barley cultivar Abed Binder 12 (Nover and Scholz, 1969). Screening of Abed Binder 12 found it was highly resistant (McNeal score 1) to *P. striiformis* f. sp. *hordei* isolate B01/2, whereas cultivar Russell was highly susceptible (McNeal score of 8). Similar differential phenotypes were observed after inoculating Abed Binder 12 and Russell with *P. striiformis* f. sp. *tritici* isolates 08/501 and 08/21, although Russell rarely showed pustules but had a clear microscopic phenotype of colonisation (Fig. 8A-D). We wanted to understand the genetic architecture of *P. striiformis* f. sp. *tritici* resistance within Abed Binder 12 and to determine whether *rps2* contributes to resistance. We independently inoculated two Abed Binder 12 x Russell F_2 populations with *P. striiformis* f. sp. *hordei* isolate B01/2 (AxR-*Psh*) and *P. striiformis* f. sp. *tritici* isolate 08/501 (AxR-*Pst*). In both experiments, the parents, F_1 and 92 F_2 plants were phenotyped using macroscopic phenotyping, and in the case of *P. striiformis* f. sp. *tritici*, the microscopic evaluation of colonisation (pCOL). In the AxR-*Psh*, F_2 population, F_1 and segregation of F_2 individuals suggested the presence of a single recessive resistance gene conditioning pustule formation (28 resistant: 65 susceptible, model 1:3; $\chi^2 = 1.29$, $p = 0.26$; Fig. A1). Pustule formation was not observed for the AxR-*Pst* F_2 population, although segregation was observed for chlorosis and pCOL (Fig. 8E-F). A strong correlation between chlorosis and pCOL was observed ($r^2 = 0.88$) (Fig. 8G). The F_1 displayed similar resistant phenotype to Abed Binder 12, although it is difficult to ascertain the mode of inheritance without understanding the number of loci contributing to resistance.

To map resistance to *P. striiformis* f. sp. *tritici*, we genotyped the AxR-*Pst* F_2 population with the barley oligonucleotide assay (BOPA1), which interrogates 1,536 SNP-based markers (Close et al., 2009). A total of 535 polymorphic OPA markers were identified between Abed Binder 12 and Russell and they were used to generate a genetic map with eight linkage groups. Chromosome 7H was the only chromosome that spanned two linkage groups. A total of 26 CAPS markers and 28 Sequenom MassARRAY markers were used to

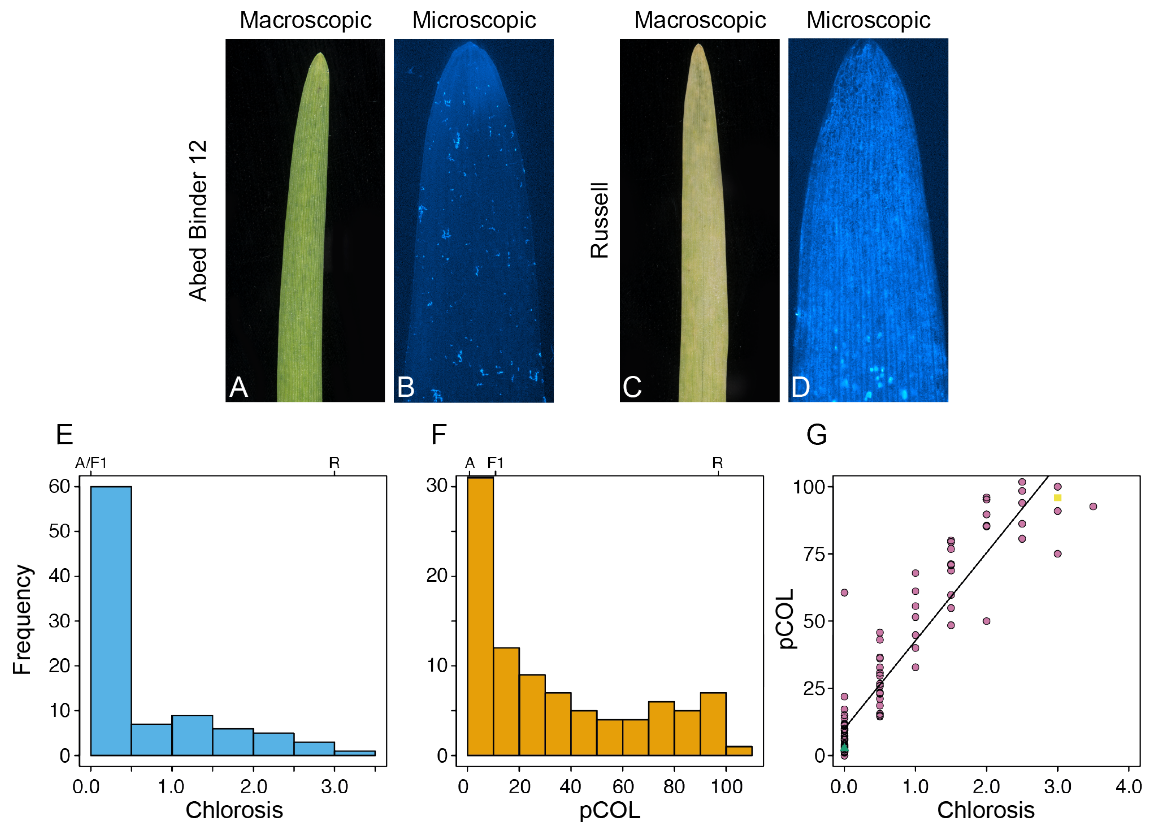


Figure 8: Chlorosis and pCOL segregate in the Abed Binder 12 x Russell F_2 mapping population inoculated with *P. striiformis* f. sp. *tritici* race 08/21. Macroscopic phenotypes showing immune reaction of (A) Abed Binder 12 and (C) chlorosis on Russell. Microscopic phenotypes of Abed Binder 12 (B) and Russell (D) collected by staining leaves with wheat germ agglutinin (WGA) conjugated to fluorescein isothiocyanate (FITC) and visualised using fluorescence microscopy. Histograms of individual lines for (E) macroscopic and (F) microscopic phenotyping, and (G) correlation between microscopic and macroscopic phenotypes on the Abed Binder 12 x Russell F_2 population.

bridge gaps between unlinked chromosome arms and increase marker density. The final map consists of 589 markers over seven linkage groups, representing 362 non-redundant marker haplotypes and a total genetic distance of 1,131 cM (Fig. 9 and Fig. A4). On average, each non-redundant marker was separated by approximately six recombination events that equated to a mean distance of 3.1 cM. Only 21 regions had genetic distances greater than 10 cM and the greatest distance was 27.9 cM. The quality of the genetic map was assessed using two point linkage tests between markers (Fig. A4). The majority of the genetic map did not exhibit segregation distortion, with only a slight reduction in heterozygosity on the long arm of chromosome 2H (marker 1_0214; $\chi^2 = 9.65$, $p = 0.003$).

We performed QTL analysis using composite interval mapping with chlorosis and pCOL phenotypes on the AxR-*Pst* population. We identified a major effect locus on the long arm of chromosome 7H that was contributed by Abed Binder 12 consistent with the observation of *Rpst2* in the previous chapter (Fig. 10 and Table 5). The QTL accounted for 57.7% and 69.4% of the phenotypic variation for chlorosis and pCOL, respectively. In both instances, marker U32_7356_p1, positioned at 169.7 cM, was the most strongly linked marker. Phenotype by genotype plots using this marker showed better clustering of the

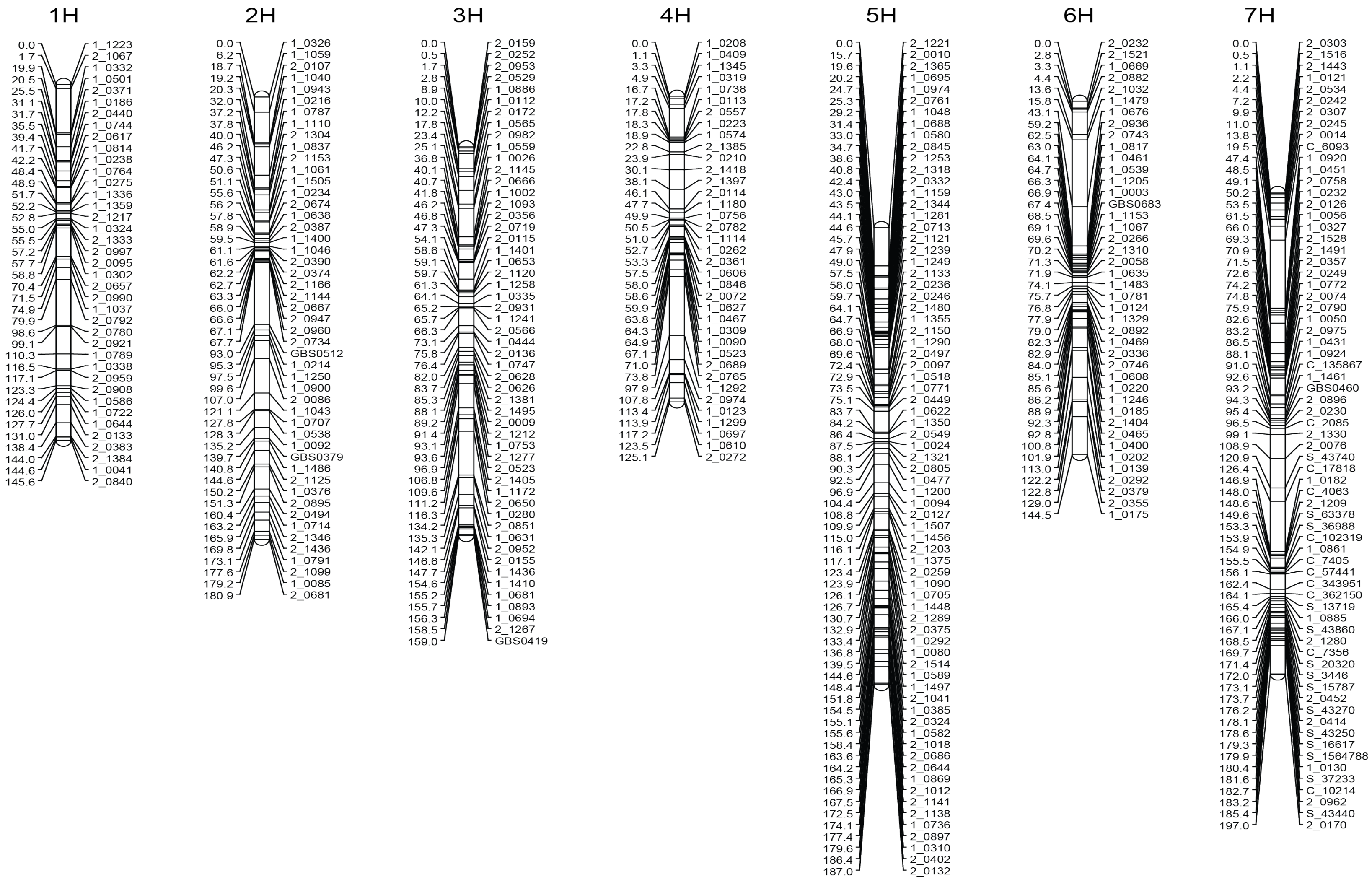


Figure 9: Genetic map of the Abed Binder 12 x Russell F_2 population using 362 non-redundant markers. Genetic distances were calculated using the Kosambi map function in cM.

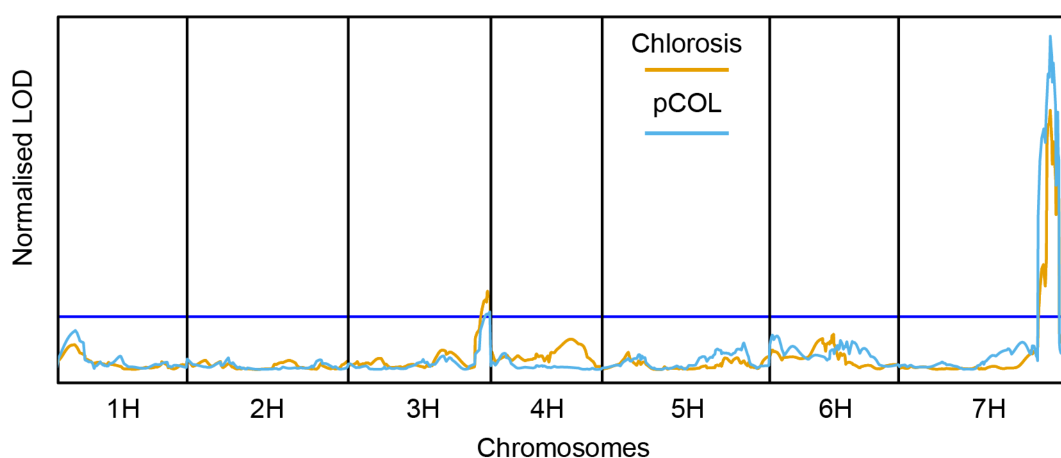


Figure 10: Composite interval mapping of chlorosis and pCOL phenotypes in the Abed Binder 12 x Russell F_2 population. QTL analyses identified a major effect locus on chromosome 7H that was *Rpst2* based on previous mapping studies (previous chapter). A small effect QTL was observed on chromosome 3H and was provisionally designated as *Qrpst3HL*. The experiment wide threshold (EWT; dark blue line) was determined using 1,000 permutations. The y-axis represents a normalised scale to the EWT for each trait.

Table 5: Composite interval mapping in the Abed Binder 12 x Russell F_2 population using chlorosis and pCOL.

Trait	Chr[1]	cM	Peak Marker	EWT[2]	LRTS[3]	LOD	AEE[4]	DEE[5]	DEE / AEE	PVE[6]
CHL	3H	155.7	1_0893	20.2	30.0	6.5	0.47	-0.07	-0.15	13.3
pCOL	3H	158.3	1_0694	19.3	21.2	4.6	0.12	0.03	0.21	7.7
CHL	7H	169.7	C_7356	20.2	99.3	21.6	-0.99	-0.51	0.52	57.7
pCOL	7H	169.7	C_7356	19.3	122.5	26.6	-0.40	-0.15	0.39	69.4

[1]Chromosome
[2]Experiment-wide threshold.
[3]Likelihood ratio test statistic.
[4]Allelic effect estimate, negative and positive values indicate resistance is contributed by the A and B alleles, respectively.
[5]Dominance effect estimate.
[6]Percent of the phenotypic variation explained.

susceptible lines using pCOL than chlorosis (Fig. A3A-C). However, despite these differences, the one and two LOD confidence intervals were consistent between the two datasets. A second minor effect QTL was identified on chromosome 3H that explains 13.3% and 7.7% of the phenotypic variation for chlorosis and pCOL, respectively (Table 5). Interestingly, the chromosome 3H QTL is contributed by Russell. A multiple QTL model was used to test for epistasis between the QTLs on chromosomes 3H and 7H, but no significant interactions could be observed (data not shown). The observation of a single major effect locus in Abed Binder 12 conditioning resistance to *P. striiformis* f. sp. *tritici* prompted us to investigate potential linkage with resistance to *P. striiformis* f. sp. *hordei*. We tested the SNP marker 2_0962 near the peak of the chromosome 7H QTL on both the

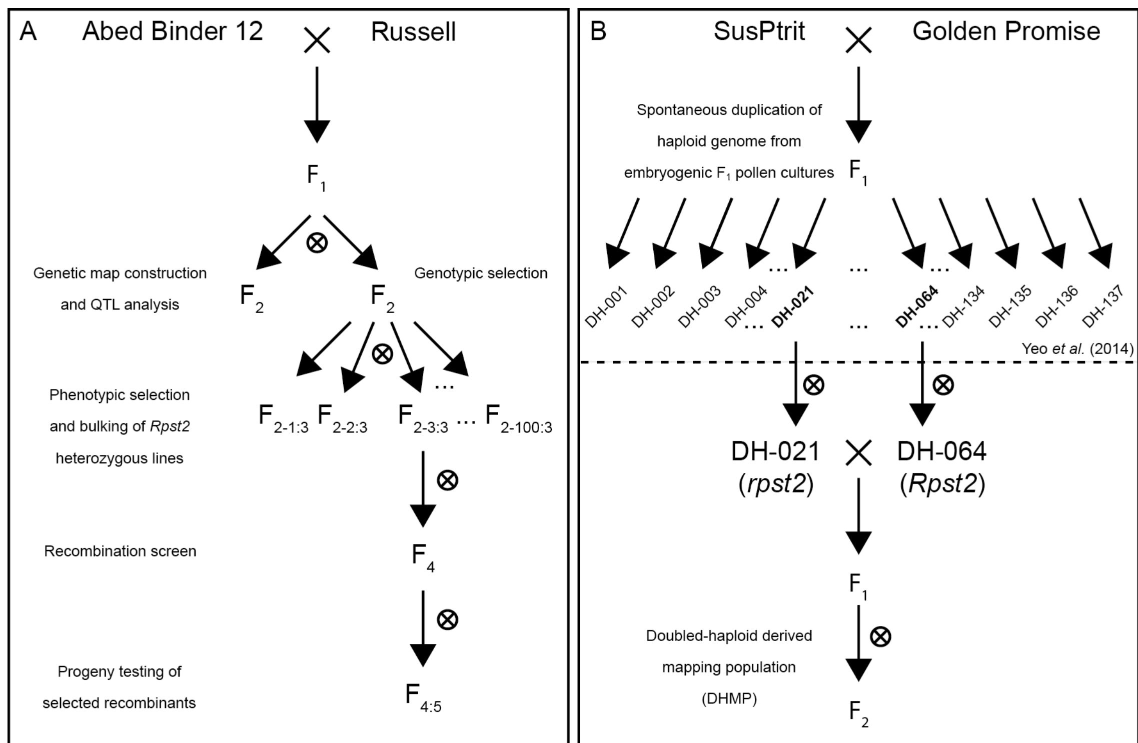


Figure 11: Isolation of *Rpst2* in two independent mapping populations. (A) An F_3 family derived from an individual F_2 plant ($F_{2:3}$) was identified using phenotypic and genotypic selection. Lines heterozygous for *Rpst2* in this family were then bulked and used for the recombination screen. (B) *Rpst2* had previously been identified in the SusPtrit x Golden Promise DH population along with *Rpst1* and *Rpst3* (previous chapter). Selected lines isogenic for *Rpst2* or *rpst2* were crossed to create a DH derived mapping population (DHMP).

AxR-*Pst* and AxR-*Psh* F_2 populations. Strong linkage was observed in the AxR-*Pst* F_2 population, whereas no linkage was observed on the AxR-*Psh* F_2 population (Fig. A2). Uncoupling of resistance to *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* indicates that the *Rpst2* locus is not linked to *rps2.*

5.2.2. Isolation of *Rpst2* using genotypic and phenotypic selection on $F_{2:3}$ families.

The presence of a minor effect QTL in the AxR-*Pst* F_2 population necessitated additional selection to isolate *Rpst2*. We used a combination of phenotypic and genotypic selection on a third AxR F_2 population (Fig. 11A). The F_2 population (N=96) was genotyped using markers flanking *Rpst2* and the minor effect QTL on chromosome 3H. Subsequently, eight plants from every $F_{2:3}$ family were macroscopically phenotyped using *P. striiformis* f. sp. *tritici* isolate 08/21. Similar significance and effect sizes were observed for *Rpst2* and the chromosome 3H QTL (Table A7). A single $F_{2:3}$ family was selected that was heterozygous for *Rpst2*, absent for the chromosome 3H QTL, and showed clear macroscopic segregation for resistance. In an initial screen, 96 $F_{2:3}$ plants derived from this family were inoculated with *P. striiformis* f. sp. *tritici* isolate 08/21, genotyped with markers flanking *Rpst2*, and phenotyped for chlorosis and pCOL. Distinct clustering was observed for *Rpst2* with the marker U32_4671_p1 in contrast to the overlapping clustering within the original AxR-*Pst*

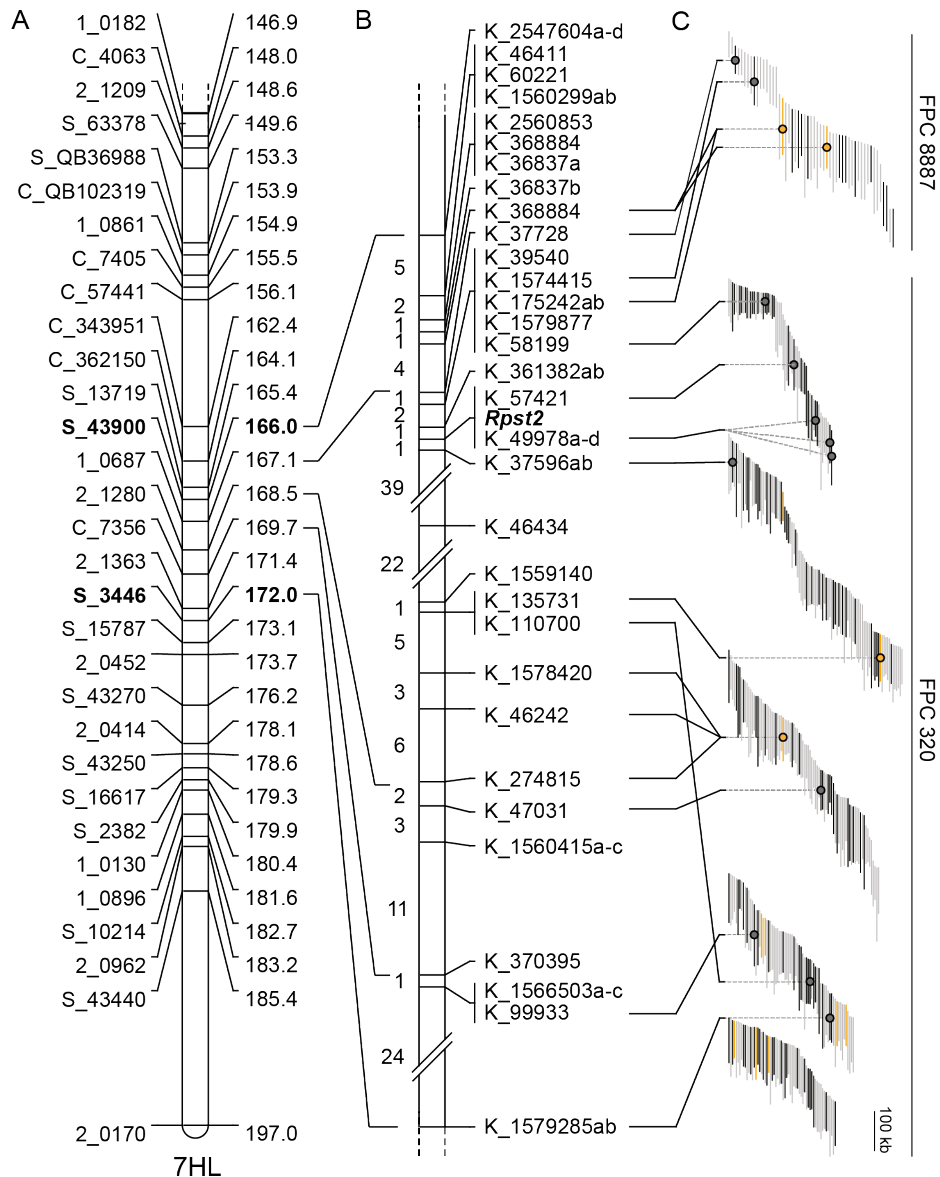


Figure 12: Fine mapping of *Rpst2*. (A) The distal end of chromosome 7H based on non-redundant markers harbouring *Rpst2* in the Abed Binder 12 x Russell F_2 population. Sequenom markers S_43900 and S_3446 were converted into KASP markers K_2547604b and K_1579285b and were used as flanking markers for the recombination screen. (B) High-resolution genetic map based on a recombination screen including 2,894 gametes. Numbers shown on left are the number of recombination events between markers. Marker names are shown on the right, with letters after marker names indicating cosegregating KASP markers derived from a single WGS contig. (C) Physical map anchoring based on the high-resolution genetic map. BACs that are sequenced or have BES available are orange or black, otherwise BACs are shown in grey. A truncated FPC 320 is shown based on the anchoring of markers.

F_2 population (Fig. A3A-D). *Rpst2* is additive in its contribution to chlorosis and pCOL, however, transgressive segregation was found within this selected $F_{2:3}$ family for pustule formation. *Rpst2* is dominant for conditioning resistance to pustule formation, suggesting that in a fully susceptible background it would be considered dominant (Fig. AE).

5.2.3. Fine mapping delimits the *Rpst2* locus to a 0.01 cM region.

To fine map *Rpst2*, we carried out a recombination screen and saturated the locus with markers based on the genomic resources available in barley. The recombination screen

was carried out using seed bulked from F_3 plants that were heterozygous for *Rpst2* in the previously characterised $F_{2:3}$ family (Fig. 11). The KASP markers K_2547604b and K_1579285b were generated from Sequenom markers S_43900 and S_3446, respectively, and used as flanking markers that span a 6.0 cM region encompassing *Rpst2* (Fig. 12A). In total, 2,894 gametes were characterised, identifying 135 recombination events between the flanking markers (Fig. 12B). Progeny tests were performed using individuals with recombinant chromosomes and scored homozygous or segregating for resistance, or homozygous susceptible. Additional marker saturation was required to resolve *Rpst2*, so we adopted two strategies for the development of markers. In the first instance, we compared genomic contigs derived from cultivars Barke, Bowman, and Morex to identify SNPs. In parallel, we performed RNAseq on Abed Binder 12 and Russell and aligned reads to WGS contigs anchored to the *Rpst2* region (IBGSC, 2012, Mascher et al., 2013). These analyses were performed twice; initially using the anchored contigs from the IBGSC reference anchoring that included 78 contigs between 127.12 cM and 129.21 cM (IBGSC, 2012). Later, a larger interval was investigated including 1,345 contigs between 126.20 cM and 131.44 cM based on an updated anchoring (Mascher et al., 2013). RNAseq data was aligned to WGS contigs and manually curated to identify SNPs polymorphic between Abed Binder 12 and Russell. A total of 102 SNPs were successfully converted into KASP markers and surveyed on recombinant individuals in the *Rpst2* region. In total, 49 KASP markers representing 30 WGS contigs mapped between the *Rpst2* flanking markers (Fig. 12B). At a fine scale, contigs mapped in a different order relative to their current anchoring in the barley POPSEQ anchored contigs, although at the rough scale the general order was preserved (Fig. 13A-C). The markers collapsed into 18 marker bins and positioned *Rpst2* in a 0.1 cM region, flanked by K_361382 (proximal) and K_37596 (distal) (Fig. 12B-C). *Rpst2* is located 0.07 cM from the proximal marker with only two recombination events to be resolved between them. Contrastingly, only a single recombination event differentiates the distal marker with *Rpst2*.

5.2.4. *Rpst2* anchors to FPC 320 in the barley physical map.

To anchor the *Rpst2* locus to the barley physical map, we used the available BES and shotgun sequenced BACs in the *Rpst2* region (IBGSC, 2012). In the proximal region, several KASP markers map to the physical map on FPC 8887 based on BES and sequenced BACs (Fig. 12B-C). Using currently available information it is unclear if FPC 8887 is correctly orientated based on our marker order. Marker K_58199 defines a boundary on FPC 320, indicating that K_361382 is located on the physical sequence between K_58199 and K_57421. *Rpst2* cosegregates with markers K_57421 and K_49978, which both map to proximal region of FPC 320. The entire region, from K_58199 to K_1579285, is well

anchored to FPC 320. Unequal rates of recombination were observed based on the physical map of barley, with extremely high rates of recombination observed between markers K_37596 and K_135731 (0.15 Mb / cM), whereas substantially lower rates of recombination were observed between markers K_58199 and K_37596 (2.58 Mb / cM). Annotated genes in the region include MLOC_18254 on contig 1579877 and two NLRs present on contigs 49978 and 37596. The high confidence gene model MLOC_65262 is present on contig 49978 and cosegregates with *Rpst2* based on the resolution of our recombination screen, whereas the NLR on contig 37596 is separated by a critical recombination event. MLOC_65262 is preferentially expressed in roots, with little or no expression in leaves in Morex (IBGSC, 2012). BAC sequencing along the minimal tiling path of FPC 320 will be critical for delimiting the genetic and physical interval harbouring *Rpst2*, in addition to permitting the full annotation of the gene content in the region.

5.2.5. NLR motif analysis identifies five candidate genes at the *Rpst2* locus.

To identify candidate NLR genes at the *Rpst2* locus we utilised existing barley genomics resources in combination with transcriptome assemblies from six barley accessions. We selected a region harbouring *Rpst2* based on linkage mapping of the region (Fig. 13A-C). Using marker colinearity, we identified 665 WGS contigs anchored to the *Rpst2* region derived from the cultivars Morex, Barke, and Bowman (IBGSC, 2012) (Fig. 13C and Table 6). The contigs were anchored in 16 genetic bins and each bin contained a variable number of contigs ranging from 1 to 442 (Fig. 13C). The mean contig length ranged from 3,239 to 4,008 bp (Table 6). The total length of the anchored sequence information for each accession ranged from 690-900 kbp. To check for the presence of candidate genes in the anchored sequence information, we searched for motifs associated with NLRs using the motif alignment and search tool (MAST) according to the parameters and motifs reported by Jupe et al. (2012). Eleven WGS contigs contained NLR motifs (Table 7). These contigs were compared to identify whether similar contigs had been identified in each parent. Indeed, we observed redundancy between accessions and the contigs collapsed into 5 homologous groups. We provisionally designated the putative *NLRs* harboured in the contigs as *NLR-A*, *NLR-B*, *NLR-C*, *NLR-D*, and *NLR-E*. The only Morex contig identified via MAST analysis grouped with the *NLR-D* homologous group and was the high confidence gene MLOC_65262 cosegregating with *Rpst2* in the high-resolution genetic map (Table 7). The second annotated NLR identified from Morex contig 37596 in the barley physical map was not identified using MAST analysis as only contigs contained in the original anchoring of IBGSC (2012) were interrogated. However, BLAST analysis indicated that the annotated NLR on contig 37596 grouped with the *NLR-E* homologous group. This

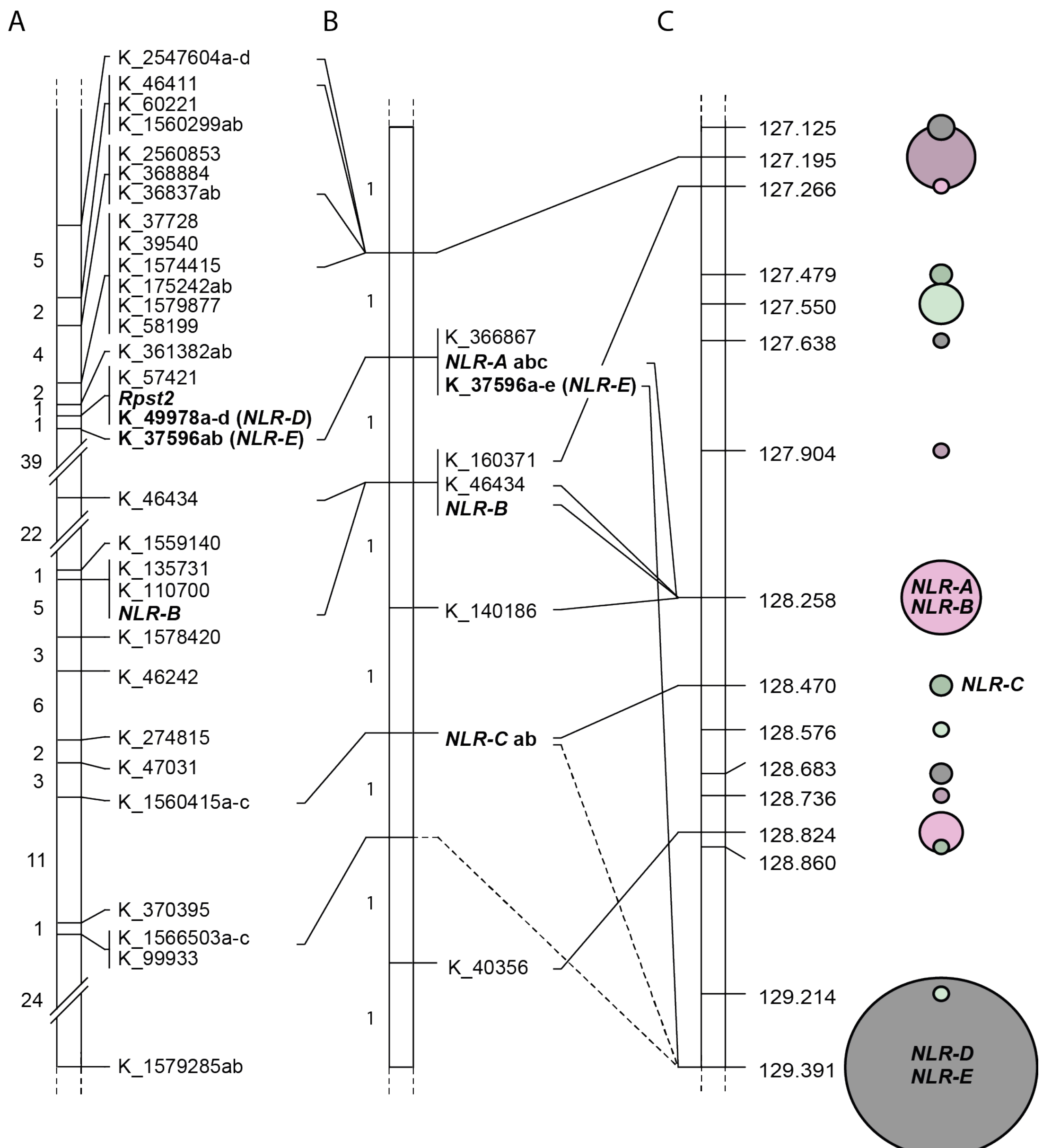


Figure 13: Identification and genetic mapping of five NLR candidate genes in the *Rpst2* region. (A) Abed Binder 12 x Russell high-resolution F_2 map used for fine mapping *Rpst2*. (B) Doubled haploid derived F_2 mapping population (DHMP). Numbers on left of linkage group show the number of recombination events between markers (N=91). (C) Linkage group representing the POPSEQ data published by Mascher et al. (2013). Bubbles show anchoring of WGS contigs in each bin and bubble size represents the number of contigs in each bin (log scale). The presence of five candidate NLRs identified via MAST analysis are shown. Unbroken black lines between linkage groups shows identical markers. Broken lines illustrate that marker position can be inferred.

Table 6: Anchored WGS contigs in the *Rpst2* region.

Accession	Contigs[1]	Mean length (bp)	Total Length (kb)
Barke	213	3,239	690
Bowman	215	4,008	860
Morex	237	3,782	900

[1]Number of contigs anchored in POPSEQ data between 127.13 and 129.39 cM (Mascher et al. 2013).

contig was separated from *Rpst2* by a critical recombination event in the high-resolution genetic map.

5.2.6. Gene expression profiling supports *NLR-A* as a candidate gene for *Rpst2.*

To investigate the five NLR candidate genes in more detail we established expression profiles for each gene using transcript assemblies for six key accessions harbouring *Rpst2* or *rpst2,* identified in Chapter 3 (Table 7). The WGS contigs harbouring NLR motifs were used to search the assemblies for transcripts corresponding to the NLR candidates. Strikingly, transcripts corresponding to *NLR-A* were only detected in accessions harbouring *Rpst2*, showing a clear expression polymorphism between resistant and susceptible genotypes (Table 7). No allelic diversity was evident in the *NLR-A* transcripts found in the *Rpst2* accessions. Conversely, *NLR-E* exhibited an inverse expression polymorphism between *Rpst2* and *rpst2* accessions. A comparison of the *NLR-E* transcripts derived from Russell and Manchuria (*rpst2*), and the Barke/Bowman (*Rpst2*) WGS contigs, revealed a single synonymous substitution. This, coupled with the critical recombination event separating *NLR-E* (K_37596ab) from *Rpst2* in the high-resolution genetic map, excluded *NLR-E* as a candidate gene. We did not detect any expression of *NLR-B* or *NLR-D*. This was consistent with the annotation of *NLR-D* (MLOC_65262) as a root-expressed gene in the barley high confidence gene assembly (IBGSC, 2012). In the case of *NLR-C,* we did not observe any expression polymorphisms differentiating *Rpst2* from *rpst2* accessions.

5.2.7. *NLR-A* is a CNL containing a large intron.

The clear differentiation of *Rpst2* and *rpst2* accessions by *NLR-A* expression profiling provided evidence that supported *NLR-A* as the causal gene underlying *Rpst2*. Therefore, we investigated *NLR-A* further by constructing a gene model using the available genomic information. We aligned the Abed Binder 12 *NLR-A* transcript to Barke contig 2780081, originally identified as harbouring *NLR-A* using MAST analysis. (Table 7). We observed a perfect alignment with the exception of approximately 600bp of sequence at the 5’ of the Abed Binder 12 transcript. Therefore, we searched the anchored Barke WGS contigs using the unaligned 5’ sequence and identified an additional Barke contig (contig 54347) to which the 5’ sequence aligned (Fig. 14A). We assembled a full gene model for *NLR-A* by concatenating the Barke contigs and re-aligning the Abed Binder 12 *NLR-A* transcript (Fig. 14B). This model proposed that *NLR-A* consisted of four exons and three introns. Interestingly, the second intron was approximately 8.8 kbp in length. However, according to POPSEQ anchoring of the Barke contigs, contig 54347 was anchored 4.4 cM distal to contig 2780081. This suggested that an extremely large physical distance existed between the contigs and we wanted to understand the relationship of these contigs in Abed Binder

Table 7: Identification of five candidate NLR genes in the *Rpst2* region.

	MAST analysis on POPseq		RNAseq expression analysis[1,2]						
			Rpst2			*rpst2*			
	WGS contigs[4]	**E- value**	**A**	**G**	**B**	**S**	**R**	**M**	**MLOC**[3]
NLR-A	barke_contig_2780081	1.50E-43	H	H	H	ND	ND	ND	-
	bowman_contig_200425	6.80E-43							
NLR-B	barke_contig_268211	2	ND	ND	ND	ND	ND	ND	-
	bowman_contig_856833	0.11							
NLR-C	barke_contig_1788934	5.40E-36	L	L	H	L	ND	ND	-
	bowman_contig_874416	9.30E-37							
NLR-D	barke_contig_417389	5.70E-47	ND	ND	ND	ND	ND	ND	MLOC_65262
	bowman_contig_852986	3.60E-47							
	morex_contig_49978	1.40E-40							
NLR-E	barke_contig_480243	2.20E-49	L	L	L	H	H	H	-
	bowman_contig_859170	3.10E-47							
	morex_contig_37596[1]								

[1]Genotypes are abbreviated as follow: (A) Abed Binder 12; (G) Golden Promise; (B) Barke; (S) SusPtrit; (R) Russell; and (M) Manchuria.
[2]Gene expression is abbreviated either as (H) high expression based on the recovery of full-length transcripts in RNAseq data, (L) low expression as observed by the recovery of only partial transcripts in RNAseq data, or (ND) based on no detectable expression of gene.
[3]MLOC identified in barley high confidence gene set (Mayer et al. 2012).
[4]Contig identified by BLAST search as not anchored in first iteration of POPseq data.

12. To do this, we performed long range PCR on Abed Binder 12 gDNA using primers that spanned the two contigs. We observed successful amplification showing that they were physically linked in Abed Binder 12 (Fig. 14C-D). Furthermore, Sanger sequencing of the amplicons revealed that a single guanine residue separated the contigs. These results demonstrated that POPSEQ anchoring of WGS contigs is imperfect and highlights the need for a cautious approach when utilising such data.

5.2.8. Genetic mapping supports *NLR*-A as a candidate gene for *Rpst2*.

Genetic mapping had excluded *NLR-E* as a candidate gene and showed that *NLR-D* co-segregated with *Rpst2*. We also wanted to map *NLR-A*, *NLR-B*, and *NLR-C* into the high-resolution genetic map to see where they were positioned relative to the *Rpst2* locus. We initiated a PCR based strategy for identifying SNP markers for *NLR-A*. To do this, we designed PCR primers along the length of the concatenated Barke contigs 54347 and 2780081. PCR amplification using these primers on Abed Binder 12 and Russell gDNA, showed a clear presence/absence polymorphism between Abed Binder 12 and Russell (Fig. 15A). This result was concurrent with the absence of an *NLR-A* transcript in the Russell transcriptome. However, failure to amplify *NLR-A* from Russell gDNA meant it

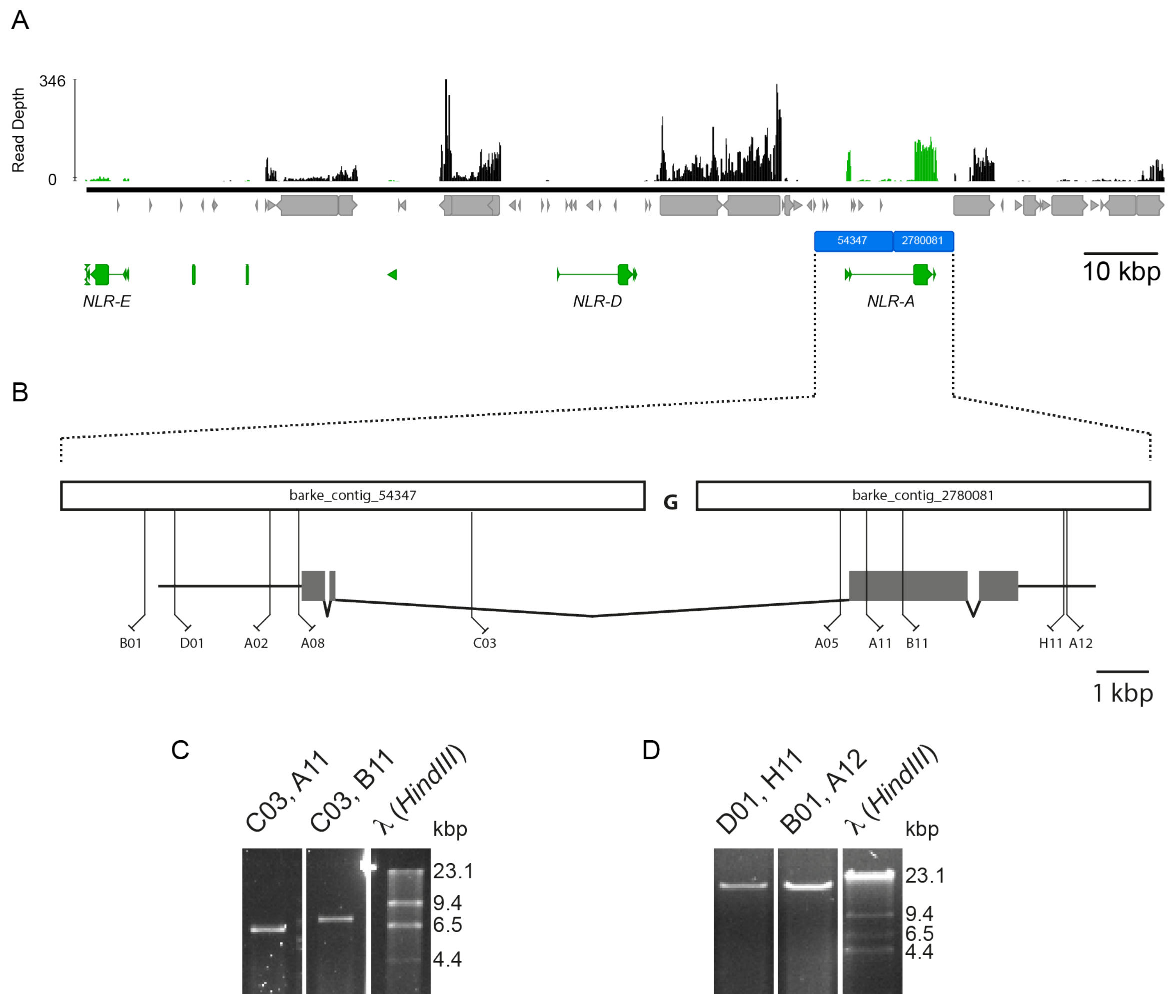


Figure 14: Physical mapping and gene model of the candidate gene *NLR-A.* (A) Alignment of Abed Binder 12 RNAseq reads to the Abed Binder 12 BAC clone harbouring *NLR-A*. The BAC clone was obtained by PCR screening using primer combinations A02/A08 and A05/A11. Grey boxes and peaks represent retrotransposon elements (Table A8), green boxes and peaks represent NLR candidates *NLR-E*, *NLR-D*, and *NLR-A* (left to right), and blue boxes represent Barke contigs. (B) Gene model obtained by aligning *NLR-A* transcript with concatenated Barke contigs. A single guanine (G) residue separates the contigs in Abed Binder 12. Lines represent the position of primers. (C and D) Long range PCR bridging of the contigs using Abed Binder 12 gDNA. DNA fragment sizing was estimated relative to *HindIII* digested Lambda DNA. Primer combinations used for amplification are shown above the gel lanes.

would not be possible to precisely map *NLR-A* in the Abed Binder 12 x Russell mapping population as it is a dominant marker. Therefore, we hypothesised that it may be possible to amplify *NLR-A* in different accessions known to harbour *Rpst2* or *rpst2*.

In the previous chapter, we detected *Rpst1*, *Rpst2*, and *Rpst3* in the SusPtrit x Golden Promise DH population. Using the SusPtrit x Golden Promise DH genetic map we identified two DH lines that harboured *Rpst2* or *rpst2* in the absence of *Rpst1* and *Rpst3* (Fig. 11). Using these lines, and the key accessions used for RNAseq analysis, we PCR amplified gDNA using eight primer pairs that amplified the 5’ and 3’ ends of *NLR-A*. We observed three *NLR-A* haplotypes, in the eight different accessions, which we named *NLR-A*$_1$, *NLR-A*$_2$ and *nlr-a* (Table 8). The *Rpst2* containing accessions Abed Binder 12, Barke, Golden Promise, and SusPtrit x Golden Promise DH line 64 (DH-064) represented the *NLR-A*$_1$

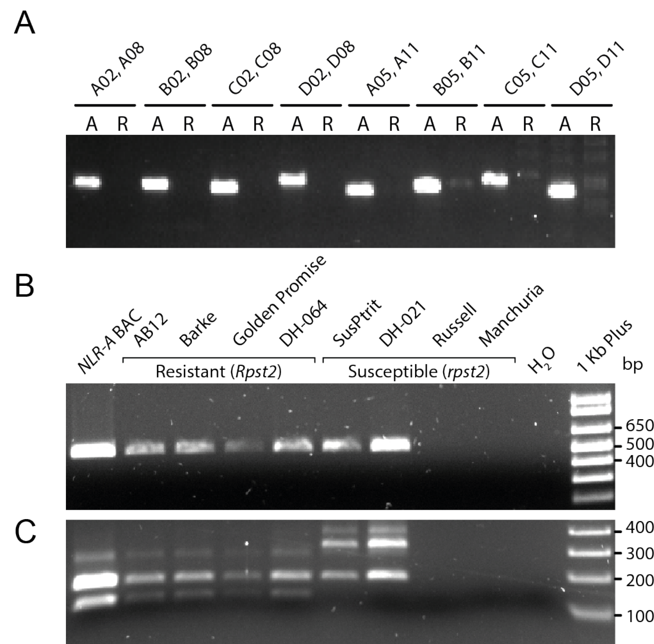


Figure 15: A single nucleotide polymorphism differentiates three *NLR-A* haplotypes. (A) Eight PCR primer pairs specific for *NLR-A* amplified with gDNA from Abed Binder 12 'A' and Russell 'R'. (B) Primer pair A05/A11 amplified with gDNA from *Rpst2* and *rpst2* accessions. (C) A CAPS marker designed on a single nucleotide polymorphism identified at the beginning of the third exon of *NLR-A* differentiates three *NLR-A* haplotypes in eight *Rpst2* differential accessions.

haplotype exhibiting 100% amplification of all primers tested. Russell and Manchuria, both susceptible to *P. striiformis* f. sp. *tritici*, showed no amplification of any of the primers (*nlr-a* haplotype). However, SusPtrit and SusPtrit x Golden Promise DH line 21 (DH-021) showed successful amplification of 50% of the primers tested and represented the *NLR-A*$_2$ haplotype. Sanger sequencing of the PCR amplicons revealed a SNP in the putative NBS domain of *NLR-A* between the SusPtrit and Golden Promise alleles. Using this SNP, we developed a CAPS marker that showed clear differentiation of the different haplotypes (Fig. 15B-C). The absence of any amplicons in *nlr-a* accessions suggested that *NLR-A* was deleted in these lines. In order to map *NLR-A,* we crossed DH-021 with DH-064 to create a DH derived F_2 mapping population (DHMP) (Fig. 11). A linkage map was constructed using 43 KASP markers including markers for *NLR-A* and *NLR-E*. *NLR-A* and *NLR-E* co-segregated with K_366867. This positioned *NLR-A* and *NLR-E* at the *Rpst2* locus based on marker colinearity with the Abed Binder 12 x Russell high-resolution mapping population (Fig. 13A-B). *NLR-D* markers were not polymorphic on the DHMP population and we were unable to determine the position of *NLR-D* in the DHMP population.

In order to map *NLR-B* and *NLR-C*, we designed markers based on SNPs identified between Barke and Morex WGS contigs harbouring the genes. *NLR-B* mapped distal to *Rpst2* and excluded the gene as a candidate gene for *Rpst2* (Fig 13A-B). *NLR-C* markers were not polymorphic in the high-resolution genetic map but we were able to map *NLR-C* distal to *Rpst2* in the DHMP population (Fig. 13B). Taken together, these results excluded *NLR-B*,

Table 8: Eight primer pairs differentiate three *NLR-A* haplotypes.

Accession	Allele	PCR primer combination[1,2]								Haplotype
		1	2	3	4	5	6	7	8	
Abed Binder 12	*Rpst2*	■	■	■	■	■	■	■	■	*NLR-A1*
Barke	*Rpst2*	■	■	■	■	■	■	■	■	*NLR-A1*
Golden Promise	*Rpst2*	■	■	■	■	■	■	■	■	*NLR-A1*
DH-064	*Rpst2*	■	■	■	■	■	■	■	■	*NLR-A1*
SusPtrit	*rpst2*					■	■	■	■	*NLR-A2*
DH-021	*rpst2*					■	■	■	■	*NLR-A2*
Russell	*rpst2*									*nlr-a*
Manchuria	*rpst2*									*nlr-a*

[1]*Paired primer combinations include 1= A02/A08, 2= B02/B08, 3= C02/C08, 4= D02/D08, 5= A05, A11, 6= B05/B11, 7= C05/C11, and 8= D05/D11.*
[2]Shaded and white boxes represent amplification or no amplification of gDNA, respectively.

NLR-C, and *NLR-E* as candidate genes for *Rpst2*. The presence of a putative deletion region harbouring *NLR-A* and a clear expression polymorphism between *Rpst2* and *rpst2* harbouring accessions supports *NLR-A* as a candidate gene for *Rpst2*.

5.2.9. Isolation of an *NLR-A* harbouring BAC clone from an Abed Binder 12 library reveals the presence of three CNLs encoding genes, *NLR-A*, *NLR-D*, and *NLR-E*.

We initiated physical mapping of the *Rpst2* region using an Abed Binder 12 BAC library. A PCR screen, using two sets of primers, identified a single BAC clone harbouring *NLR-A* (primer pairs A02/A08 and A05/A11; Fig. 14A-B). We sequenced the BAC clone with Pacific Biosciences long read sequencing using a SMRT cell with C4-P6 chemistry and were able to construct and annotate a single contiguous BAC sequence (Fig 14A). The clone mostly consisted of repetitive and low complexity sequence (Table A8). However, the clone harboured three *CNL* genes: *NLR-A*, *NLR-D*, and *NLR-E* (Fig. 14A and Table A8). Signatures of three additional genes annotated in the barley low confidence gene set were identified on the BAC: MLOC_8985.1, MLOC_41646.1, and MLOC_19985.1. All three were annotated as unknown proteins although InterPro scan revealed MLOC_19985.1 contains an F-box domain. These genes were not considered as candidate genes for *Rpst2*. Comparison of *NLR-A*, *NLR-D*, and *NLR-E* showed high homology between the genes at the amino acid level (~60%) and in the DNA coding sequence (~70%) (Table A9 and A10). Despite the similarity, alignment of the Abed Binder 12 RNAseq reads to the BAC contig differentiated *NLR-A*, *NLR-D,* and *NLR-E* and demonstrated that *NLR-A* was highly expressed compared to *NLR-E* and *NLR-D* (Fig. 14A). This result confirmed the earlier expression analysis performed using *de novo* assembly of the RNAseq data. The physical linkage of *NLR-A*, *NLR-D*, and *NLR-E* was consistent with the genetic linkage of these genes

in the high-resolution and DHMP mapping populations. We anchored the BAC clone to the Abed Binder 12 x Russell genetic map using markers K_49978 (*NLR-D*) and K_37596 (*NLR-E*). A single recombination event separates these markers and defines the distal physical region of the *Rpst2* locus. The *Rpst2* proximal marker, K_361382, does not reside within the BAC clone and the boundary of the proximal physical region has yet to be defined.

5.3. Discussion.

The barley nonhost resistance locus *Rpst2* was previously mapped to the long arm of chromosome 7H using linkage mapping. It was shown to condition colonisation resistance to wheat stripe rust and was prevalent in European spring barley accessions. In this study, we used phenotypic and genotypic selection on $F_{2:3}$ families to isolate *Rpst2* as a single Mendelian locus for fine mapping. Marker saturation and the identification of recombinant gametes delimited the locus to a 0.1 cM genetic interval that encompasses approximately 267 kbp. Using a candidate gene approach tailored towards *NLRs*, we identified a candidate for *Rpst2* based on high-resolution mapping, expression analysis, and presence/absence variation between *Rpst2* and *rpst2* accessions. Transformation of *NLR-A*, our prioritised candidate, into susceptible barley lines and the identification of mutants is now underway to validate its role as *Rpst2*.

5.3.1. *Rpst2* colocalises with several resistance specificities effective against host and nonhost pathogens.

Several resistance specificities to *P. striiformis* f. sp. *tritici* have been mapped to the distal region on chromosome 7H. Using the consensus maps that integrate multiple genotyping platforms developed by Szűcs et al. (2009), Aghnoum et al. (2010), and Muñoz-Amatriaín et al. (2011) we inferred the position of previously mapped genes (Fig. 16). We found that *Rpst2* colocalises with *YrpstY1*, a gene that confers resistance to a Chinese isolate of *P. striiformis* f. sp. *tritici* in barley (Sui et al., 2010). Mapping of *YrpstY1* was achieved using nine simple sequence repeat (SSR) markers and delimited the *YrpstY1* locus to a region spans 40 cM. *Rpst2* and *YrpstY1* colocalise based on the position of *EBmac0755*, the most closely linked marker to *YrpstY1*, relative to the position of *Rpst2* in the AxR-*Pst* F_2 map (Fig. 16). *Rpst2* has independently identified and found to provide resistance in barley to *P. striiformis* f. sp. *tritici* isolates from the U.S. (J. Dubcovsky, personal communication). Taken together, these observations suggest that this locus is an integral component of resistance in barley to *P. striiformis* f. sp. *tritici* in distinct regions around the world.

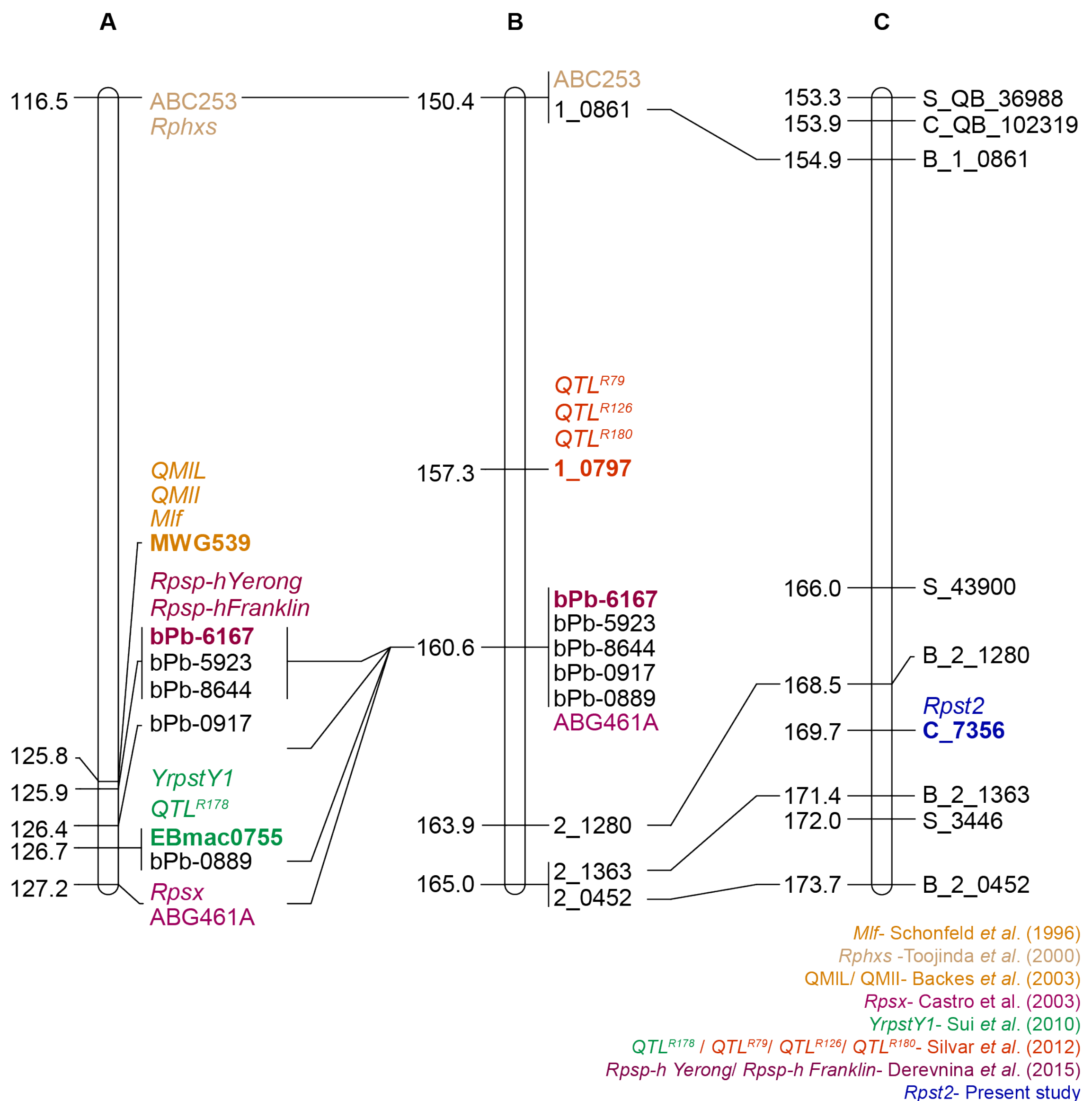


Figure 16: *Rpst2* colocalises with previously mapped resistance loci. Relative positions between loci have ben inferred by comparing the Marcel, 2009 consensus map data (Aghnoum et al. 2008) and the Oregon Wolfe Barley consensus map integrating DArT and OPA markers (Szűcs et al. 2009) (both available on GrainGenes: http://wheat.pw.usda.gov/browse?class=mapdata;query=*barley*) with the *Rpst2* recombinant map developed in this study. Lines between linkage groups indicate relative marker positions in each map. Genetic distances on the left of each group are in cM. The coloured markers represent the closest marker to the resistance specificity in linkage mapping or the peak marker for QTLs. Names of resistance genes or QTLs are given above the corresponding marker ID. Redundant markers or markers that do not link the genetic maps have been removed.

In addition to resistance to *P. striiformis* f. sp. *tritici*, several resistance specificities to host and nonhost pathogens have been mapped near *Rpst2* (Fig. 16). Adult plant resistance to the host pathogen *P. striiformis* f. sp. *hordei* has been mapped to the *Rpst2* region (Castro et al., 2003). Castro et al. (2003) identified *Rpsx* using the restriction fragment length polymorphism (RFLP) markers. RFLP marker ABG461A was the closest linked marker to *Rpsx* and maps in close proximity to *Rpst2* based on marker colinearity between maps. Similarly, $Rphx_S$, an adult plant resistance specificity to *P. hordei* also mapped to the *Rpst2* region on chromosome 7HL (Toojinda et al., 2000). This locus is distal to RFLP marker ABC253 and accounted for 84% of the phenotypic variance. Derevnina et al. (2015)

mapped *Rpsp-hYerong*, a QTL conferring resistance to *P. striiformis* f. sp. *pseudo-hordei* (barley grass stripe rust; BGYR), in the vicinity of *Rpst2*. The DArT marker bPb-6167 was the marker underlying the peak of *Rpsp-hYerong*. BGYR is a contemporary *formae speciales* of *P. striiformis* (Wellings et al., 2000). It is an adapted pathogen of wild *Hordeum* spp. (barley grass) and as such its interaction with barley can be considered an intermediate host pathosystem according to terminology proposed by Bettgenhaeuser et al. (2014). The observation of nonhost resistance specificities in this region also coincides with barley powdery mildew resistance including the resistance gene *Mlf* and several QTLs (Schönfeld et al., 1996, Backes et al., 2003, Silvar et al., 2012). The association of resistance at the *Rpst2* locus to multiple diseases extends to *M. oryzae*, wherein a minor effect QTL maps to the region (Inukai et al., 2006). It is unclear whether these specificities are due to linkage rather than pleiotropy based on current map positions as the large mapping intervals observed in most of the studies hinders our ability to draw conclusions from this data. Additional fine mapping and cloning of the genes underlying resistance will be required to conclusively define whether colocalisation of these loci are due to genetic linkage or pleiotropy.

5.3.2. Sufficient recombination for map-based cloning of *Rpst2*.

The success of map-based cloning is determined by the chromosomal location and physical structure of the region encompassing the gene of interest. In barley, as with many other plants, recombination rates vary along the length of the chromosome and significantly reduced rates of recombination can be observed in pericentromeric regions when compared to distal regions (IBGSC, 2012). Recombination is essential for map-based gene isolation as it influences the degree to which the locus can be delimited using recombination breakpoints and the ratio of physical to genetic distance in the region. This was highlighted during the anchoring of the barley BAC-based physical map when it was estimated that the ratio of physical to genetic distance in pericentromeric regions was 10 to 500 times greater than in distal regions (IBGSC, 2012). In the case of *Rpst2*, the chromosomal localisation was favourable for mapping due to its distal location on chromosome 7H. Indeed, we observed recombination that was sufficient to delimit *Rpst2* to a 0.1 cM region. Based on our current markers, we have been able to anchor *Rpst2* to FPC 320 (IBGSC, 2012).

5.3.3. Barley genomics information facilitated the isolation a candidate gene for *Rpst2*.

The advent of genomics information within the Triticeae tribe promises to facilitate crop improvements by aiding gene isolation studies (Schulte et al., 2009, IBGSC, 2012). Using available genomics tools in barley we have been able to implement a candidate gene

approach for identifying a candidate gene underlying *Rpst2* resistance. Any candidate gene approach is dependant upon existing knowledge, which is used to generate *a priori* assumptions about the gene being isolated. For *Rpst2*, we tailored our candidate gene approach to specifically look for genes that code for NLRs. We were motivated to do this for two reasons: 1) Most resistance genes that have been cloned to date in plants have coded for NLR type proteins (Krattinger et al., 2009a) and 2) An increasing body of literature suggests a role for NLRs in nonhost immunity (Thordal-Christensen, 2003, Schulze-Lefert and Panstruga, 2011). After interrogating WGS contigs anchored in the proximity of the *Rpst2* locus we were able to identify five candidate genes. The identification of *NLR* genes in this region of chromosome 7H is congruent with the clustering of *NLR* genes in the distal region of chromosome 7H (IBGSC, 2012). One of the five candidates, *NLR-A*, was supported as *Rpst2* based on expression polymorphism between *Rpst2* and *rpst2* harbouring accessions. Furthermore, the development of SNP markers linked to the five candidates excluded *NLR-B*, *NLR-C*, and *NLR-E* from the *Rpst2* locus. Comparatively, *NLR-A* and *NLR-D* mapped between key recombinants in our linkage map positioning them at high resolution at the *Rpst2* locus. We have not been able to exclude *NLR-D* as a candidate gene based on genetic mapping. However, the identification of three haplotypes defining resistance and susceptibility at the locus; with Abed Binder 12, Golden Promise and Barke harbouring $NLR\text{-}A_1$, SusPtrit harbouring $NLR\text{-}A_2$ and Russell and Manchuria being devoid of the gene altogether (*nlr-a*) provides additional evidence that *NLR-A* is *Rpst2*. Russell and Morex appear to share the same haplotype structure at the *Rpst2* locus based on the absence of *NLR-A*. However, they do harbour *NLR-D* based on mapping of this gene in the high resolution mapping population. To fully validate *NLR-A* as the causal gene underlying *Rpst2* resistance it will be necessary to 1) observe resistance in a susceptible barley accession after transformation with *NLR-A*, 2) demonstrate loss of function of *NLR-A* in a resistant accession, and 3) delimit recombination breakpoints in the genetic map and relate this to the physical map (ideally down to the resolution of a single gene).

Map-based cloning of *Rpst2* will open up the possibility of transferring a nonhost resistance gene into the host species, wheat. Whether *Rpst2* would retain functionality in wheat is unclear and would depend upon the species conservation of the mechanisms underlying immunity (Tai et al., 1999). Wheat and barley diverged from a common ancestor approximately 11.6 million years ago (Wicker et al., 2009). Encouragingly, alleles of *Mla* from barley retained functionality when transferred to an immuno-compromised *A. thaliana* accession (Maekawa et al., 2012). This demonstrated conservation of the underlying immune systems in two species that evolutionarily separated ~200 million

ago. Since the separation of wheat and barley occurred, they have split into many subspecies and modern day bread wheat exists as a hexaploid due to polyploidisation events that occurred between three ancestral genomes (Chalupska et al., 2008). Due to its polyploid nature, the wheat genome has remarkable plasticity. This is demonstrated by the numerous examples of alien introgressions from wild relatives that have proven beneficial for introducing new diversity into the wheat gene pool (Wulff and Moscou, 2014). Encouragingly, a series of fertile wheat-barley chromosome addition lines (ditelosomic and disomic) exist, suggesting compatibility between the two species. Barley cvs. Betzes and Igri have both been used to generate introgression lines in wheat (Islam et al., 1981, Islam and Shepherd, 2000, Szakács and Molnár-Láng, 2007). In the previous chapter, we demonstrated that Betzes harbours *Rpst1* and *Rpst3* and as such the functionality of these genes may be tested in wheat using the corresponding introgression lines. We do not currently know the genetic architecture of resistance to *P. striiformis* f. sp. *tritici* in Igri and it may be possible to use the Igri chromosome addition lines for validating *Rpst2* functionality in wheat. Successful intergenera transfer of *Rpst2* and other genes contributing to the intermediate host status of barley will establish if barley may be used as a resource for the improvement of wheat.

6. Fine mapping of *rps2*: A recessive host resistance gene in barley effective against barley stripe rust.

6.1. Introduction.

Plants have a sophisticated immune system that provides resistance to pathogenic microbes. In host systems, the most common form of resistance manifests as the hypersensitive response (HR): a rapid, localised cell death at sites of infection, which is thought to limit pathogen ingress (Heath, 2000). This form of resistance is often mediated via intracellular or membrane localised immune receptors, particularly the NLR class of protein (Jones and Dangl, 2006). NLR immune receptors function by recognising, either directly or indirectly, secreted proteins (effectors) that are delivered into the host plant by a would-be pathogen (Jones and Dangl, 2006, van der Hoorn and Kamoun, 2008). Recognition of pathogen-derived molecules by host plants was first proposed in the gene-for-gene hypothesis (Flor, 1956a). This concept was derived from the observation of dominant modes of inheritance for resistance in the host plant, flax, and, conversely, recessive inheritance of avirulence in the pathogen, flax rust (Flor, 1956a, Flor, 1956b). The flax-flax rust pathosystem has since been used as a model system for deciphering the molecular mechanisms underpinning gene-for-gene interactions. We now know of 31 resistance specificities in flax that map to five dominant resistance loci, namely; *K*, *L*, *M*, *N*, and *P*. Several *R* genes have been cloned from these loci and all encode NLR proteins (Ellis et al., 2007).

In the Triticeae tribe, biotrophic fungal resistance follows a similar inheritance pattern to the flax-flax rust pathosystems, with most loci having a major dominant inheritance (McIntosh et al., 1995). To date, 108 genes have been recognised in wheat that confer resistance to rust pathogens (*Puccinia* spp.) and the majority are dominant, major *R* genes (McIntosh et al., 1995). Of these genes, eight have been cloned and six encode NLR proteins (Krattinger et al., 2009a). However, occasionally, recessive resistance genes can also confer resistance to rust fungi in the Triticeae. Recessive resistance genes are less well understood and occur at much lower frequencies than their dominant counterparts. This is highlighted by the fact that of the 108 recognised genes in wheat to *P. striiformis* f. sp. *tritici* only three are recessive (Chen et al., 2012b, Feng et al., 2014, Ren et al., 2015).

In the context of host resistance, the interaction between barley and *P. striiformis* f. sp. *hordei* is a unique system, as recessive resistance genes condition the majority of race-specific immunity (Chen and Line, 2002). Early publications reported three recessive specificities in barley to *P. striiformis* f. sp. *hordei* in Europe (Nover and Scholz, 1969).

Shortly after, Bakshi and Luthra (1970) reported on 11 specificities in India, three of which were recessive. However, the most comprehensive investigation of *P. striiformis* f. sp. *hordei* resistance to date, was conducted in the U.S. by Chen and Line where 28 resistance specificities were reported with 25 conditioning race-specific recessive resistance (Chen and Line, 1999, Chen and Line, 2002). To date, only two of the genes reported by Chen and Line (2002) have been genetically mapped: *rps1.a* to chromosome 3HL and *rpsGZ* to chromosome 4HL. None of the recessive *P. striiformis* f. sp. *hordei R* genes have been cloned and nothing is known about the molecular mechanisms underlying these resistance specificities in barley.

Research into recessive *R* genes frequently uncovers novel mechanisms involved in plant immunity. The most well characterised systems for studying recessive *R* genes are derived from plant-virus interactions, where approximately half of the *R* genes are recessively inherited (Truniger and Aranda, 2009). Viruses have small genomes that encode a small number of proteins. In isolation, these proteins are not sufficient for viral replication; as a consequence, they interact closely with host factors to complete their life cycle (Boevink and Oparka, 2005). It is postulated, that mutations to host factors, required for pathogenicity, will impede the interaction between virus and host and give rise to recessively inherited resistance specificities (Diaz-Pendon et al., 2004). Fourteen recessive virus *R* genes have been characterised, including *rym4*, *rym5,* and *rym6* from barley (Kanyuka et al., 2005). All have been shown to code for, or map in the proximity of, eukaryotic translation initiation factors (eIFs), predominantly eIF4E, eIF4G and their isoforms (Wang and Krishnaswamy, 2012). It is hypothesised that viruses encode proteins that bind eIFs to recruit the necessary protein complexes for translation of viral mRNAs into protein (Wang and Krishnaswamy, 2012). Mutations in eIFs act as recessive *R* genes (or dominant susceptibility genes) as they attenuate the binding of viral proteins to eIFs and inhibit viral replication and spread in the host. Targeting and manipulation of host factors is not a phenomenon unique to viruses and similar principles, although mechanistically distinct, apply to interactions with fungi, bacteria, and oomycetes (Jones and Dangl, 2006, Dodds and Rathjen, 2010). Two recessive resistance genes, *xa5* and *xa13*, conferring resistance to *Xanthomonas oryzae* pv. *oryzae*, appear to support these claims in bacterial interactions with rice. *X. oryzae* pv. *oryzae* utilises transcription activator like (TAL) effectors that specifically target disease-promoting genes in the host and function by manipulating host transcription machinery to up-regulate gene expression (Boch et al., 2009, Moscou and Bogdanove, 2009). *xa5* was cloned and shown to code for the gamma subunit of the general eukaryotic transcription factor 2 IIA (TFIIAγ) (Iyer and McCouch, 2004). Two amino acid variations differentiate *xa5* from *Xa5*. *xa5* is hypothesised to inhibit

transcriptional activation of TAL effector susceptibility targets by disrupting the binding between TALs and the pre-initiation complex of eukaryotic transcription (Gu et al., 2009). Contrastingly, *xa13* is a direct target of TAL effector *PthXo1* and encodes Os8N3, a membrane bound protein implicated in rice development (Chu et al., 2006, Yang et al., 2006a). This gene was shown to be a sugar transporter (SWEET gene) that localises to the phloem plasma membrane (Chen et al., 2012a). It is now accepted that SWEET genes play a role in sucrose efflux; a process which pathogens manipulate to increase the apoplasmic carbon available for growth and proliferation (Chen, 2014). Mutations in the promoter-binding element confer resistance by attenuating binding of *PthXo1* and subsequently preventing transcriptional regulation of the gene by the pathogen (Antony et al., 2010). As well as up-regulating disease promoting genes in the host, it is also documented that bacterial pathogens will target transcription factors that regulate defence responses, particularly WRKY transcription factors (Dong et al., 2003). *rrs1* is the only known recessive *NLR* and harbours a WRKY transcription factor at its C terminal (Deslandes et al., 2002). *rrs1* works in tandem with *RPS4* to mediate race specific resistance to two bacterial pathogens and one fungal pathogen (Narusaka et al., 2009). In two recent publications, it was proposed that the WRKY domain present in *rrs1* is an integrated decoy that is necessary for the detection of bacterial effectors that target WRKY transcription factors to suppress disease responses (Le Roux et al., 2015, Sarris et al., 2015). It is difficult to reconcile the recessive mode of inheritance for *rrs1* in light of this new data, but it has been proposed that it may be due to haploinsufficiency: a quantitative decrease in the phenotypic response due to a lower protein abundance in the heterozygous state (Deslandes et al., 2003). Interestingly, *rrs1* acts as a dominant gene when transformed in transgenic plants (Deslandes et al., 2002).

In the Triticeae tribe, two recessive *R* genes conferring resistance to fungal pathogens have been characterised. Resistance to *P. graminis* f. sp. *tritici* in barley is conferred by *rpg4* that was genetically defined to a 1 kbp region harbouring a single actin depolymerising factor-like gene (*Adf2*) (Brueggeman et al., 2008, Brueggeman et al., 2009). *rpg4* was genetically mapped close to *Rpg5*: a dominant *R* gene conferring resistance to *P. graminis* f. sp. *secalis* (Brueggeman et al., 2008). Unfortunately, the precise nature of *rpg4*-mediated resistance is unclear as no distinguishing polymorphisms were observed between *Adf2* alleles from resistant and susceptible lines. However, the mechanism was linked to the concerted action of *Adf2*, *Rpg5,* and a second *NLR* gene (Wang et al., 2013). The second recessive *R* gene characterised in the Triticeae is *mlo*, conferring resistance to all known isolates of *B. graminis* f. sp. *hordei* in barley. Map-based cloning of *mlo* identified a single gene on chromosome 4H that encodes a seven

transmembrane domain containing protein that binds to the Ca^{2+} sensor, calmodulin (Büschges et al., 1997, Kim et al., 2002). *Mlo* is a negative regulator of *B. graminis* f. sp. *hordei* immunity and functions by inhibiting vesicle-mediated and SNARE protein-dependent resistance (Kim et al., 2002, Collins et al., 2003). In Europe, *mlo* is extensively used in elite barley cultivars to control mildew disease pressure in the field (Jørgensen, 1992).

Our investigation into the recessive resistance to *P. striiformis* f. sp. *hordei* in barley is directed towards addressing two critical questions: 1) Where does *rps2* map in the barley genome? And 2) What is the molecular mechanism of recessive resistance in barley to *P. striiformis* f. sp. *hordei*? To address these questions we investigated host resistance in barley to *P. striiformis* f. sp. *hordei* by inoculating a panel of European barley accessions. Some of the accessions contained previously reported recessive *R* genes. One of these genes, *rps2,* appeared to function in cv. Abed Binder 12, a line that harbours the nonhost resistance specificity *Rpst2* (Chapter 3 and Chapter 4). We leveraged the genetic and genomic resources developed in the previous chapters to map *rps2* to the long arm of chromosome 2H showing that it is distinct from *Rpst2* (7H). We anchored the *rps2* genetic map to the physical map of barley and delimited the locus to an ~330 kbp region. We identified an NLR gene in the vicinity of the *rps2* locus but were unable to identify a strong candidate within the *rps2* locus itself. The on going sequencing of the barley minimal tiling path will help to define the gene content in the region to identify the gene underlying *rps2* resistance.

6.2. Results.

6.2.1. Evaluation of *P. striiformis* f. sp. *hordei* resistance in barley.

To investigate host resistance to *P. striiformis* f. sp. *hordei* we inoculated 107 barley accessions with isolate B01/2 and scored the phenotypic responses using the McNeal scale (McNeal et al., 1971). The majority of the accessions exhibited pustule formation, to varying degrees, and were classified as intermediate or susceptible infection types (Table. A11). Five susceptible accessions contained previously reported *R* genes, suggesting that the genes do not function against isolate B01/2. Only 15 accessions gave a resistant reaction type and the majority contained previously reported recessive *R* genes. One of these genes, *rps2*, contained within cv. Abed Binder 12, conditions race specific resistance in Europe, U.S., and India (Nover and Scholz, 1969, Upadhyay and Prakash, 1977). We wanted to investigate whether the resistance observed in this study to race B01/2, in Abed Binder 12, was mediated by *rps2*. To do this, we inoculated the F_1 and an F_2 population comprised of 93 individuals derived from the cross between Abed Binder 12

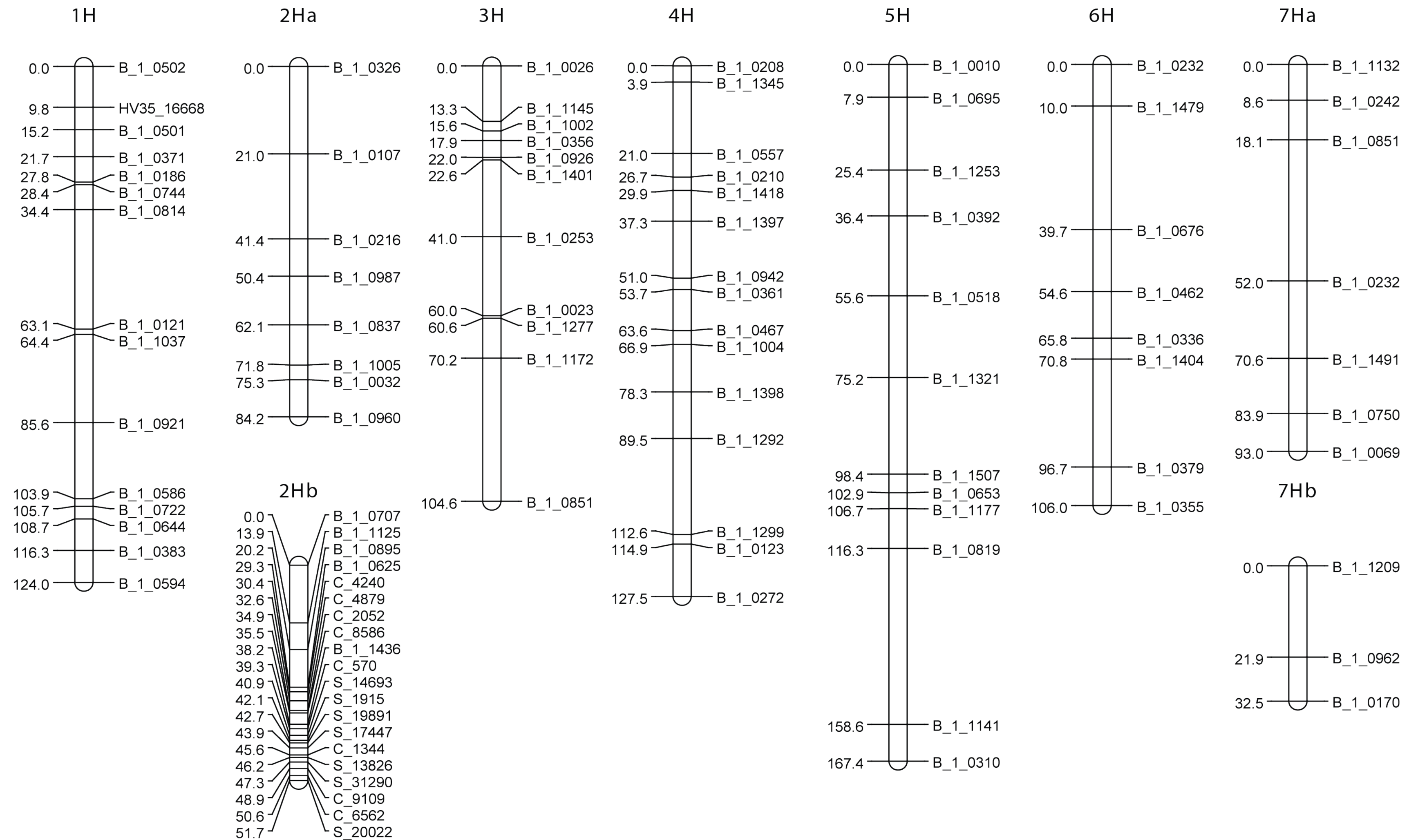


Figure 17: Genetic map of the Abed Binder 12 x Russell F_2 population. Chromosome names are given above each linkage group. cM positions are denoted on the left using the Kosambi function and marker names on the right of each group. Chromosome 2H and 7H were fragmented into two linkage groups designated 'a' and 'b'.

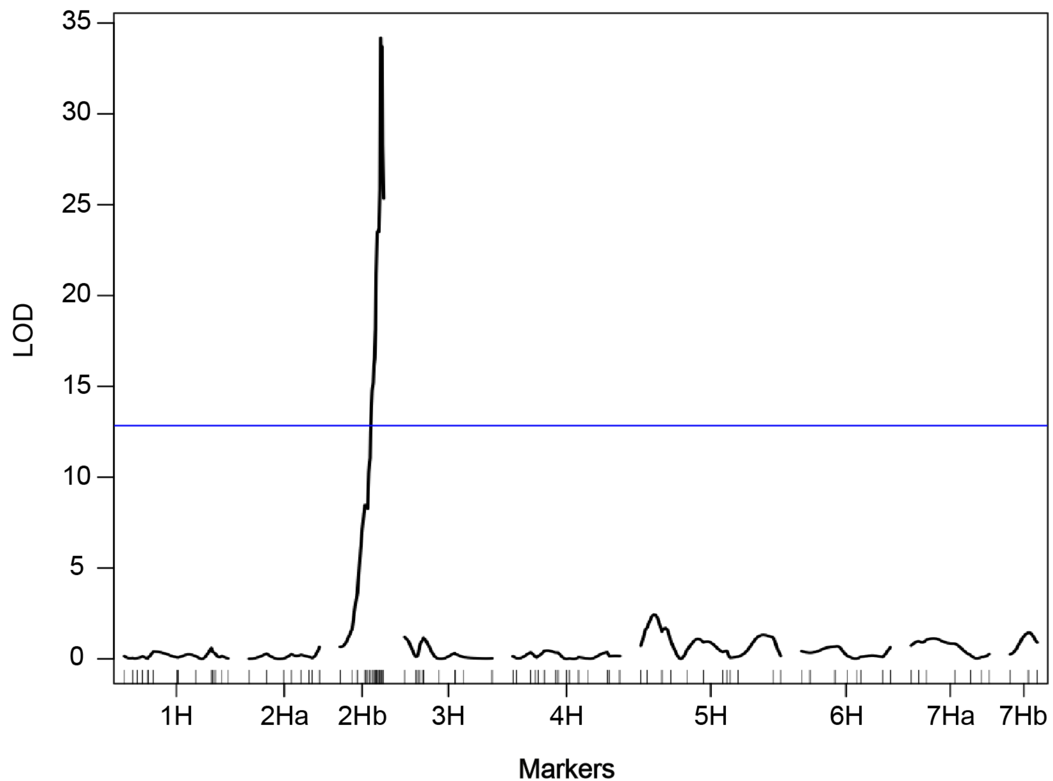


Figure 18: *rps2* maps to the long arm of chromosome 2HL in the Abed Binder 12 x Russell F_2 population. The estimate wide threshold (EWT; dark blue line) was determined using 1,000 permutations of the data. The x-axis represents the markers from the genetic map and the y-axis represents the logarithm of odds (LOD) score.

Table 9: Interval mapping localises *rps2* to the long arm of chromosome 2H.

Trait	LG[1]	cM	Peak Marker	EWT[2]	LRTS[3]	LOD	AEE[4]	DEE[5]	DEE / AEE	PVE[6]
McNeal	2Hb	50.6	C_6562	13.1	130.1	28.3	-3.4	1.3	-0.4	75.3

[1]Linkage group.
[2]Experiment-wide threshold.
[3]Likelihood ratio test statistic.
[4]Allelic effect estimate, negative and positive values indicate resistance is contributed by the A and B alleles, respectively.
[5]Dominance effect estimate.
[6]Percent of the phenotypic variation explained.

and the phenotypically differential accession Russell. The F_1 plant displayed a susceptible phenotype (McNeal score: 7; Fig. A1) and the F_2 population showed a bimodal distribution of the phenotypic data suggesting the presence of a single recessive resistance gene conditioning pustule formation (28 resistant: 65 susceptible, model 1:3; $\chi^2 = 1.29$, $p = 0.26$; Fig. A1). Together, these observations were consistent with the recessive mode of inheritance previously reported for *rps2*.

Figure 19: *rps2* is a Mendelian trait in the Abed Binder 12 x Russell F_2 population. C_6562 shows clear cosegregation of the phenotypic and genotypic data in the Abed Binder 12 x Russell F_2 population. F_2 individual plants are represented by hollow black circles. Error bars represent means with standard deviation. x-axis represents the genotypic call and y-axis the phenotypic score.

6.2.2. *rps2* is located on the long arm of chromosome 7H.

We genetically mapped *rps2* using a linkage mapping approach. To do this, we extracted DNA from phenotyped 14 day old F_2 seedlings and genotyped them using the Sequenom MassARRAY platform. Molecular markers were developed using the barley OPA consensus map by selecting markers at ~10 cM intervals across the genetic map (Muñoz-Amatriaín et al., 2011). The resulting map consisted of nine linkage groups representing the seven chromosomes of barley (Fig. 17). Chromosome 2H and 7H were fragmented into two linkage groups due to a lack of sufficient marker coverage. Nevertheless, a preliminary analysis detected significant linkage to the resistance phenotype on the long arm of chromosome 2H (data not shown). We saturated this region with additional markers using cleaved amplified polymorphic sequences (CAPS) and Sequenom MassARRAY markers. The final genetic map constituted 157 markers, contained within 99 bins and spanned a total genetic distance of 891 cM (Fig. 17). We used this genetic map to perform interval mapping and identified a single major effect locus close to the telomere of chromosome 2HL (Fig. 18). We designated this locus as *rps2*. The locus accounted for 75.3% of the phenotypic variation and the peak marker was C_6562 (Table 9). A plot using this marker showed clear cosegregation of the phenotypic and genotypic values expected of a Mendelian trait (Fig. 19). In the heterozygous condition, a range of scores was observed

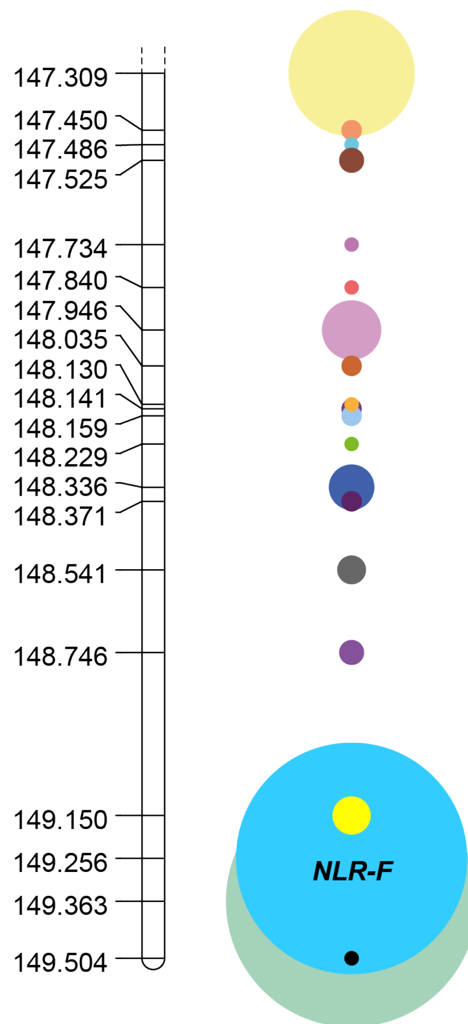


Figure 20: Identification of the candidate gene *NLR-F* in the *rps2* region. An approximate 2 cM region in the POPSEQ data published by Mascher et al. (2013) at the telomeric end of chromosome 2HL was selected for candidate gene analysis. Genetic distances shown to the left of the linkage group represent genetic bins in cM. Coloured bubbles represent number of contigs anchored to each bin (log scale). A single candidate, *NLR-F* was identified in the turquoise bin at 149.256 cM.

Table 10: Anchored WGS contigs in the *rps2* region.

Accession	Contigs[1]	Mean Length (bp)	Total Length (Mb)
Barke	315	3,013	0.95
Bowman	313	4,028	1.26
Morex	370	3,613	1.34

[1]Number of contigs anchored in POPSEQ data between 127.13 and 129.39 cM (Mascher et al. 2013).

across the phenotypic scale. This indicated that *rps2* functions in an additive manner as opposed to being truly recessive based on the McNeal scale, as was previously reported by Nover and Scholz (1969).

6.2.3. Identification of an *NLR* close to the *rps2* locus.

rps2 confers race specific resistance and appears to function in an additive manner (Nover and Scholz, 1969, Upadhyay and Prakash, 1977). We hypothesised that *rps2* resistance

Table 11: Identification of a single NLR candidate gene in the *rps2* locus.

			Expression analysis						High confidence genes			
			R[1]	*I*[2]			*S*[3]					
WGS contigs	**cM**	**E- value**	**A[4]**	**G[5]**	**B[6]**	**S[7]**	**R[8]**	**M[9]**	**MLOC[10]**	**Annotation**	**% match**	**E- value**
morex_contig_62011	149.256	1.10E-37							MLOC_72627.1	NLR	85	0
bowman_contig_1989289	149.256	2.30E-28	H	H	H	H	H	H	MLOC_54830.2	NLR	90	0
barke_contig_1828911	149.256	2.30E-26							MLOC_19010.2	NLR	90	0
									MLOC_77972.2	NLR	97	0

[1]Resistant phenotype
[2] Intermediate phenotype
[3] Susceptible phenotype
[4] Abed Binder 12
[5] Golden Promise
[6] Barke
[7] SusPtrit
[8] Russell
[9] Manchuria
[10] MLOC identified in barley high confidence gene set (Mayer et al. 2012).

may be conferred by an *NLR* and we initiated a candidate gene approach to search for *NLRs* at the *rps2* locus. To do this, we identified WGS contigs anchored to the telomeric end of chromosome 2HL in an ~2 cM region according to POPSEQ data (Mascher et al., 2013) (Fig. 20 and Table 10). We identified 315, 313, and 370 WGS contigs with average lengths of 3,013, 4,028 and 3,613 bp for the cultivars Barke, Bowman, and Morex respectively (Table 10). The cumulative length of all contigs in the region, for each accession, ranged from 0.95-1.34 Mbp. We searched the anchored contigs for motifs associated with *NLR*s using the motif alignment and search tool (MAST) according to the parameters published by (Jupe et al., 2012). We identified three contigs positioned at 149.256 cM that harboured NLR motifs (Table 11). The contigs collapsed into one homologous group representing a single *NLR* candidate in the region. We provisionally designated the gene as *NLR-F*. Morex contig 62011 harboured a full length *NLR* whereas Barke contig 1828911 and Bowman contig 1989289 appeared to harbour motifs associated with the NB domain only (data not shown). We retrieved a single transcript for *NLR-F* by performing BLAST using the WGS contigs against the Abed Binder 12 assembled transcriptome. NLR-F was highly expressed in all barley accessions tested (Table 11). Four high confidence genes previously annotated by the Barley Genome Sequencing Consortium were identified using the largest open reading frame in the Abed Binder 12 *NLR-F* transcript (IBGSC, 2012). All of the high confidence genes had similarity to NLR proteins (Table 11).

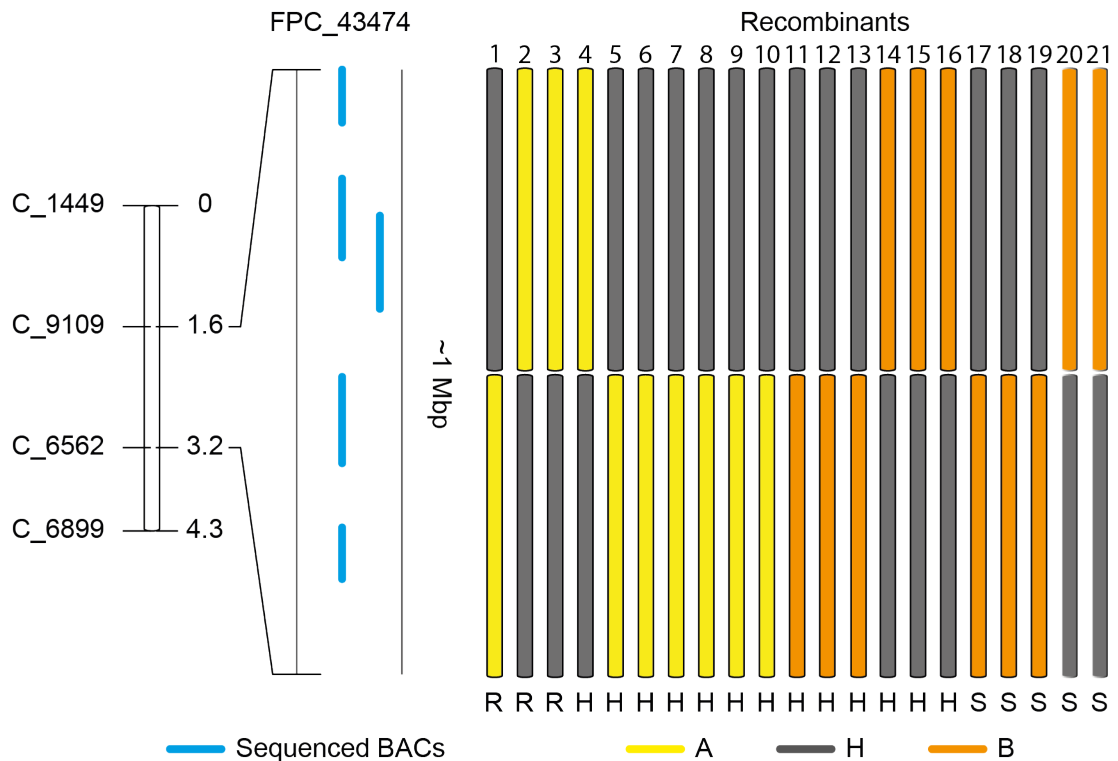


Figure 21: A 1.6 cM *rps2* region harbouring 21 unresolved recombination events anchors to the physical map of barley. Four CAPS markers were used to delimit the locus. Blue vertical lines represent five BAC clones anchored in the region and sequenced by the International Barley Genome Sequencing Consortium (Mayer et al. 2012). Yellow, grey, and orange colouring of recombinant lines represent Abed Binder 12, heterozygous, and Russell allele calls, respectively. R, H, and S denote resistant, segregating, and susceptible phenotype calls for F_3 families (2 x 8 leaves for each family).

Table 12: Anchoring of the *rps2* locus to the barley physical map.

Marker	Unigene[1]	Morex contig	FPC[2]	Sequenced BACs[3]	BAC ID
				HVVMRX83KHA0070D03	D03
C_1449	1449	contig_140602	-	HVVMRX83KHA0069A19	A19
C_9109	9109	contig_135650	43474	HVVMRX83KHA0063F09	F09
C_6562	6562	contig_44855	43474	HVVMRX83KHA0124C21	C21
C_6899	6899	contig_200660	-	HVVMRXALLHA0113O23	O23
				HVVMRX83KHA0028K08	-

[1]Unigenes derived from assembly 32 (http://harvest.ucr.edu).
[2]Fingerprinted contig.
[3]BACs all reside within FPC 43474.

6.2.4. Fine mapping *rps2* to a 0.85 cM region excludes *NLR-F* as a candidate gene.

To fine map *rps2* we identified recombination events between markers C_1449 (proximal) and C_6562 (distal) in Abed Binder 12 x Russell F_2 seedlings (Fig. 21 and Table A12). We screened a total of 1,536 gametes and identified 49 recombination events delimiting the *rps2* locus. Using CAPS markers, anchored in the F_2 genetic map, we delimited the locus to a 1.6 cM region, containing 21 unresolved recombination events between marker C_9109 (proximal) and C_6562 (distal) (Fig. 21 and Table A12). We anchored the *rps2* locus to the

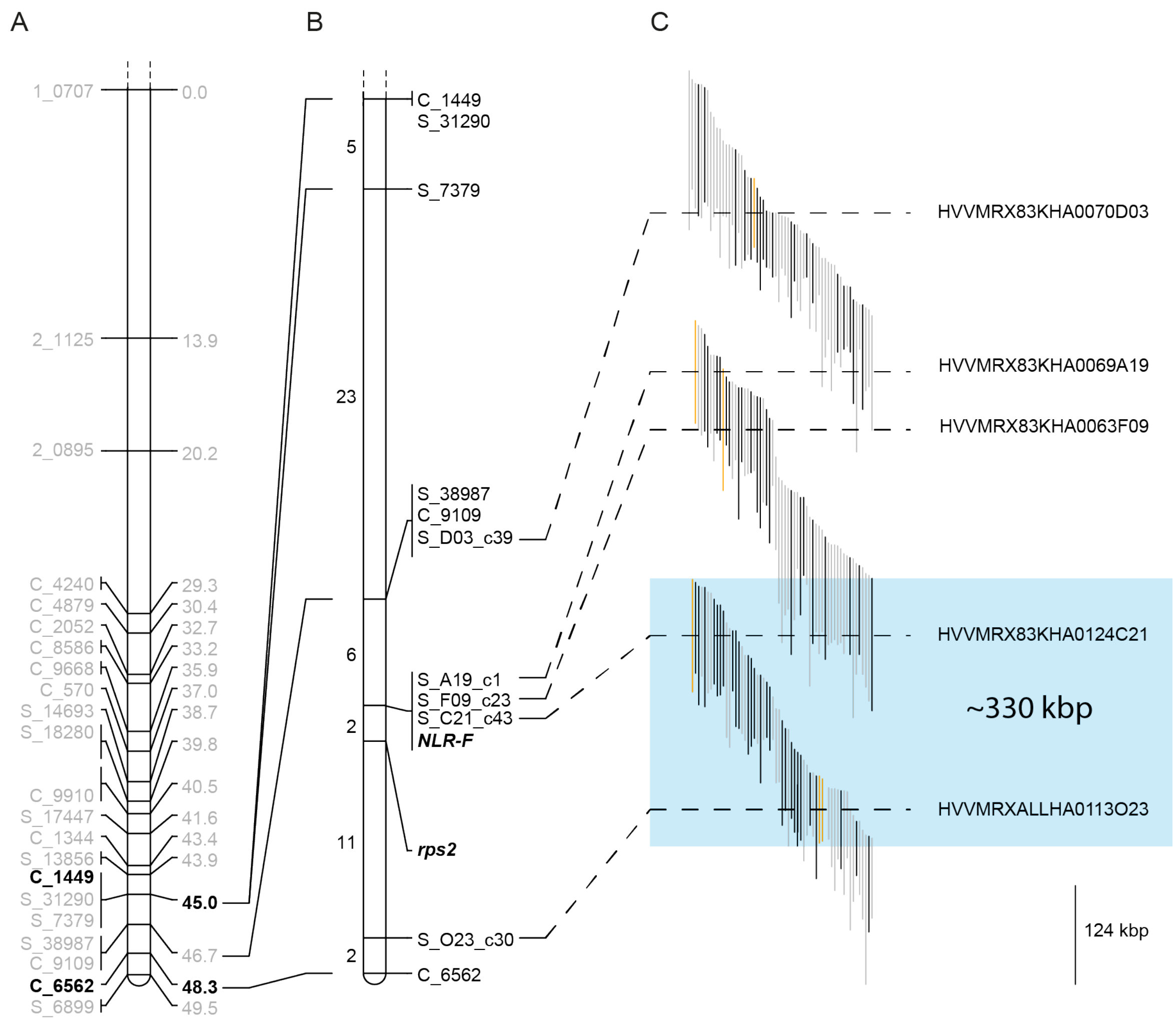


Figure 22: Marker saturation delimits the *rps2* locus to a 330 kbp region. (A) Distal region of chromosome 2HL based on mapping in Abed Binder 12 x Russell F_2 population. (B) High resolution linkage map generated from 1,536 recombinant gametes. (C) Anchoring to the barley physical map (Mayer et al. 2012). BACs that are sequenced or have BES available are orange or black, otherwise BACs are shown in grey.

barley physical map by performing BLAST using the unigenes that were used to develop the flanking markers against the POPSEQ anchored WGS contigs (Table 12). Morex contig 135650 and contig 44855 corresponding to the *rps2* flanking markers were anchored to FPC 43474. Six BAC clones in the FPC had been previously sequenced by the Barley Genome Sequencing Consortium (IBGSC, 2012) (Fig. 21 and Table 12). We used the sequence information derived from these BACs to design Sequenom markers for saturating the *rps2* locus along with an *NLR-F* specific marker. We were able to position *rps2* in a 0.85 cM region flanked by markers derived from BACs A19, F09, C21 (proximal), and BAC O23 (distal). *NLR-F* mapped within the cluster of proximal markers positioned two recombination events from *rps2*, excluding it as a candidate gene (Fig. 22). For markers that were not separated by recombination events we inferred the position relative to the physical map. *rps2* is flanked by BACs C21 and O23 (Fig. 22). The physical distance underlying this region is approximately 330 kbp (IBGSC, 2012).

6.3. Discussion.

Host resistance to *P. striiformis* f. sp. *hordei* was previously shown to be mediated by a large proportion of recessive resistance specificities in barley (Nover and Scholz, 1969, Bakshi and Luthra, 1970, Chen and Line, 2002). Yan and Chen mapped *rps1.a* and *rpsGZ* to the long arms of chromosomes 3H and 4H, respectively (Yan and Chen, 2006, Yan and Chen, 2007). However, additional knowledge on the genomic location of these specificities is lacking and even less is known about the molecular mechanisms underlying them. In this study, we showed that cv. Abed Binder 12, a barley accession previously shown to contain a recessive *R* gene called *rps2*, was resistant to European *P. striiformis* f. sp*. hordei* isolate B01/2. The resistance appeared to be recessive at the F_1 stage suggesting *rps2* was the underlying resistance gene. We mapped *rps2* to the long arm of chromosome 2H and showed that host resistance to *P. striiformis* f. sp. *hordei* did not colocalise with nonhost resistance to *P. striiformis* f. sp. *tritici* in Abed Binder 12. We anchored the *rps2* region to the barley physical map and defined the locus with three Morex BAC clones. These clones formed the minimal tiling path (MTP) between the *rps2* flanking markers, encompassing a region of ~330 kbp.

6.3.1. *rps2* is located in a favourable genomic location for map based cloning.

The genomic location of *rps2* on the long arm of chromosome 2H was extremely favourable for mapping. Higher rates of recombination are known to occur in distal regions of barley chromosome arms especially towards the telomere (IBGSC, 2012). The rate of recombination in a given region can influence the success or failure of a mapping study as it influences the amount of recombination breakpoints that can be identified for delimiting the locus (Krattinger et al., 2009a). In the case of *rps2*, we performed a relatively small recombination screen, surveying 1,536 gametes, and were able to delimit *rps2* to an approximately 0.85 cM region. *rps2* is positioned two recombination breakpoints from marker *NLR-F* (proximal) and eleven from marker S_O23_c30 (distal).

6.3.2. Utilisation of sequenced BACs from the barley physical map for marker development.

A rate-limiting step in any mapping study is the ability to identify markers that are used to resolve recombination breakpoints that delimit the locus. We used the barley OPA genetic map published by Muñoz-Amatriaín et al. (2011) to identify markers ~10 cM apart to develop a Sequenom assay that would provide sufficient resolution for interval mapping. The position of *rps2* toward the telomere of chromosome 2HL highlighted the importance of selecting markers in the telomeric portions of chromosomes. Failure to do so could

result in the failure to detect loci, like *rps2*, positioned in such regions. For marker saturation at the *rps2* locus we first exploited all known markers in the region based the consensus maps of barley (Potokina et al., 2008, Close et al., 2009, Moscou et al., 2011, Muñoz-Amatriaín et al., 2011). We achieved additional resolution by anchoring *rps2* to the barley physical map. A total of five BAC clones, anchored to the *rps2* region, had been sequenced by the International Barley Genome Sequencing Consortium (IBGSC, 2012). This provided an invaluable resource for marker development directly at the locus. Two of the sequenced BAC clones, HVVMRX83KHA0124C21 and HVVMRXALLHA0113O23, flanked *rps2* and enabled the delimitation of the locus to an approximately 330 kbp region containing 13 unresolved recombination events. Assuming recombination rates are constant across the locus we can infer that marker *NLR-F* (proximal) is no more than ~50 kbp, and marker S_O23_c30 (distal) no more than ~280 kbp, from *rps2*.

6.3.3. Development of a minimal tiling path encompassing *rps2.*

Recently, Zang et al. (2015) reported a candidate gene for *Un8*, a true loose smut resistance gene, after sequencing two overlapping BAC clones from the physical map of barley. Key to this endeavour was the assembly and anchoring of three large BAC fragments that spanned the majority of the *Un8* MTP (~151, 52, and 45 kbp). While the BAC clones in this study were invaluable for marker development they had some limitations for physical mapping of the *rps2* locus. HVVMRX83KHA0124C21 consisted of 11 unordered fragments ranging from 509 to 52,067 bp and HVVMRXALLHA0113O23 consisted of 46 unordered fragments ranging from 508 to 14,720 bp. The existing *rps2* fine map did not contain sufficient marker density or recombinant breakpoints for obtaining the linear order, and orientation, of each BAC fragment. Similarly, the BACs flanked *rps2* according to ordering of the BACs in FPC 43474 but they did not overlap. Therefore, additional BAC clones need to be sequenced in order to obtain a MTP for the *rps2* locus. We intend to sequence four BAC clones, HVVMRX83KHA0124C21 and HVVMRXALLHA0113O23 plus two additional BAC clones, HVVMRX83KHA0215K06 and HVVMRX83KHA0179H16, that form the MTP for *rps2*, using Pacific Biosciences SMRT cell long read sequencing. In the previous chapter, we used a similar strategy to sequence a BAC clone containing *NLR-A* and a high amount of repetitive sequence information. We demonstrated that a single contiguous sequence representing the entirety of the BAC clone could be obtained using this technology. The longer read length permits assembly of the repetitive sequence often present in cereal genomes (Schulte et al., 2009, IBGSC, 2012). The development of an *rps2* MTP will permit further marker development at the *rps2* locus to resolve the remaining recombination breakpoints in the region. Moreover, it

will facilitate the identification of candidate genes, albeit in an accession not known to harbour *rps2*.

6.3.4. Determining the mode of inheritance for *rps2*.

In this study, we demonstrated that *rps2* functions in an additive manner, similar to *Rpst2* (Chapter 4). These conclusions were based on the distribution of phenotypes across the phenotypic scale in plants heterozygous for *rps2*. A truly recessive *R* gene would be characterised by susceptible phenotypes in the heterozygous state comparable to the susceptible parent. *rps2* was previously reported to be recessive based on segregation ratios in F_2 populations that were phenotyped using a five point scale (Nover and Scholz, 1969). Calculating segregation ratios depends upon the qualitative separation of the data into discreet classes (resistant and susceptible). This process is not clearly defined: classifications may be arbitrary and the best fit of the data. Similarly, phenotypic characterisation of the mode of inheritance can change depending on the growth stage and genetic background of the plant. An example of growth stage dependence includes the genes conferring resistance to *P. striiformis* f. sp. *hordei* in cvs. Cambrinus and Mazurka, were dominant based on the reaction at the first leaf stage and recessive at the second leaf stage (Chen and Line, 2002). Similarly, *rrs1*, the only known recessive *NLR* acts as a dominant *R* gene when transformed into susceptible plants (Deslandes et al., 2002). This highlights the difficulty in interpreting the mode of inheritance for *R* genes solely on phenotypes and segregation ratios and caution should be exercised when interpreting such data.

6.3.5. Candidate gene approach fails to identify high priority genes.

We initiated a candidate gene approach for *rps2* tailored towards *NLRs*. We were motivated to do this for three reasons. First, *rps2* appeared to function in an additive manner similar to *Rpst2*, a nonhost specificity for which a candidate *NLR* was discovered in the previous chapter. Second, we can reconcile the additive nature of *rps2* with respect to *rrs1* from *A. thaliana* where the recessive nature is hypothesised to be due to haploinsufficiency, wherein the presence of only one functional allele leading to a reduced amount of functional protein, as opposed to a qualitative change leading to complete loss of function (Deslandes et al., 2002, Deslandes et al., 2003). Lastly, the majority of host resistance genes cloned to date have coded for NLRs, particularly CNLs in the Triticeae (Krattinger et al., 2009a). After interrogating anchored WGS contigs for NLR motifs we were able to identify a single NLR candidate in the vicinity of *rps2* (*NLR-F*). However, mapping *NLR-F* positioned it two recombination events from *rps2*, excluding it as a candidate gene. *NLRs* are known to exist in gene clusters often in close proximity with

members of the same *NLR* family (Wei et al., 1999, Zhou et al., 2001, Yahiaoui et al., 2004, Hurni et al., 2013). In the previous chapter, we identified *NLR-A* as a candidate for *Rpst2* and showed that is was located in a gene cluster with two gene family members: *NLR-D* and *NLR-E*. The close proximity of these genes was surprising given their distal genetic proximity to *NLR-A* according to POPSEQ anchoring of WGS contigs (Mascher et al., 2013). This result highlighted the cautionary approach needed when interrogating POPSEQ data. Given the close proximity of *NLR-F* (identified in this study) to *rps2*, we cannot rule out the possibility that we will discover other, as of yet unidentified, *NLR*s at the *rps2* locus after physical mapping.

This study has shown that host resistance to *P. striiformis* f. sp. *hordei* does not colocalise with nonhost resistance to *P. striiformis* f. sp. *tritici* in cv. Abed Binder 12. To see whether this observation holds true, more generally, it will be necessary to scrutinise additional host resistance specificities in barley particularly focusing on previously characterised recessive genes. We have made significant progress towards map based cloning *rps2*. Despite the prominence of recessive resistance in barley to *P. striiformis* f. sp. *hordei* this would be the first cloned gene and may provide significant insight in to this anomalous observation. Physical mapping using the barley physical map will assist in delimiting the locus and assessing potential candidates in the region. This may be combined with the existing RNAseq data for Abed Binder 12 and Russell to identify expression polymorphisms for candidates in the region and for marker development. It would be advantageous to build a physical map for the resistant haplotype using the Abed Binder 12 BAC library generated in the previous chapter for eventual cloning of the gene underlying *rps2* resistance.

7. General discussion.

In this thesis, I have described the genetic architecture of intermediate host resistance in barley to *P. striiformis* f. sp. *tritici* and the efforts made to identify a causal gene underlying this specificity. The genetic architecture is relatively simple and involves the concerted action of three major effect loci, *Rpst1*, *Rpst2*, and *Rpst3*, which act as Mendelian traits when isolated independently of each other. Using classical map-based genetics, in combination with more contemporary genomics information, it was possible to fine map *Rpst2* and to identify a candidate *NLR* gene underlying this specificity. The identification of a candidate *NLR* gene supports the evolutionary model for nonhost (Schulze-Lefert and Panstruga, 2011) resistance proposed by Schulze-Lefert and Panstruga that suggests the role of NLRs will decrease as a function of the evolutionary separation from the host species of the pathogen (Schulze-Lefert and Panstruga, 2011). Further work is needed to fully validate the role of *NLR-A* in *Rpst2* mediated resistance. However, the observation of *NLR* genes in the *Rpst2* region provides compelling evidence for a role of *NLRs* in intermediate host resistance. To further validate the model of Schulze-Lefert and Panstruga, it will be necessary to identify additional pathosystems that are more evolutionary distant, to the host plant wheat, than barley. The interaction of *P. striiformis* f. sp. *tritici* with *B. distachyon* (diverged form wheat 32-39 Myr ago) and *O. sativa* (50 Myr ago) present well-resourced model systems to define this landscape (Vogel et al., 2010, Bossolini et al., 2007, Wei et al., 1999). According to the model, *NLR* mediated resistance will be less frequently observed and alternative modes of resistance uncovered during these interactions. Preliminary work studying the interaction of *B. distachyon* with *P. striiformis* f. sp. *tritici* seems to support this hypothesis (Jan Bettgenheauser, unpublished data).

7.1.1. Multiple pathogen recognition specificity.

In this thesis, I was able to uncouple host and nonhost resistance to *P. striiformis* in barley by studying the genetic architecture of resistance in Abed Binder 12 to both pathogens. However, another key theme suggests that resistance loci to Ascomycota and Basidiomycota host and nonhost pathogens colocalise. The genetic resolution of this colocalisation is often limited. Therefore it is difficult to determine whether or not the mechanisms will be conditioned by the same or different genes (i.e. genetic linkage or pleiotropy). However, research into *Rpst1,* not presented in this thesis, has uncovered an interesting result relevant to this discussion. Notably, that nonhost resistance to *P. striiformis* f. sp. *tritici* is in complete genetic coupling with the *Mla* locus that confers host resistance to *B. graminis* f. sp. *hordei* (Matthew Moscou, unpublished data). The *Mla* locus

has also been associated with resistance to *M. oryzae* (Inukai et al., 2006). As the majority of plant *R* genes encode NLR proteins, the genetic coupling of host, intermediate host, and nonhost resistance with a locus harbouring NLR encoding genes prompts a direct association. If true, this would link *R* genes typically involved in host-pathogen interactions in determining the host species specificity of intermediate host and nonhost resistance. Further characterisation of these loci will be required to determine a causal association. However, given that *P. striiformis* f. sp. *tritici* and *B. graminis* f. sp*. hordei* resistance are in complete genetic coupling we can generate three hypotheses that may explain multiple pathogen recognition specificity at the *Mla* locus:

Hypothesis 1: Resistance to host and nonhost pathogens is conferred by distinct *NLR* genes at the *Mla* locus. Three NLR encoding gene families exist at the *Mla* locus, with functional alleles of *Mla* belonging to the *RGH1* family (Wei et al., 1999). It is possible that the other two gene families, *RGH2* and *RGH3*, may encode functional resistance genes against *P. striiformis* f. sp. *tritici* and *M. oryzae*. This model is supported by the observation that different *Mla* haplotypes can express more than *NLR* at the locus (Seeholzer et al., 2010). This would be similar to the hypothesised gene combination at the *Sr31*/*Lr26*/*Yr9* locus on the 1BL.1RS rye translocation in wheat (Mago et al., 2005). In this study, the three specificities were separated using genetic recombination and mutational analysis. However, given the supressed recombination reported at the *Mla* locus only a mutational approach may be viable for separating recognition specificities in this scenario (Wei et al., 1999).

Hypothesis 2: Resistance to host and nonhost pathogens is conferred by genes other than *NLR*s at the *Mla* locus. Under this hypothesis, it is proposed that one or more genes are present at the *Mla* locus that condition *Rpst1*-mediated resistance that do not encode NLR-type *R* genes. These genes could include known genes at the *Mla* locus in the cultivar Morex, such as the *chymotrypsin inhibitor 2* (*CI2*) gene family, which are protease inhibitors that negatively affect the growth of insects through the inhibition of gut proteases (Heath et al., 1997) and several other genes with unknown function (Wei et al., 1999). Alternatively, novel, as of yet unidentified, gene(s), that vary based on the host haplotype, may contribute to resistance and may work in cohort with *NLRs*. This hypothesis is not without merit, as recent evidence from studying the stem rust resistance locus *rpg4*/*Rpg5* in barley has found a complex set of novel genes and fusion products conditioning resistance. For *Rpg5*, this involves a novel gene fusion that encodes an NLR-kinase, whereas other susceptible variants are lacking the kinase domain and are flanked by a gene encoding a putative protein phosphatase (PP2C) (Brueggeman et al., 2008). The same locus harbours a recessive resistance gene to wheat stem rust, *rpg4*, which requires

three genes: a NLR encoding gene, *Rpg5*, and a gene encoding an actin depolymerising factor (Wang et al., 2013).

Hypothesis 3: Resistance to host and nonhost pathogens is conferred via the pleiotropic action of the *RGH1* family members currently associated with resistance to *B. graminis* f. sp. *hordei*. It is possible that the same genes conferring resistance to *B. graminis* f. sp. *hordei* also confer resistance to *P. striiformis* f. sp. *tritici* and *M. oryzae*. Rusts, blasts, and mildews belong to entirely different phyla (Basidiomycota and Ascomycota), therefore it is compelling that a single gene may recognise all pathogens. Based on this hypothesis, there are broader implications in terms of the mode of pathogen recognition. NLRs are known to perceive effector proteins either through direct or indirect recognition (Jones and Dangl, 2006, van der Hoorn and Kamoun, 2008). Under the model of direct recognition, *Mla* could recognise either two different effector proteins or a conserved effector between barley powdery mildew and wheat stripe rust. In contrast, under the indirect recognition model, *Mla* would guard a host protein in barley that is a conserved effector target for both barley powdery mildew and wheat stripe rust, and recognition occurs as a result of a modification to this host protein.

Of the three hypotheses presented, the idea of multiple pathogen recognition specificity conferred by the pleiotropic effect of single NLR family members (Hypothesis 3) is the most compelling. At present, there are only three examples of resistance to multiple pathogens being conditioned within the same haplotype. These examples include the *RPS4*/*RRS1* resistance locus in the model plant *A. thaliana* (Deslandes et al., 2002), the *Mi-1* gene in tomato (Vos et al., 1998, de Ilarduya et al., 2003), and the adult plant resistance gene *Lr34* in wheat (Krattinger et al., 2009b). All three genes are intensively studied: *RPS4/RRS1* as a genetic system to understand the early signal transduction cascade leading to immunity, *Mi-1* for its broad recognition of nematodes, aphids, and whitefly, and *Lr34* for its role as an adult plant resistance gene, its nonspecific resistance to multiple pathogens, and its current use in breeding durable disease resistance. Therefore, the colocalisation of host and nonhost resistance specificities in barley may present an ideal system for studying the role of multiple recognition specificity in determining host status. Studying the colocalisation of multiple resistance specificities has the potential to expand our current understanding of plant-pathogen interactions by moving away from single species complexes towards multifactorial ecological systems. This may help us to understand how plants are able to resist the multiplicity of pathogens to which they are exposed while only encoding a finite number of immune receptors.

7.1.2. Recommendations for future research.

- Validate the role of *NLR-A* in *Rpst2* mediated resistance. To fully validate *NLR-A* it will be necessary to see resistance mediated by *NLR-A* in a susceptible barley accession and to generate and identify mutations in *NLR-A* in resistant accessions that compromise the resistance response. Mutagenesis would be practical using the lines from the SusPtrit x Golden Promise doubled haploid population monomorphic for *Rpst2*, such as DH-064 (Yeo et al., 2014). Assuming *NLR-A* is the causal gene it should be straightforward to recover mutants using an EMS suppressor screen as has been demonstrated for numerous *NLRs* in the Triticeae to date (Krattinger et al., 2009a)(Table 1). Transforming *NLR-A* into a susceptible barley accession presents more of a challenge. To date, only a handful of barley accessions have been transformed with the most prominent line being Golden Promise, an accession highly resistant to *P. striiformis* f. sp. *tritici*. Therefore it will be necessary to identify transformable, susceptible accessions. Recently, Yeo et al. (2014) successfully transformed lines from the SusPtrit x Golden Promise DH population suggesting that it may be possible to identify a line that has the transformability of Golden Promise but the susceptibility to *P. striiformis* f. sp. *tritici* of SusPtrit. The size of *NLR-A* (~17 kbp) also presents some challenges for cloning the gene prior to transformation. While it should be feasible to clone the cDNA excluding the introns it is not known whether the absence of such large intronic regions will affect the functionality of the gene.
- Test the functionality of *Rpst2* in wheat. Assuming that *NLR*-A is confirmed as *Rpst2*, a major area of future research in this field should focus on testing the functionality of *NLR-A* in wheat. Retained functionality would open up the possibility of transforming multiple intermediate host resistance specificities into the host plant to engineer potentially durable disease resistance.
- Pathogen recognition. At present, there is a dearth of information about effector recognition in the Triticeae tribe. This is largely due to the lack of a robust transient assay for interrogating large numbers of effector candidates in monocot species such as members of the Triticeae tribe. While using the type III secretion system of bacterial pathogens may hold some promise as a transient assay for monocot species (Upadhyaya et al., 2014), alternative approaches may be required for identifying functional effectors in the Triticeae. One such approach may be to mutate the pathogen and screen for gain of virulence. The identification of pathogen virulence/avirulence determinants would be a major break through for understanding Triticeae immunity. It would also facilitate breeding efforts for

durable resistance, through the use of effectoromics to integrate stacks of *R* genes in transgenic lines.

8. Appendices.

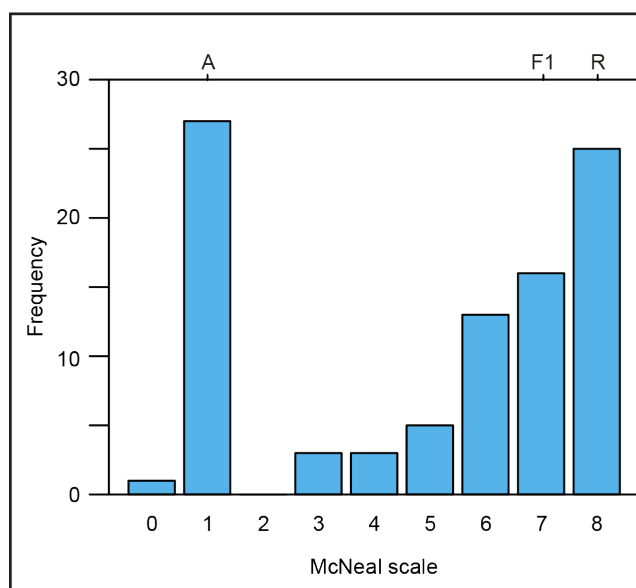


Figure A1: Resistance to *P. striiformis* f. sp. *hordei* shows a bimodal distribution in the Abed Binder 12 x Russell F_2 population. y-axis represents frequency of observation and x-axis represents McNeal scores: 0 (immune; no visible symptoms), 1 (necrotic/chlorotic flecks without sporulation), 2 (necrotic/chlorotic stripes (NCS) without sporulation), 3 (trace sporulation with NCS), 4 (light sporulation with NCS), 5 (intermediate sporulation with NCS), 6 (moderate sporulation with NCS), 7 (abundant sporulation with NCS), 8 (abundant sporulation with chlorosis), and 9 (abundant pustule formation, without chlorosis). Relative position of the parents and F_1 are indicated.

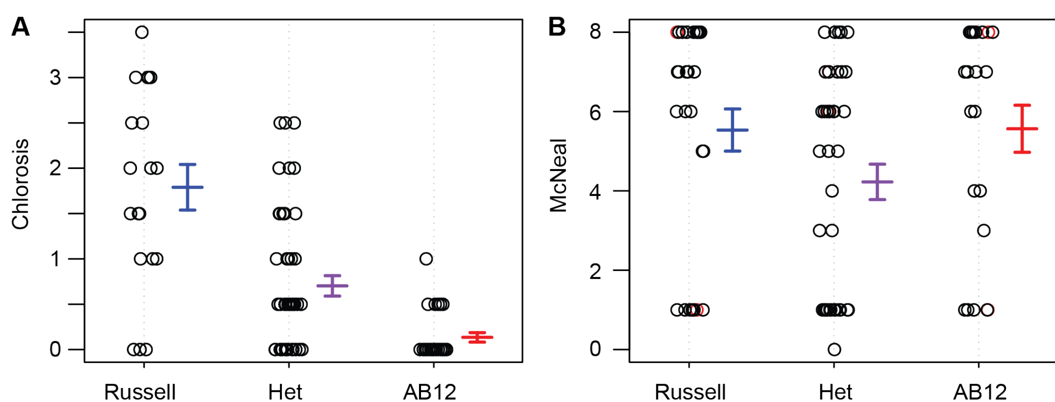


Figure A2: Uncoupling of *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* resistance in two independent Abed Binder 12 x Russell F_2 populations. Phenotype x genotype plots using marker 2_0692 on (A) AxR-*Pst* phenotyped using chlorosis and (B) AxR-*Psh* phenotyped using the McNeal scale.

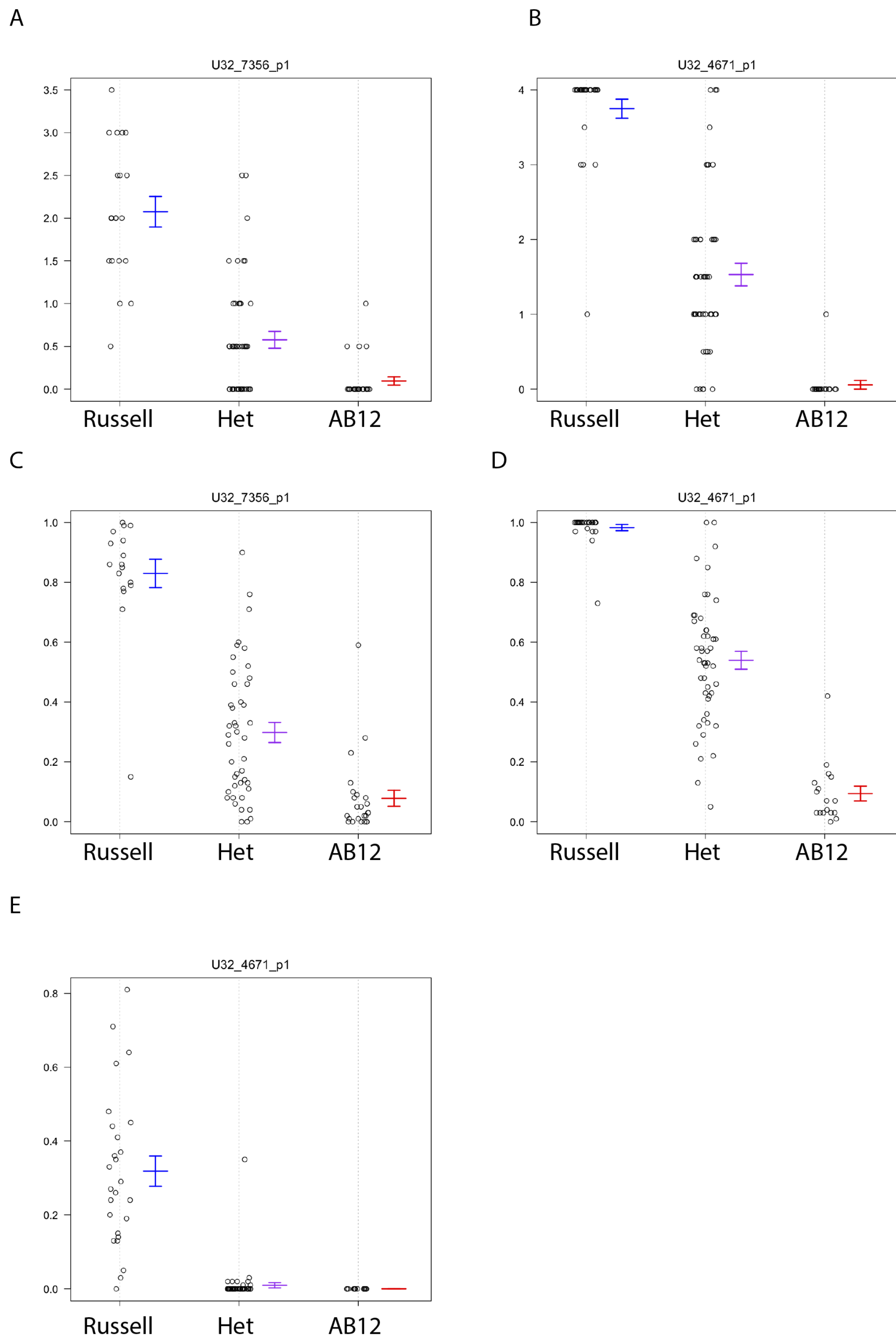


Figure A3: Isolation of *Rpst2* in an $F_{2:3}$ family. Phenotype by genotype plots. x-axes represent allele call using marker C_7356 and C_4671 for the Abed Binder 12 x Russell F_2 population and $F_{2:3}$ population, respectively. y-axes represent phenotypic scale used. For (A and B) chlorosis, (C and D) pCOL, and (E) pPUST.

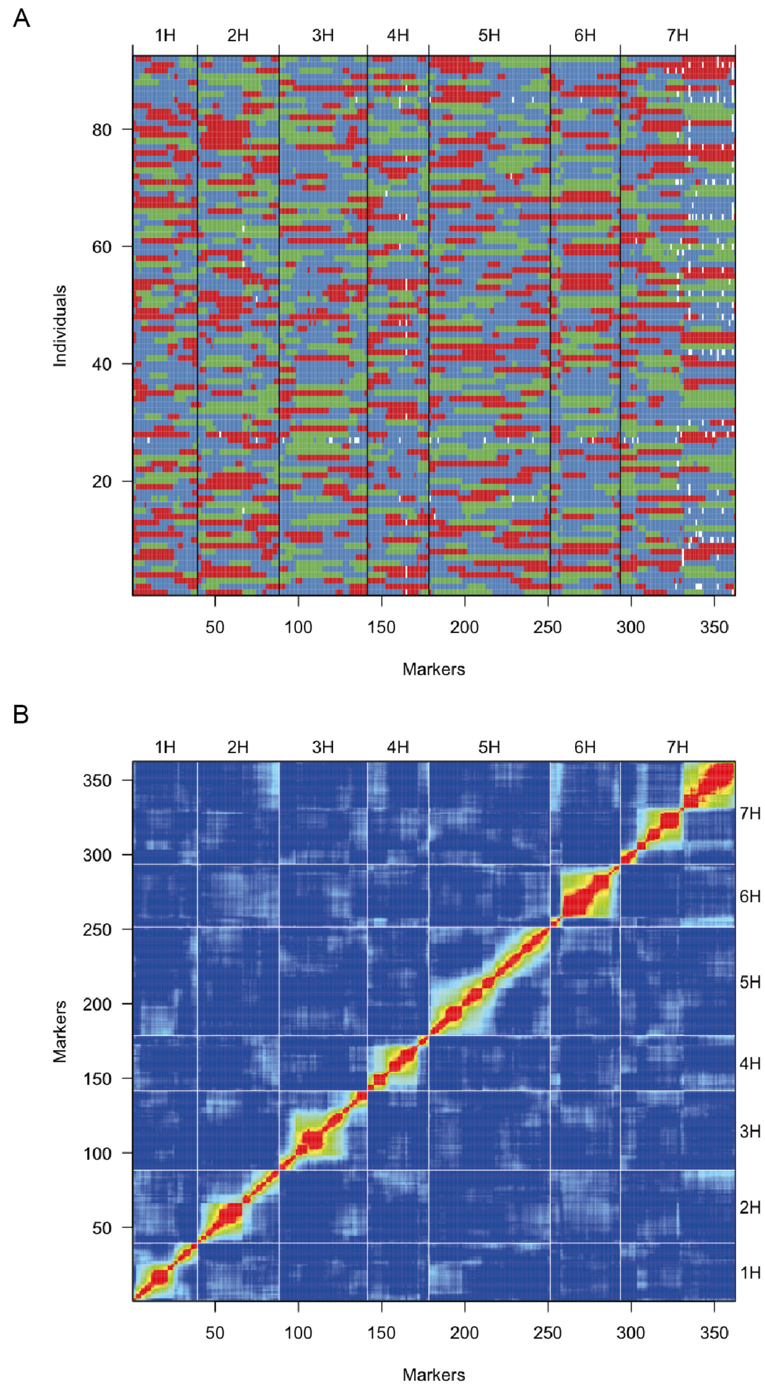
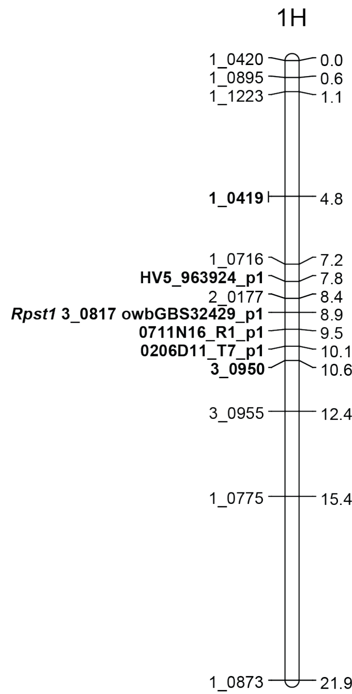
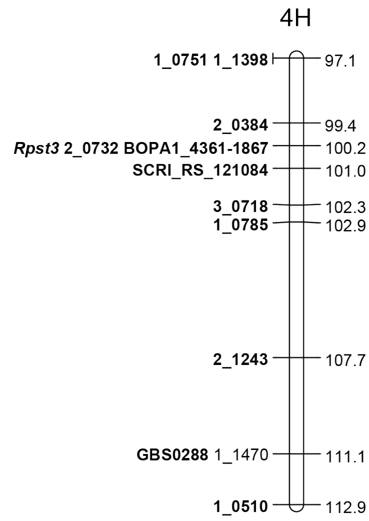


Figure A4: Genetic map of the Abed Binder 12 x Russell F_2 population using 362 non-redundant markers. (A) Recombination map (red= Abed Binder 12, green= Russell, blue= heterozygous, and white= missing data). (B) Two-point linkage association heat map between markers.

A



B



C

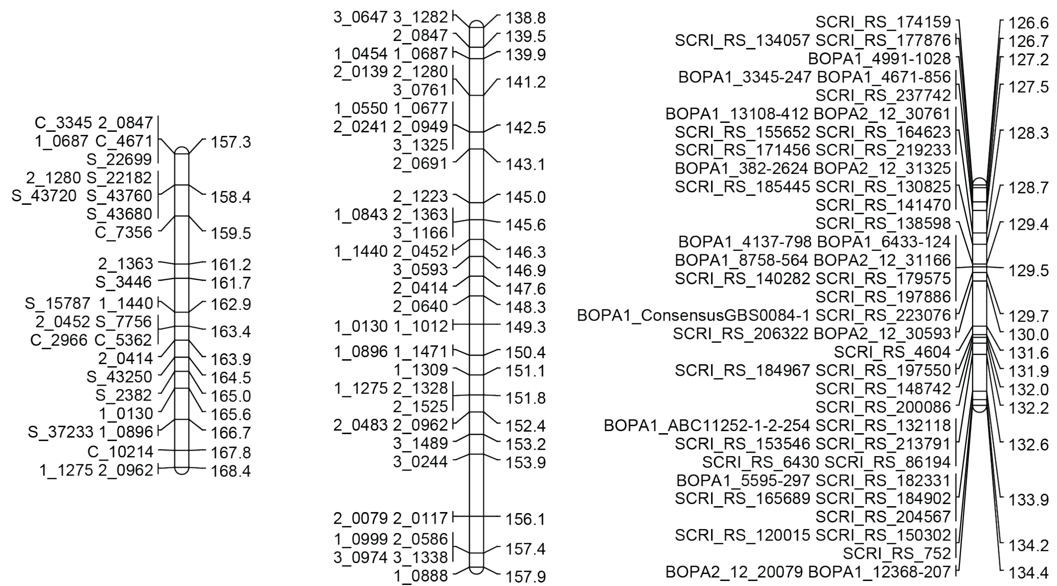


Figure A5: Consensus linkage groups for *Rpst1* (1H), *Rpst2* (7H), and *Rpst3* (4H). Relative positions of all markers used for assaying the (A) *Rpst1*, (B) *Rpst3*, and (C) *Rpst2* loci in barley.

Table A1: Accessions of barley used for inoculation with *P. striiformis* in Chapter 3.

Accession	Alternate ID	Experiment used[1]	Source
Abed Binder 12	PI 327961	*Pst*	USDA-GRIN
ADONIS		*Psh, Pst*	James Hutton Institute
AGENDA		*Psh, Pst*	James Hutton Institute
Akashinriki	PI 467400	*Pst*	USDA-GRIN
AKITA		*Psh, Pst*	James Hutton Institute
ALABAMA		*Psh, Pst*	James Hutton Institute
Algerian	PI 539104	*Pst*	USDA-GRIN
ALLIOT		*Psh, Pst*	James Hutton Institute
ALUMINIUM		*Psh, Pst*	James Hutton Institute
ANACONDA		*Psh, Pst*	James Hutton Institute
ANAIS		*Psh, Pst*	James Hutton Institute
ANNABELL		*Psh, Pst*	James Hutton Institute
APPALOOSA		*Psh, Pst*	James Hutton Institute
ARAMIR		*Psh, Pst*	James Hutton Institute
Armelle	PI 410855	*Pst*	John Innes Centre
ASB 04-18		*Psh, Pst*	James Hutton Institute
ASPEN		*Psh, Pst*	James Hutton Institute
ASTORIA		*Psh, Pst*	James Hutton Institute
ATEM		*Psh, Pst*	James Hutton Institute
ATHENA		*Psh, Pst*	James Hutton Institute
AVEC		*Psh, Pst*	James Hutton Institute
Barke		*Pst*	IPK- Gatersleben
Baronesse		*Pst*	Oregon State University
BCD47		*Pst*	Oregon State University
BEATRIX		*Psh, Pst*	James Hutton Institute
BERAC		*Psh, Pst*	James Hutton Institute
BERWICK		*Psh, Pst*	James Hutton Institute
BERYLLIUM		*Psh, Pst*	James Hutton Institute
Betzes		*Pst*	John Innes Centre
BLENHEIM		*Psh, Pst*	James Hutton Institute
Bowman	PI 483237	*Pst*	USDA-GRIN
BRAEMAR		*Psh, Pst*	James Hutton Institute
BRAHMS		*Psh, Pst*	James Hutton Institute
BRAZIL		*Psh, Pst*	James Hutton Institute
CALICO		*Psh, Pst*	James Hutton Institute
CAMPALA		*Psh, Pst*	James Hutton Institute
CANASTA		*Psh, Pst*	James Hutton Institute
CECILIA		*Psh, Pst*	James Hutton Institute
CELEBRA		*Psh, Pst*	James Hutton Institute
CELLAR		*Psh, Pst*	James Hutton Institute
CENTURION		*Psh, Pst*	James Hutton Institute
CENTURY		*Psh, Pst*	James Hutton Institute
CHAD		*Psh, Pst*	James Hutton Institute
CHALICE		*Psh, Pst*	James Hutton Institute
CHARIOT		*Psh, Pst*	James Hutton Institute
CHARM		*Psh, Pst*	James Hutton Institute

CHASER		*Psh, Pst*	James Hutton Institute
Chevalier		*Pst*	John Innes Centre
CHIEFTAIN		*Psh, Pst*	James Hutton Institute
CHIME		*Psh, Pst*	James Hutton Institute
CI 16153	CI 16153	*Pst*	USDA-GRIN
CIho 4196	CIho 4196	*Pst*	USDA-GRIN
CLARET		*Psh, Pst*	James Hutton Institute
CLARITY		*Psh, Pst*	James Hutton Institute
CLASS		*Psh, Pst*	James Hutton Institute
COCKTAIL		*Psh, Pst*	James Hutton Institute
COLADA		*Psh, Pst*	James Hutton Institute
COLSTON		*Psh, Pst*	James Hutton Institute
COOPER		*Psh, Pst*	James Hutton Institute
CORK		*Psh, Pst*	James Hutton Institute
CORNICHE		*Psh, Pst*	James Hutton Institute
CORSICA		*Psh, Pst*	James Hutton Institute
CPBT B67		*Psh, Pst*	James Hutton Institute
CPBT B75		*Psh, Pst*	James Hutton Institute
CPBT B76		*Psh, Pst*	James Hutton Institute
CPBT B80		*Psh, Pst*	James Hutton Institute
CRUSADER		*Psh, Pst*	James Hutton Institute
CSBC 5466-27		*Psh, Pst*	James Hutton Institute
DALLAS		*Psh, Pst*	James Hutton Institute
DECANTER		*Psh, Pst*	James Hutton Institute
DELIBES		*Psh, Pst*	James Hutton Institute
DERKADO		*Psh, Pst*	James Hutton Institute
DEW		*Psh, Pst*	James Hutton Institute
DH46		*Pst*	Estación Experimental de Aula Dei
Dopla		*Pst*	Estación Experimental de Aula Dei
DOYEN		*Psh, Pst*	James Hutton Institute
DRAUGHT		*Psh, Pst*	James Hutton Institute
DRAY		*Psh, Pst*	James Hutton Institute
DRUM		*Psh, Pst*	James Hutton Institute
Duplex	CIho 12420	*Pst*	USDA-GRIN
Durani	PI 125311	*Pst*	USDA-GRIN
FAIRYTALE		*Psh, Pst*	James Hutton Institute
FELTWELL		*Psh, Pst*	James Hutton Institute
FERMENT		*Psh, Pst*	James Hutton Institute
FONTANA		*Psh, Pst*	James Hutton Institute
Foster		*Pst*	Washington State University
FOXTROT		*Psh, Pst*	James Hutton Institute
FRACTAL		*Psh, Pst*	James Hutton Institute
Franger	PI 180669	*Pst*	USDA-GRIN
GEORGIE		*Psh, Pst*	James Hutton Institute
GLEN		*Psh, Pst*	James Hutton Institute
GLOBAL		*Psh, Pst*	James Hutton Institute
Golden Promise	PI 343079	*Pst*	John Innes Centre
GOLDIE		*Psh, Pst*	James Hutton Institute

GRANTA		*Psh, Pst*	James Hutton Institute
GUNDEL		*Psh, Pst*	James Hutton Institute
GZ		*Psh, Pst*	James Hutton Institute
H602		*Pst*	Okayama University
HANKA		*Psh, Pst*	James Hutton Institute
Hanna	CIho 906	*Pst*	USDA-GRIN
HARRIOT		*Psh, Pst*	James Hutton Institute
Haruna Nijo		*Pst*	Okayama University
HEATHER		*Psh, Pst*	James Hutton Institute
HENLEY		*Psh, Pst*	James Hutton Institute
HERON		*Psh, Pst*	James Hutton Institute
HOPPER		*Psh, Pst*	James Hutton Institute
HOR 3401	PI 327764	*Pst*	USDA-GRIN
HORIZON		*Psh, Pst*	James Hutton Institute
HOST		*Psh, Pst*	James Hutton Institute
HYDRA		*Psh, Pst*	James Hutton Institute
I 5	CIho 11619	*Pst*	USDA-GRIN
Igri	PI 428488	*Pst*	USDA-GRIN
INDOLA		*Psh, Pst*	James Hutton Institute
ISABELLA		*Psh, Pst*	James Hutton Institute
JIVE		*Psh, Pst*	James Hutton Institute
KASSIMA		*Psh, Pst*	James Hutton Institute
KLAXON		*Psh, Pst*	James Hutton Institute
Kwan	PI 39367	*Pst*	USDA-GRIN
KYM		*Psh, Pst*	James Hutton Institute
LANDLORD		*Psh, Pst*	James Hutton Institute
LINDEN		*Psh, Pst*	James Hutton Institute
LITHIUM		*Psh, Pst*	James Hutton Institute
LIVET		*Psh, Pst*	James Hutton Institute
Long Glumes	CIho 6168	*Pst*	USDA-GRIN
LP1124.8.98		*Psh, Pst*	James Hutton Institute
MACARENA		*Psh, Pst*	James Hutton Institute
MACAW		*Psh, Pst*	James Hutton Institute
MADRAS		*Psh, Pst*	James Hutton Institute
Manas		*Pst*	John Innes Centre
Manchuria	CIho 2330	*Pst*	USDA-GRIN
MANDOLIN-1418		*Psh, Pst*	James Hutton Institute
MAYPOLE		*Psh, Pst*	James Hutton Institute
MELITTA		*Psh, Pst*	James Hutton Institute
MELTAN		*Psh, Pst*	James Hutton Institute
MIKADO		*Psh, Pst*	James Hutton Institute
MINSTREL		*Psh, Pst*	James Hutton Institute
MONIKA		*Psh, Pst*	James Hutton Institute
Morex	CIho 15773	*Pst*	USDA-GRIN
Multan	PI 57956	*Pst*	USDA-GRIN
NATASHA		*Psh, Pst*	James Hutton Institute
NERUDA		*Psh, Pst*	James Hutton Institute
Nigrate	CIho 2444	*Pst*	USDA-GRIN

NORD 03/2408		*Psh, Pst*	James Hutton Institute
NOVELLO		*Psh, Pst*	James Hutton Institute
NSL 04-4622		*Psh, Pst*	James Hutton Institute
NSL 95-1257		*Psh, Pst*	James Hutton Institute
NSL 97-4552		*Psh, Pst*	James Hutton Institute
NSL 98-5065		*Psh, Pst*	James Hutton Institute
NSL 99-5363		*Psh, Pst*	James Hutton Institute
ONYX		*Psh, Pst*	James Hutton Institute
OPTIC		*Psh, Pst*	James Hutton Institute
OWB Dominant	GSHO 3450	*Pst*	USDA-GRIN
OWB Recessive	GSHO 3451	*Pst*	USDA-GRIN
PARAMOUNT		*Psh, Pst*	James Hutton Institute
PENTHOUSE		*Psh, Pst*	James Hutton Institute
PEWTER		*Psh, Pst*	James Hutton Institute
PITCHER		*Psh, Pst*	James Hutton Institute
POLYGENA		*Psh, Pst*	James Hutton Institute
POTTER		*Psh, Pst*	James Hutton Institute
POWER		*Psh, Pst*	James Hutton Institute
PRAGUE		*Psh, Pst*	James Hutton Institute
PRESTIGE		*Psh, Pst*	James Hutton Institute
PRISMA		*Psh, Pst*	James Hutton Institute
Psaknon	CIho 6305	*Pst*	USDA-GRIN
PUBLICAN		*Psh, Pst*	James Hutton Institute
PUTNEY		*Psh, Pst*	James Hutton Institute
QUARTET		*Psh, Pst*	James Hutton Institute
QUENCH		*Psh, Pst*	James Hutton Institute
RAINBOW		*Psh, Pst*	James Hutton Institute
RAKAIA		*Psh, Pst*	James Hutton Institute
RANGOON		*Psh, Pst*	James Hutton Institute
REGGAE		*Psh, Pst*	James Hutton Institute
RENATA		*Psh, Pst*	James Hutton Institute
RIA		*Psh, Pst*	James Hutton Institute
RICARDA		*Psh, Pst*	James Hutton Institute
RUMMY		*Psh, Pst*	James Hutton Institute
Rupee	CIho 4355	*Pst*	USDA-GRIN
Russell	PI 483127	*Pst*	USDA-GRIN
S02		*Pst*	John Innes Centre
S03		*Pst*	John Innes Centre
S06		*Pst*	John Innes Centre
S07		*Pst*	John Innes Centre
S09		*Pst*	John Innes Centre
S10		*Pst*	John Innes Centre
S11		*Pst*	John Innes Centre
S12		*Pst*	John Innes Centre
S13		*Pst*	John Innes Centre
S15		*Pst*	John Innes Centre
S17		*Pst*	John Innes Centre
SABEL		*Psh, Pst*	James Hutton Institute

SACHA		*Psh, Pst*	James Hutton Institute
SALOON		*Psh, Pst*	James Hutton Institute
SCANDIUM		*Psh, Pst*	James Hutton Institute
SCARLETT		*Psh, Pst*	James Hutton Institute
SEBASTIAN		*Psh, Pst*	James Hutton Institute
SHAKIRA		*Psh, Pst*	James Hutton Institute
SILICON		*Psh, Pst*	James Hutton Institute
Siri		*Pst*	John Innes Centre
SKAGEN		*Psh, Pst*	James Hutton Institute
SKITTLE		*Psh, Pst*	James Hutton Institute
SPEY		*Psh, Pst*	James Hutton Institute
SPIKE		*Psh, Pst*	James Hutton Institute
SPIRE		*Psh, Pst*	James Hutton Institute
Spiti	CIho 14349	*Pst*	USDA-GRIN
SPLASH		*Psh, Pst*	James Hutton Institute
STARLIGHT		*Psh, Pst*	James Hutton Institute
STATIC		*Psh, Pst*	James Hutton Institute
Steptoe	CIho 15229	*Pst*	USDA-GRIN
SusPtrit		*Pst*	Wageningen University
SW 2808		*Psh, Pst*	James Hutton Institute
SW MACSENA		*Psh, Pst*	James Hutton Institute
SW SCANIA		*Psh, Pst*	James Hutton Institute
SW STELLA		*Psh, Pst*	James Hutton Institute
TABORA		*Psh, Pst*	James Hutton Institute
TANKARD		*Psh, Pst*	James Hutton Institute
TAPHOUSE		*Psh, Pst*	James Hutton Institute
TARDUS		*Psh, Pst*	James Hutton Institute
TARTAN		*Psh, Pst*	James Hutton Institute
TAVERN		*Psh, Pst*	James Hutton Institute
Tipple		*Pst*	John Innes Centre
Trebi	PI 537442	*Pst*	USDA-GRIN
UNKNOWN		*Psh, Pst*	James Hutton Institute
URSA		*Psh, Pst*	James Hutton Institute
VEGAS		*Psh, Pst*	James Hutton Institute
VELVET		*Psh, Pst*	James Hutton Institute
VISKOSA		*Psh, Pst*	James Hutton Institute
VIVENDI		*Psh, Pst*	James Hutton Institute
VORTEX		*Psh, Pst*	James Hutton Institute
WAGGON		*Psh, Pst*	James Hutton Institute
WEITOR		*Psh, Pst*	James Hutton Institute
West China	CIho 7556	*Pst*	USDA-GRIN
WESTMINSTER		*Psh, Pst*	James Hutton Institute
WICKET		*Psh, Pst*	James Hutton Institute
WIDRE		*Psh, Pst*	James Hutton Institute
WIKINGETT		*Psh, Pst*	James Hutton Institute
WREN		*Psh, Pst*	James Hutton Institute
Z91-103-21		*Psh, Pst*	James Hutton Institute

[1]*Pst*: *P. striiformis* f. sp. *tritici* and *Psh*: *P. striiformis* f. sp. *hordei*

Table A2: Accessions of barley used for inoculation with *P. striiformis* f. sp. *tritici* in Chapter 4.

Accession name	PI/CI Number	Row	Status	Isolate	CHL[1,2]	INF[1,3]
Commander		2	cultivar	08/21	0.00	0.00
Hindmarsh		2	cultivar	08/21	0.00	0.00
Maritime		2	cultivar	08/21	1.21	0.00
Clipper	PI 349366	2	cultivar	08/21	1.00	0.00
Finniss		2	cultivar	08/21	1.38	0.00
Q21861	PI 584766	2	breeding	08/21	2.00	0.00
Bancroft	PI 605474	2	cultivar	08/21	0.50	0.00
Betzes		2	cultivar	08/21	0.00	0.00
SM89010		2	cultivar	08/21	0.50	0.00
Bowman	PI 483237	2	cultivar	08/21	0.00	0.00
BCD47	PI 659444	2	genetic	08/21	0.50	0.00
SEBASTIAN		2	cultivar	08/21	0.00	0.00
RAINBOW		2	cultivar	08/21	0.00	0.00
FRACTAL		2	cultivar	08/21	0.00	0.00
OPTIC		2	cultivar	08/21	0.00	0.00
FELTWELL		2	cultivar	08/21	0.00	0.00
SALOON		2	cultivar	08/21	0.00	0.00
POWER		2	cultivar	08/21	0.00	0.00
HENLEY		2	cultivar	08/21	0.00	0.00
Tipple		2	cultivar	08/21	0.00	0.00
HERON		2	cultivar	08/21	0.00	0.00
ATEM		2	cultivar	08/21	0.00	0.00
ONYX		2	cultivar	08/21	0.00	0.00
Barke		2	cultivar	08/21	0.00	0.00
RIA		2	cultivar	08/21	0.00	0.00
PRISMA		2	cultivar	08/21	0.00	0.00
TRIUMPH		2	cultivar	08/21	0.00	0.00
Trumpf	PI 548762	2	cultivar	08/501	0.00	0.00
Diamant	CIho 15226	2	cultivar	11/08	1.00	0.00
DERKADO		2	cultivar	08/21	0.00	0.00
HEATHER		2	cultivar	08/21	0.00	0.00
TARDUS		2	cultivar	08/21	0.00	0.00
Zephyr	PI 339815	2	cultivar	08/21	0.50	0.00
Carlsberg II	CIho 15218	2	cultivar	11/08	0.00	0.00
Maythorpe		2	cultivar	08/21	0.00	0.00
Golden Promise	PI 343079	2	cultivar	08/21	0.00	0.00
Pallas	CIho 11313	2	cultivar	11/08	0.00	0.00
Siri		2	cultivar	08/21	0.00	0.00
Sultan 5		2	cultivar	11/08	0.00	0.00
Cambrinus	PI 321779	2	cultivar	08/21	1.00	0.00
Ingrid		2	cultivar	08/501	0.00	0.00
M1460		-	genetic	08/21	0.00	0.00
Haisa	CIho 9855	2	cultivar	08/501	0.00	0.00
Varunda	PI 410865	2	cultivar	08/501	0.00	0.00
Emir	CIho 13541	2	cultivar	11/08	0.00	0.00
Mazurka	PI 399501	2	cultivar	08/21	1.00	0.00

Minerva		2	cultivar	08/21	0.00	0.00
Vada		2	cultivar	08/501	0.00	0.00
Baronesse	PI 568246	2	cultivar	08/21	0.00	0.00
JIVE		2	cultivar	08/21	0.00	0.00
Apex		2	cultivar	08/501	0.00	0.00
Armelle	PI 410855	2	cultivar	08/21	0.00	0.00
Chevalier	CIho 156	2	cultivar	08/21	0.00	0.00
Stauffers Obersulzer	PI 467580	2	cultivar	08/501	0.00	0.00
Malteria Heda	CIho 15224	2	cultivar	11/08	0.50	0.00
Probstdorfer Vollkorn	CIho 15222	2	cultivar	08/501	0.00	0.00
Heils Franken	PI 327917	2	cultivar	11/08	0.00	0.00
Haruna Nijo		2	cultivar	08/21	0.00	0.00
CIho 4196	CIho 4196	2	landrace	08/21	0.00	0.00
L94		2	landrace	08/501	1.00	0.00
Grannenlose Zweizeilige	PI 548740	2	landrace	08/21	1.50	0.00
Hiproly	PI 60693	2	landrace	08/501	1.00	0.00
Abyssinian 14	CIho 7202	2	landrace	08/501	3.00	1.00
Benton	PI 539105	6	cultivar	08/21	0.00	0.00
HOR 2926	PI 548734	2/6	-	11/08	0.00	0.00
HOR 1428	PI 548708	2	landrace	11/08	0.00	0.00
SusPtrit		6	genetic	08/21	3.00	4.00
OWB Recessive	GSHO 3451	6	genetic	08/21	2.00	0.00
Trebi	PI 537442	6	cultivar	08/21	0.00	0.00
Steptoe	CIho 15229	6	cultivar	08/21	3.50	0.00
Algerian	PI 539104	6	cultivar	08/21	1.00	0.00
Abed Binder 12	PI 327961	6	cultivar	08/21	0.00	0.00
Duplex	CIho 12420	6	landrace	08/21	0.00	0.00
Cebada Capa		6	cultivar	NA	NA	NA
Psaknon	CIho 6305	6	breeding	08/21	0.00	0.00
WBDC 350		2	wild	08/21	0.00	0.00
WBDC 045		2	wild	08/21	0.00	0.00
Spontaneum I	PI 293413	2	wild	08/501	0.00	0.00
WBDC 259		2	wild	08/21	1.00	0.50
WBDC 241		2	wild	08/21	3.00	1.00
22	PI 466309	2	wild	08/501	2.50	1.00
WBDC 085		2	wild	08/21	0.00	0.00
WBDC 148		2	wild	08/21	3.00	1.00
WBDC 013		2	wild	08/21	0.00	0.00
WBDC 334		2	wild	08/21	2.00	3.00
WBDC 247		2	wild	08/21	3.00	2.00
32	PI 466444	2	wild	08/501	1.00	0.00
WBDC 072		2	wild	08/21	3.00	0.50
20007	PI 284752	2	wild		0.50	0.00
WBDC 038		2	wild	08/21	3.00	3.00
WBDC 199		2	wild	08/21	0.00	0.00
55	PI 466279	2	wild	08/501	3.50	0.00
WBDC 253		2	wild	08/21	0.00	0.00
WBDC 008		2	wild	08/21	0.00	0.00

WBDC 110		2	wild	08/21	0.50	0.00
WBDC 068		2	wild	08/21	0.50	0.00
WBDC 109		2	wild	08/21	2.00	0.00
WBDC 112		2	wild	08/21	0.50	0.00
OWB Dominant	GSHO 3450	2	genetic	08/21	4.00	0.00
OUH602		2	wild	08/21	4.00	3.00
WBDC 343		2	wild	08/21	1.50	0.00
G-88	PI 466211	2	wild	08/501	1.00	0.00
Spiti	CIho 14349	6	landrace	08/21	1.50	0.00
Black Hull-less	PI 24849	6	landrace	08/21	3.00	3.00
West China	CIho 7556	6	breeding	08/21	0.00	0.00
Nigrate	CIho 2444	6	landrace	08/21	0.50	0.00
Durani	PI 125311	6	landrace	08/21	0.50	1.00
WBDC 172		2	wild	08/21	0.00	0.00
Multan	PI 57956	6	landrace	08/21	0.00	0.00
Kwan	PI 39367	6	landrace	08/21	0.00	0.00
Rupee	CIho 4355	6	landrace	08/21	0.00	0.00
I 5	CIho 11619	6	-	08/21	0.50	0.00
Long Glumes	CIho 6168	2	genetic	08/21	0.00	0.00
HOR 3401	PI 327764	6	landrace	08/21	0.00	0.00
Hanna	CIho 906	2	breeding	08/21	0.00	0.00
Dopla		6	cultivar	08/21	0.50	0.00
Regina		6	cultivar	NA	NA	NA
Igri	PI 428488	2	cultivar	08/21	0.00	0.00
DH46		-	genetic	08/21	2.00	1.00
Parasol		-	cultivar	NA	NA	NA
Manas		-	cultivar	08/21	0.50	0.00
Franger	PI 180669	6	cultivar	08/21	0.50	1.00
Astrix	PI 339826	6	cultivar	08/21	0.50	0.00
Fong Tien		6	-	11/08	3.50	2.50
Manchuria	CIho 2330	6	cultivar	08/21	3.00	0.50
Bigo	CIho 13611	6	cultivar	08/21	0.00	0.00
Foster		6	cultivar	08/21	1.50	0.00
Morex	CIho 15773	6	cultivar	08/21	1.50	0.00
Russell	PI 483127	6	cultivar	08/21	1.00	0.00

[1]Phenotypic scores represent average score of six leaves.
[2]CHL: Macroscopic observation of chlorosis.
[2]INF: Macroscopic observation of infection (pustule formation).

Table A3: Barley CAPS and SSLP markers for mapping *Rpst2* and *rps2*.

Marker name	Synonym	Chr.	Design source	Source identifier	Forward PCR primer	Reverse PCR primer	Restriction Enzyme
QB_36988	C_36988	7HL	Silvar et al. (2012)	QB_36988	CTCACCACTGGCTCTCCTTC	TGGATGTGGCTTTGTGACAT	*Mse*I
QB_102319	C_102319	7HL	Silvar et al. (2012)	QB_102319	GCATTTCCCTTTGCTCTTCA	GAGCTGGGACCTCTTGGATT	*Hpy*188I
QB_1562518	C_1562518	7HL	Silvar et al. (2012)	QB_1562518	AGAAGCATGGGCTGAAACTG	TCAGCCAATCATTCAAACCA	*Mbo*I
U32_6093_p1	C_6093	7HL	Muñoz-Amatriaín et al., (2011)	U32_6093	CAAGGCAACATGTGAAGCTG	AGCAGCCACCACCATAAACT	*Rsa*I
U32_7023_p1	C_7023	7HL	Muñoz-Amatriaín et al., (2011)	U32_7023	CAGGAAGAGGCTCTCCAAGA	CCAAGTGAGTGCATCTGTGC	*Bsa*HI
U32_3345_p1	C_3345	7HL	Muñoz-Amatriaín et al., (2011)	U32_3345	ACCGAGGGTGAGAGATCCTT	ACGGAACAGCCCGTATACAA	*Pst*I
U32_4671_p1	C_4671	7HL	Muñoz-Amatriaín et al., (2011)	U32_4671	AGGGAAGGCTCCTCCTACAA	AAGGCTACGGTTGTTGATGC	*Hind*III
U32_7356_p1	C_7356	7HL	Muñoz-Amatriaín et al., (2011)	U32_7356	TGTGGACAGGGAGAAGACAC	GGCCTCTGAAACTGAAAGCC	*Bcl*I
U32_2966_p1	C_2966	7HL	Muñoz-Amatriaín et al., (2011)	U32_2966	TGTCTCAGGGACAGCATCAG	TACAGTGCGATCCCTTGTTG	*Hinf*I
U32_5362_p1	C_5362	7HL	Muñoz-Amatriaín et al., (2011)	U32_5362	GCGCCATGGAATGTTTTATT	CCACCACCACATTCATACCA	*Apo*I
U32_10214_p1	C_10214	7HL	Muñoz-Amatriaín et al., (2011)	U32_10214	GCATCCTCTGCGTCATCTTC	ACCAGAGACAGGGTGAATGG	*Bam*HI
U35_45798_p1	C_45798	7HL	Moscou et al. (2011)	U35_45798	TGGGAAGGTTTGTCGTTTTC	TGGAATTCCGCACATAAACA	*Dra*I
U35_2085_p2	C_2085	7HL	Moscou et al. (2011)	U35_2085	AGTTCTGCTGCGCCAGTTAT	TTTGCCTGCTGACAAATGAG	*Xcm*I
U35_17818_p2	C_17818	7HL	Moscou et al. (2011)	U35_17818	GCGACAGCTAGCTCTGTGTG	GGTTCGGAATTGCTGTTTGT	*Bsa*BI
U35_5309_p1	C_5309	7HL	Moscou et al. (2011)	U35_5309	TTATTTGCTTCCCGTTGACC	GCAAATTAGGGCATCCGTTA	*Mva*I
U35_4063_p1	C_4063	7HL	Moscou et al. (2011)	U35_4063	TGGCACATGGTCATGAATCT	GATGTCTGCCTTGGGGTTTA	*Eco*NI
c_7405_p2	C_7405	7HL	IBGSC (2012)	morex_contig_7405	ACCAGCGACGTCATGTGG	ACACTACCCTCGGGCACT	*Afl*II
c_57441_p4	C_57441	7HL	IBGSC (2012)	morex_contig_57441	TACTGTGCCCGTGCTGTG	GCCAACCGCCAATCCTCT	*Ava*I
c_343951_p3	C_343951	7HL	IBGSC (2012)	morex_contig_343951	TCGGCACAACCACTCGAC	ATGTCCTGAACACGGGCG	*Msp*I
c_362150_p4	C_362150	7HL	IBGSC (2012)	morex_contig_362150	ACGCACTGATGGTCTTCCC	GAAAAGGCAGCGCTCGTG	*Ase*I
U32_4240_p2	C_4240	2HL	Muñoz-Amatriaín et al., (2011)	U32_4240	ATGCACCTCCAAGTGAAACC	CAGAACCCCCAAGCTGATAA	SSLP
U32_4879_p3	C_4879	2HL	Muñoz-Amatriaín et al., (2011)	U32_4879	CTCCATTTCTTTGGCCATGT	GCAAAAGGCTCTTCTTGGTG	*PstI*
U32_2052_p2	C_2052	2HL	Muñoz-Amatriaín et al., (2011)	U32_2052	AACCGATGCCTTTATGGTTG	CGGTAAACGATGCCAAAATC	*NgoMIV*
U32_8586_p1	C_8586	2HL	Muñoz-Amatriaín et al., (2011)	U32_8586	GTTTGGGCCACTGACAAGAT	TCCCTGTTAGGATTCCATGC	*HaeIII*
U32_9668_p2	C_9668	2HL	Muñoz-Amatriaín et al., (2011)	U32_9668	TGCCATGCGATTTACAACAT	TAAGCCCTCAGTGGTGGAAC	*BglII*

U32_570_p1	C_570	2HL	Muñoz-Amatriaín et al., (2011)	U32_570	ACGACATTTCCAACGTTTCC	ATTCCGTGCCTAACACCAAG	*HaeIII*
U32_9910_p1	C_9910	2HL	Muñoz-Amatriaín et al., (2011)	U32_9910	CTGTGTCCAGCATTTTCAGC	GACCGACAAGGAGTGGTTTC	SSLP
U32_1344_p2	C_1344	2HL	Muñoz-Amatriaín et al., (2011)	U32_1344	ATGGGTATGGACAAGGGTGA	TCGCATCCTTAGCGGTAACT	*HindIII*
U32_4100_P1	C_4100	2HL	Muñoz-Amatriaín et al., (2011)	U32_4100	GGTGGCCCATCAACATTAAG	CGCTTCAGAAAACAACGTGA	*MspA1I*
U32_1449_p1	C_1449	2HL	Muñoz-Amatriaín et al., (2011)	U32_1449	CACCTCCGTCTTCGTCATTT	ATGCCCATGGTTTCATTCAT	*DraI*
U32_9109_p3	C_9109	2HL	Muñoz-Amatriaín et al., (2011)	U32_9109	AAAGGCGATGGCTATGTTTG	AGCAAAACTGTCCGCTTGTT	*MluI*
U32_6562_p3	C_6562	2HL	Muñoz-Amatriaín et al., (2011)	U32_6562	GCTCACAACGTGTTCGACAA	GCACCATGGAATTGGCTAGT	*RsaI*

Table A4: Sequence used for Sequenom marker development in the *Rpst2* and *rps2* region.

Marker	Synonym	Chr.	Design strategy	Source identifier	Template sequence and SNP
253_Oz_43740_p1	S_43740	7HL	Rice synteny	Os06g43740	TCCTTTGCAGGGTTAATGTACGGCAGCCAAAATTGCCCATTGACATAATTCTCATCGTCG[T/C]GTCTGTGTACTCCTGGTGCGCATCGACAAGATCATCCTTNCCCATGCTGACCAACAGCAA
262_Oz_43900_p1	S_43900	7HL	Rice synteny	Os06g43900	NGGTCATGGTTGTAGTTGGTTGCTCGTTGTTTTGCGGCTGGGTGTGTGCTCATTGTGTTTNTATACTTTTTGTGCTTCATTNTGAAACAA[T/C]AAAATTCACTCTATCGGAGGNAAAAATGAAAACGCCTAAACATCACANTATGTTTGCAAA
264_Oz_43900_p1	S_43900	7HL	Rice synteny	Os06g43900	ATTGTGTTTNTATACTTTTTGTGCTTCATTNTGAAACAANAAAATTCACTCTATCGGAGGNAAAAATGAAAACGCCTAAACATCACA[T/C]TATGTTTGCAAACTGGCAAAACACTCCATCGCCTAAGACAGGCCCCTCAAGCATGGTACT
255_Oz_43860_p1	S_43860	7HL	Rice synteny	Os06g43860	GNTGGTTCNGTATTATATAGTCCNTGACTGTTGCGCGTGGAAGCTAATTGCACTAGACGC[G/A]GTAAGAGAAGCTTTATCAGGACAGCTTAGCTAAGATTATGAACGCGAGGTACCGTCAGTT
244_Oz_43720_p1	S_43720	7HL	Rice synteny	Os06g43720	TATTGTCTCGTTAAAAACCTTATATGAAAACCCATCATGAAAAAATAATAAAGGAAAAAANAGTN[A/C]AATATGAATGACCTGAGATAACCAATAGGTTATAACTTTGTATTTTACTCCCCCAGAAAT
240_Oz_43720_p1	S_43720	7HL	Rice synteny	Os06g43720	GGAAAAAAATTGAGGAGAAGCCATATAATAATATGCCATGAAAAACTCGTTTAAAACCCA[G/C]TGGGGAAAATATAAGGAGAAACCAATATGGCATACATCCGCNCTTGTATTTATATTGTCT
254_Oz_43760_p1	S_43760	7HL	Rice synteny	Os06g43760	AACTGAGCAACAACCATTGACTTTCAAAGAGACACCACTTACCGCTCCGTATTCATCAAT[G/A]ACTTCCATAGGTGAAGGGTAATTCTGTTTACTCTTGCTCATTTTCTTCCCATCCTCTGCC
236_Oz_43680_p1	S_43680	7HL	Rice synteny	Os06g43680	AACATAAACTGTCTACTGTCCAATACATCTTCAGAAGAGGTGATAAGTTGATAGCTACATTTGATCACAT[G/A]TAGGCACATATAACATGACTGAAAAGCCTACCTTGCCAACGCAATTAGATACCCAGGGGCAGTGATGGTC
208_Oz_43270_p1	S_43270	7HL	Rice synteny	Os06g43270	TTTTACTATGCCCTGGTAGTAGTTTCCTCTGCATGTTTCCCTAAATTTGTTTTCTTTTTT[T/C]ATTTCTTCAGTGCTAAAGATGTGGATTATGACAACAATGGCTGGGATAGTTCTGAAAACC
205_Oz_43250_p1	S_43250	7HL	Rice synteny	Os06g43250	CTGCTCCCCTACAAGGCCTGGTTTTAATGTAGAATTTTCCTGTCCTCCACATCCTGGAGA[C/A]AATAAGATTGAACCGAGGTTAGCTGAGATAGCAAGATGAAGCAATTGTTCTGTCGATCGT
227_Oz_43440_p1	S_43440	7HL	Rice synteny	Os06g43440	TTTAATCATGGTGTTTTGCGCATAAAGTTTTTCTAGCTAGAGTTTGCCTATTTTTTGTAA[T/A]GGCCTAAAAGGAGCCTCTGATCATTTTCTTATTTCCAGAGTATGTATGTCGTATAACCAT
265_QB_135867	S_135867	7HL	Silvar et al. (2012)	QB_135867	ACATCACGCACCCAATTTATTTCCACAAGACAAGATGTTGCCTTGTGTACATAT[T/G]CTGTCTTTTGTGTCTCTAGATTCACGTAGGNGTCCGGCTCCACACAC
271_QB_36988	S_36988	7HL	Silvar et al. (2012)	QB_36988	TTAGTAGTAGCACTTCTTGCTTGGCGAGTGAAGTACACCAGTTGTATACATGTTTTGTTCTATCAACAAGTTCATCTTTG[T/C]TAATCCATCTCGTGGTGTTTTGTCGAAGTAATGCATGATCTGGAGTTCTGGACTATGCAGGCACCGGTGGACGGGGAGGT
272_QB_102319	S_102319	7HL	Silvar et al. (2012)	QB_102319	GTTCCCTTTTTTTTACGTACGAATCGAGCAGCTTTGAGCGCCACAAAGTCGACCATCTCGTCGAACACGAGCGCCATCAG[T/C]TGCCTGCAGCAGCACAACCAGAGCAAGGCGGCGGCGNNNNNNNNNAGGCCGATGACACTCACACTNGGAGAGCCACGGGA
267_QB_1562518	S_1562518	7HL	Silvar et al. (2012)	QB_1562518	ATGATAAAACTCAANTGTAATCAAATGTAGGAAACCATCAACCCACCGCTAGGCAAACCCAAACTCCTTTCCAGAAACTG[T/C]GTTTACAAATTGCATATACTGTTACACCTACTTCACTAATAGCAGCCTGGGTGCTACTCCCTCCGTTCCTAAATATAAGT
290_U32_3345_p1	S_3345	7HL	Munoz et al. (2011)	U32_3345	GAGAAAAGCTTGTGGACATCAGGGAAGAGATACTCCGGAAGCGAAGGGCCGGGAAGCTCCCGGGAGACACGGCGTCCACC[T/C]TGAAGGCTTGGTGGCAAGCCCACGCGAAATGGCCGTACCCAACTGTAAGTGACAGCCCGCAGTGCAGATACCATCGTCAC
281_U32_4671	S_4671	7HL	Munoz et al. (2011)	U32_4671	GCGCTAAAAAATGTTGTAGGTTGAAATTGCTCAGGCTTATGAAAAGCATGGAGCTGCGTGCTTGAGCATCTTGAC[T/C]GATGAGAAATACTTCCAGGTTCGTTGTTTAAAATTGTGTCTGTCTCTGAGGATGGTAGATGTTGTATTTACTGGGCAAGCTTA
285_U32_2966	S_2966	7HL	Munoz et al. (2011)	U32_2966	TGGGTTACCATTGCCTTGCACTTTA[G/A]GGCGCAAGCATTTCTACTTATTTTTTGCGATACAAGCTGCCTGACAGCATCTTATTTTCCATGACAAAAGGAACCTGAGGAACTCTT
297_U32_5362_p1	S_5362	7HL	Munoz et al. (2011)	U32_5362	TGCTTTGTTGTTGTGTAGAGGGCATAGCAAGGTTTAACAAGTCACTTGGCCCGACGAAAATCAGTTTGTTGAAAGAAAAA[T/C]TTATGCGAAAAAAATGTTCAAACTGCCTTGATGCTTAATGTTTATTGTCCTCTTATGCTGCGGTGTTGCCTGATACTTCA
302_U32_10214_p1	S_10214	7HL	Munoz et al. (2011)	U32_10214	ACATCACGCACCCAATTTATTTCCACAAGACAAGATGTTGCCTTGTGTACATAT[T/G]CTGTCTTTTGTGTCTCTAGATTCACGTAGGNGTCCGGCTCCACACAC
18_U35_13719_p1	S_13719	7HL	Moscou et al. (2011)	U35_13719	AAGGGATGGTTTCAATTCCCACGTTTCATACAACACCGGCTTCTAATTTCCGCACCGCCGCATAATTGCC[T/C]GGCGGCGCCGCTGAAAGCGAGCGGCGGTTTGTAAGGTGGCCATGTACACCCCCCCTCCTAGGTTTTGTAA
7_U35_18761_p1	S_18761	7HL	Moscou et al. (2011)	U35_18761	GGCTGGCGGCACCGTAGCTNACNGGGAACTGAGAGGGCGNTNACACAAATGATGGGTCAA[T/C]AAGTCCGGTCGTAGACGCTCTTATTTAGTATCCCACCANCACGGCTGTCATGATGTNCAA
8_U35_19936_p1	S_19936	7HL	Moscou et al. (2011)	U35_19936	CATCATCGCCCTAGCAAGAATGGACTAGGCCCGGCCGGCTGTCGACATGCCCGCGGACATCTGCTGACGGAACCTTGGGG[G/C]TCAGCGCTAGACCATTCTTGTCNCACCTTGCACATTGTTCTTGCAATGGTGAAACTGCCCTGGTTATCATTCATCAGAAA
9_U35_19936_p1	S_19936	7HL	Moscou et al. (2011)	U35_19936	CATCATCGCCCTAGCAAGAATGGACTAGGCCCGGCCGGCTGTCGACATGCCCGCGGACATCTGCTGACGGAACCTTGGGGNTCAGCGCTAGACCATTCTTGTC[G/A]CACCTTGCACATTGTTCTTGCAATGGTGAAACTGCCCTGGTTATCATTCATCAGAAA
1_U35_3415_p1	S_3415	7HL	Moscou et al. (2011)	U35_3415	TCCATATTTCTCTAGGGAATCACACTCGTACCTTTCGACGAAAGCACGCGATTCTTATGGTACCCAGTGTTGAGCACCTC[G/A]GCATTGAATTNCTTTGGGATGGTGTCGACACTNGGGATCTTGTAGACCCATGTGCTGTCGCTCACGACCAGTCCATCAGC
17_U35_22699_p1	S_22699	7HL	Moscou et al. (2011)	U35_22699	CTGGAGCATTCTTCCTCGTCAAGCAAGCGGAAGAGGACCGAGTCCGAAGAGGAGGAGCGG[G/C]GACAAAGGAAGCCGCAGCAAGCCGGTGGCGGGTTCGCGCTCCCTGACCTCAACATTCCGG
20_U35_22182_p1	S_22182	7HL	Moscou et al. (2011)	U35_22182	TTTCCTTTTCTTTTCTTTGGTCTTATTATCATCCTTGGCTGGCATTCATATATATTCATG[T/C]GACTATTCTCTTTTTCTGGTGCACATGAAAAACGCTGAGTTATGTAATAGCTGCGTTGCC
40_U35_20320_p1	S_20320	7HL	Moscou et al. (2011)	U35_20320	NGTTCGTACAGTGAAAGTAACTAAGCTAAACTCTTCTCTGCAGAAGTAAGAAGTTGTTCA[T/C]GTAATTTACTTCTTGAGAGAGAAGCAAGTACTCCCTCCGATCCATATTACNTGTTGCTCA
44_U35_3446_p2	S_3446	7HL	Moscou et al. (2011)	U35_3446	GACGAATGTCAAGGAATCACCTTCAGTTAAGGCTGCAACTGATATTCTGTTTCAAGCTGC[G/A]GTCTCAGAGGAATACTGTGTCTCATCAGTATAATTCATGTGACAATGATATGCTGCACAC
35_U35_15787_p2	S_15787	7HL	Moscou et al. (2011)	U35_15787	ACTACCAATTTACCATAGCAAAATGGTACTTGGTCATCACACAGTATTATGTGTACAGTA[C/A]AACATGCCAGTGCTGACACCACACCACACATTCCAGTAATATAACACACTGATTTATCCC
36_U35_7756_p1	S_7756	7HL	Moscou et al. (2011)	U35_7756	ATCATGTACACACAAATGCAGTTAACAGCAAGTGACAAGTATCGTCAAATTATAATCTGGCTGAAGTGACA[G/A]TAGAAGCAAATTTACAAAAGAGTTACTCCCTCCGTTCCTAAATATAAGTCTTTTTAAAGATTTCACTATG
56_U35_16617_p1	S_16617	7HL	Moscou et al. (2011)	U35_16617	TTGGNTCTAATTTATATATAAAGCAACGACCGAATAAAGTACGGGNCCGTCCTATAAAAAATGGTGAGGT[G/A]TCCCTTTTATAGAACCGGTCTCNGAATAANCAGNCCANGCAGAACACAATTACACATGCTACCGAAAGAT
30_U35_2382_p1	S_2382	7HL	Moscou et al. (2011)	U35_2382	TGAAACTTCTCNTCAAAGGACGGGTTCCTTCCTCCATCTGATTGTATAAAAAAAGACAGTAAGCTTATGGAGNTAAAATA[T/C]GTACAAGATGAAATATTTCGTTAATGTTGGAAATGTAGGCCACANANAANAACANTGTCCTAGCNNTCCNCNTTTATTNA
58_U35_37233_p2	S_37233	7HL	Moscou et al. (2011)	U35_37233	NCCCTCTATANAGTATACGGTACGGTAAAAAAAGCTCTCAAAATTGGAGACTCACATTCA[T/G]AGAATCATCATCTTTCAGGGTTTTTTCTTTCTAAAAACCGTCATTATTGTGCGGACAATT
310_c_1579096_p2	S_1579096	7HL	IBGSC et al. (2012)	morex_contig_1579096	GAAGATTCAANCCCCTTGATAAGCGCCAGATTCAGCNGATGCACAANGGAACCGAANGAATAAATACATAAACCCCAAGG[C/G]NGACATCTGATGAGTCTGGTGGCTGATTGGCAGAAGCATGCATGCCTGTACANNNNCAGGCATGCATGCATTAGTGTTTA
337_c_63378_p0	S_63378	7HL	IBGSC et al. (2012)	morex_contig_63378	AAAAAAATCAAAATACAAAATTAGAAGAAAAAAACGATGCTTACATCAAATGGACCGGATAACTTTGATCATATT[T/C]GATGCTTTAGTAAGATTTAGTTTTATTCGGGTATGAGTAGTGGAAGGCAAAGAAAGTTTGGTTTTATTTGAGATTTTT

327_c_7405_p2	S_7405	7HL	IBGSC et al. (2012)	morex_contig_7405	AAGACTATGATCGTAAGAGACCTTAACCATCTATGCATAAATGGTAACTAGTGGTTGGGGGCTGTCGTTGCGGGTCGCTT[G/A]AGAGGAAGTTATGCACATCATACCTTCATCTTAAAATTTTAAAAAAACTTACATCATGTGAACATCACATCCATCTCATA
318_c_343951_p3	S_343951	7HL	IBGSC et al. (2012)	morex_contig_343951	CGNTAGCTATAATNGCTTTTCTCGATTACTACAACTTTTTTTGTAGTCGTGACCGTCACACACTATTTGGCCAGCCTGCT[G/A]GAAGCTCCTTGTTCTAAGGATNAGATATGTGAAGCTCNTACGAAACCGACAATCCACGAAACCCCAGCTCGAGCAACCAA
328_c_136527_p8	S_136527	7HL	IBGSC et al. (2012)	morex_contig_136527	TAGCATTTTGAGAGGACAAAAAAATGCAGGAAATGACGACAGGTGCCCCAGTGTGCGGCTGTAAAAATTACTGTTACCAA[G/A]GTTATAAATTACCTGAACAACTGAGAAAAGACGGAAGGCACGGTCTTGACCAGCAGATAAGATGAATTTTCCGTTGCCAT
373_c_46411_p0	S_46411	7HL	IBGSC et al. (2012)	morex_contig_46411	AGAAAAGGTCGCGCCAACAAAAAAAGCGATGTTTCATCGCNAAGTCATCCACTGTTGCTCCTTCCCCCTCCTCTCT[G/A]TGTGGTGACAGACACGGAGAACGAGGGGCAGTTTCGTTCCGTCCAAAG
370_c_1560299_p0	S_1560299	7HL	IBGSC et al. (2012)	morex_contig_1560299	CCAAACTTCCGGCTCGTCTAGAAAACNAATCCCCGGNTCGACNGCGACAGA[T/C]TGGGTTCTGAAATCTAGAGGGAAATTCGGATGAGGAGTTGCTGAATTTTAGTCATTAGCAGCGCAGAGGCAGAGTGAGCTAATGCNTNATGATCAAACGCT
359_c_1564788_p2	S_1564788	7HL	IBGSC et al. (2012)	morex_contig_1564788	GCCATGAGAGGTCGATCGCGCCGGTCGAAGGGTTGATCGGCATNCNTNTNCCCNTCCNGGGGGTNNAGCAACAACAACTC[T/A]CCTTCCGTCACGGTGGTGACCCTGGCGACGACGCCGCCCGGGAGACCCTCCGGCCACACCCCGTCGATTACACTCCCACC
65_U35_14693_p1	S_14693	2HL	Moscou et al. (2011)	U35_14693	CCGGTGGGTCAAGTCTGAGTGGAAGAAGGACGAGAACATGGCTGGTGAATGGAACCACAC[T/A]TCTGGAAAATGGCATGGAGATGCTGAGGACAAAGGTGAATTACCCAGAGCTCTGTCTATC
126_U35_1915_p1	S_1915	2HL	Moscou et al. (2011)	U35_1915	TGAACCGACTCNTTNTNTTTAGGTACATAANGTTCGGCCTAAAATGGTGTCAGTGCTTCG[T/C]GAAAAGTTTGCACACCTGAACCTGACCTTTTCTATTGGAGGGCAGATCAGTTTTGATGTA
97_U35_1730_p2	S_1730	2HL	Moscou et al. (2011)	U35_1730	GGTTCAGACAGGGGACGCCTTTAGTTCCTCACATANNNNGTTACAAACAAAACTTTATCA[G/A]TTCAGATGCACCGCCAGTCGCTTGTAAACGCGTTTTGTGTCAGTCGCGTGAAAACAGCCG
79_U35_18280_p1	S_18280	2HL	Moscou et al. (2011)	U35_18280	AGAACTAGGGTTTNTGTGTACAAAGGGTTCGATCGCAAGATCATGTAGGGGTGCTTACCTCAGACTATGG[G/A]TGTGAGAACATATTGTAAGGTTACATACCAATTGTGCGGGGGGTGGAGGGGGCTGCTACGTTTACAGGAC
86_U35_20591_p2	S_20591	2HL	Moscou et al. (2011)	U35_20591	ACATATCAGCCTACATAGCTATATGTGCCCATGTGACGACCTCTTATTTTAGAGCTCGAT[G/A]TTATTTTACGATGTATTTCTTCCAGTCAGTATCCTGGATGCCATTCTGCCTCAGGCAGTA
72_U35_19891_p1	S_19891	2HL	Moscou et al. (2011)	U35_19891	TACAACATGTCAATTTGTATAGAAGAACGAAAGAAATTGAGAAGTGAAAGCAACCTGAGAGTAACAATC[T/A]ATAGCAGTAGTAAAATCCTTTTGCCGAAATGCACTGTCACCCTTCTTCTTTGAGGTTAATGTGTCTTGCAT
93_U35_1302_p2	S_1302	2HL	Moscou et al. (2011)	U35_1302	CCGTGCGAAATCCTCCTGATTGCCGTGTGGATTTGGTCACCACAAACGAAAAGGAAGTTTCGCTGGTCGC[C/A]CTTGGCTTCATTCACCACAACGAGTCGCGATGAACCAACAAGCAAGTATGAATACATCAACGGCGATATA
96_U35_18223_p1	S_18223	2HL	Moscou et al. (2011)	U35_18223	GAGCTTCGCTGCATATANAAGGAGAATATGTTTCAGCAAGTCACTCTTGACTAGAAANTTGCCTCTCTTTTTTATGTTGTCCGTATATAATC[C/A]GTTTCATCTAGGTGAATGCTTCAAGTCTCTTTTGTGGAATGCTTACCATGAAATGGTCGT
193_U35_17447_p1	S_17447	2HL	Moscou et al. (2011)	U35_17447	GATGGGTGAGCTGCACCTAGCCATACGATTTTCTTCAACGTCATTGGTGAACATGCTGTA[T/C]CTGTACTCCCGACCTTTGCTGCCGAAGATGCACTATGCACGTCCAATACCAGTGCTTCAG
111_U35_13826_p1	S_13826	2HL	Moscou et al. (2011)	U35_13826	GTTAGGGNCGCCCCAACCGTAANTTNCGGGCGAGTACAAATAATAAAGAGCGAATAGAATNGCTCCGCAAAAGGCCTACTAC[G/C]AGACCATCGGTCTAGCCAAAATAGGGTGCTCGCACCCGAACCAATCCTAATTGACGAGCC
129_U35_13856_p2	S_13856	2HL	Moscou et al. (2011)	U35_13856	TGATCTTTGCCAATTCGGTTTGTAGTGTATAAATATTCATGGTTTGACTTGAATATTTTG[T/C]GTATGCATTTAATACGCCCTCACAACACTATAATTTTATTTTTCATCTAAGGAAGAACCT
140_U35_31290_p1	S_31290	2HL	Moscou et al. (2011)	U35_31290	ACGCCAACATCNTTGCAGGGATGGGGGTGCTCAGCACCGACATGGAGCTCCTCAACGACCNNTTCACCA[G/A]AGATTTGGTCCATAAGTGGGCTAACGACAAGGATGATTTCTTTCGGAAGTTCAGCGACGCC
148_U35_10450_p1	S_10450	2HL	Moscou et al. (2011)	U35_10450	AAACACACCTTCTGTAAACACATCGTCACAGTGGCTGGTGGACATGGTGCGTAGTGCACTCTCCTCTTGG[C/A]AGAAGAATAATGGAAAGAGATCTCATCTTTTCCACTAGCCAGAGGCGTTACAAATATAGGAGCCGATCCC
149_U35_7379_p1	S_7379	2HL	Moscou et al. (2011)	U35_7379	NNNNNAAAATGTCATTGAGNTTGTTCNTAAGAGAAGTTGTGATGGTCTATTTAAATTTAC[G/A]CCTTTATAATACTTTCCATCCCATCCCATATTATATGTTCGTCCGTTTCAGTTAAGCATT
165_U35_38987_p1	S_38987	2HL	Moscou et al. (2011)	U35_38987	ATCGTTTTGTTTTCTCTTGTAAATTACAGGTTCGCTGCAGCTACATACATCCTTGTAAATGACA[T/C]TACTTTTGAATGCAGGNACCTTTCTTGGAACACACACTCAATGGGATAATAGGAAATCCACTTTCTACCGGAGCCGT
191_U35_43918_p2	S_43918	2HL	Moscou et al. (2011)	U35_43918	CGATGTCGCTTGTCCNNTANGNCGGTGGANGGGNGTGGGTTCACTATCGATGCATGTGCCGA[T/A]GCTCANTGGTGAGAATTACACCACGTGGNCGATCAAGGTCGAGGCAACTTGGACGCTGCTAACATC
174_U35_20022_p1	S_20022	2HL	Moscou et al. (2011)	U35_20022	CCAGTCCTGCGCGTAGATACAAATGAAATGACATGCAAAGCAACAACTAAGATGTTCTGACA[G/A]ACACTACTTGAACTTCTCGACNCACACTCTGTACTACCTGAACGAAATGCCATGAAAGACAGAAAGAAACAAAAGANNNNNT
176_U35_6899_p1	S_6899	2HL	Moscou et al. (2011)	U35_6899	AACAGGAAAGTCGACGTGACCCTTTCGAGCAATCCAGCGACGGGACTTCCCATAGGCTTC[T/G]ATGGCCACTCTCAGCNTTCCTTGTGATTCCACTGAAACCACTTTCCTCGATAGTTGAAGG
CAPS0071_AD0005_c39_186	S_D03_c39	2HL	IBGSC et al. (2012)	HVVMRX83KHA0070D03	GCGACAAACATTATTGAACGTTCCTNAATCTCGTCACTCCGTAGTAAAAGCGTC[A/C]ATTGTCNGAGCCTATAAGTGNCTCCGAATAAGACCTGCGGAAAAGTTTNAGGTNGGCAATTATCAAACACAACATCATTCCTTGAGATCATA
CAPS0071_AD0005_c39_207	S_D03_c39	2HL	IBGSC et al. (2012)	HVVMRX83KHA0070D03	TTATAGTGCGACAAACATTATTGAACGTTCCTNAATCTCGTCACTCCGTAGTAAAAGCGTCNATTGTCNGAGCCTATAAGTG[C/T]CTCCGAATAAGACCTGCGGAAAAGTTTNAGGTNGGCAATTATCAAACACAACATCATTCCTTGAGATC
CAPS0071_AD0006_c1_46	S_A19_c1	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AGTTTGNGGATGCAGATCATTGTTCCTTCAGCCATTTGATAATTTN[A/C]CTGGTCTAAAGTACTTCCTGCTTCTTCATCTTGGTTCCCATAATAAGGGATGATTTTAGGATCTAGCATCTCGCAAATCTTTTCAGGAAATGATTTTTCC
CAPS0071_AD0006_c1_202	S_A19_c1	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	CAAATCTTTTCAGGAAATGATTTTTCCACAAATTTATAAAGGCTCAGGCCNTCTTTGAACATCTCATCAGTTGGACGCTTCC[C/T]AGTGAGCATCTCTAAGATAATGATTCCATAGCTATAAACATCACCCTCCATGGAGATTTTGCTCCCTAATCCA
CAPS0071_AD0006_c6_61	S_A19_c6	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TTAGGTTTGTTGGGATCTCACCGACGATGGAGTTACTCCCAAGATCAATAACTCGAAGCTG[A/G]GAACATGAGGATAGATTGCTTGGTATCATACCGCTAAGATTGTTTGAGCTAAGGTTAAGATACCGCAACCTATTTAGTTGGCCAAGTTCAGC
CAPS0071_AD0006_c6_415	S_A19_c6	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AGTAAGACCGGGCCAAGTGCAGAACCGTTGGAGGGAATCATCAATCTTCCATGAGGCTAGGACTCCGGCAGAAGTATTGAGATGGAGTTTAAGGCAACGGAGTGCTTGGAAGTCGGT[G/T]CTAGCATCCCCGCGGAGAGCTGTGGCA
CAPS0071_AD0006_c12_290	S_A19_c12	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	ACANAATACATGNCTACATTACATTGATGAATTNGAACTAGTTTTGTGTCGCTCTAGTATGTAA[C/T]TGTTACATGATTGATGCCGTACAGTTCGAGATTTGGATGCATGGTATAAGAGATAAAACGTATTGAATAAATGAATCTAAAAA
CAPS0071_AD0006_c12_111	S_A19_c12	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	ACAACATGTGATAATAATTANAATTCTTTCTCGTGATTGTCGTAGTTTGAGGAGTGTTTATATTCATCATGTGCTAATGTTTTATTCCGGTT[C/T]CCTATTAAAAGGAGGCATTAATATCCNTTAGATTTTCTTATGGACCCCCGTGCCACAGGAGGGTA
CAPS0071_AD0006_c12_259	S_A19_c12	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GCCACAGGAGGGTAGGACAAAAGATGNCATGNAAGTTNTTATTATAAACACGTATGATTATATACANAATACATGNCTACATTACATTGATGAATT[G/T]GAACTAGTTTTGTGTCGCTCTAGTATGTAANTGTTACATGATTGATGCCGTACA
CAPS0071_AD0006_c12_189	S_A19_c12	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GGTTNCCTATTAAAAGGAGGCATTAATATCCNTTAGATTTTCTTATGGACCCCCGTGCCACAGGAGGGTAGGACAAAAGATG[C/T]CATGNAAGTTNTTATTATAAACACGTATGATTATATACANAATACATGNCTACATTACATTGATGAATTNGAACT
CAPS0071_AD0006_c21_54	S_A19_c21	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TCTTCAGCACCTCCCCTGAGACCTTTGAGACGAACACGGCGTATATATGGATAC[C/T]GCTTGAGCTTCCAGCCACTGTTACACCTACTTCTCAGTTAGAAAGTGCAGAGAATATATGATCTGAATAATACTA
CAPS0071_AD0006_c28_44	S_A19_c28	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GGCTTAATGCGTGTGATAAAATGCCAAATGAATCTCAACATTGT[C/T]GGCTTCCGTTGCAGGAATCGTAGCTGATTGGATGCTTTCGAGCTGCAGATTCAGAGCACATAGTGATTGTCNAGTGCTCATCGAG
CAPS0071_AD0006_c28_158	S_A19_c28	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TGGATGCTTTCGAGCTGCAGATTCAGAGCACATAGTGATTGTCNAGTGCTCATCGAGACGTGGGTTTGAAGCATTGAAGTGTTAG[C/G]TGGCTTTTCCATTGCGTAGTTGTAACCTGTAAAANCAAGTTGGGTTTTTGTCAAGAGCTGAAGATGAA
CAPS0071_AD0006_c28_193	S_A19_c28	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AGACGTGGGTTTGAAGCATTGAAGTGTTAGNTGGCTTTTCCATTGCGTAGTTGTAACCTGTAAAA[A/T]CAAGTTGGGTTTTTGTCAAGAGCTGAAGATGAAGAGTACTCGAGGAGCAATGTTATCTCCCCTCTGTCTCTGTGCAGTGGTTTGC

CAPS0071_AD0006_c33_50	S_A19_c33	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GGGCAGTTCGGAGGGTGTGACCCTGGGGGGNCACTCGGGTCGANTGTCAC[A/G]ATTTGCAAAAATATCAGGCAATGAACAAATAAAAACTCACGAGAGAAGAGCAGAATGCCAAGATAATCATCTACTACTGCTAATGNG
CAPS0071_AD0006_c34_259	S_A19_c34	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GATTTGGATGCCGAGTTGAGAGGATATCCAGCTTCTGATCTGAGGATATCTTGTAGTGCTGTGGCCCGATTTGATTTGC[C/G]TATCCATCCCTAAGCCACCCGCCGGATTTATTATATGCCAAAATCATACTCCTACTTGTTGAATCTGCTATGG
CAPS0071_AD0006_c37_241	S_A19_c37	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GCACCCCACACTCTCCCAACACCCAAAGACCTTACAGAAACTCATCAGCCATGAGCATAAGCAGAAGCACCGCAGACCTTGCAAACATGTCTTC[A/G]CCCTACCTCCGCACGTCGCTCCTCCGCGGAGGCTCAACACACTCCCTGGTCGGTAANCACCTAT
CAPS0071_AD0006_c37_382	S_A19_c37	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AACACACTCCCTGGTCGGTAANCACCTATCTCCACCAGTGAACATAAGTTATGCTTATGNCCCNCCGCAGATGTACAATNCCGGCTACGCGTCGCCCAGCCCGCA[C/T]GCCGGTTCTTCGTACATCTCCAACTCTGGGTACGGATCCTCATC
CAPS0071_AD0006_c37_115	S_A19_c37	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	NAGTTCACTTGCCTTCTCCGTCATCCTCATTCCGTTCGAGCGTGGGAGCATCTCCTCCCAGACCAATAAGCCGGCGTCCTTCGTC[G/T]CAGTCCATATCTCGGATCTACTCTGCTTCCGGCACCCCACACTCTCCCAACACCCAAAGACCTTACAGAAACTCA
CAPS0071_AD0006_c40_154	S_A19_c40	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TCCCTANGGCCTCTCCTCTTCCATCGACGAGAACACTTCCTCTCGCCTACCGGTTCTGAAGCCTTGTGCCACCCA[C/T]TGCTTTCTCATTTTATCCTCAACGNACAATGTGAAGTGAGAGAACATNCCTCCNAAACCTCACCTCTTGCGTTCTTGT
CAPS0071_AD0006_c40_208	S_A19_c40	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TTCTGAAGCCTTGTGCCACCCANTGCTTTCTCATTTTATCCTCAACGNACAATGTGAAGTGAGAGAACATNCCTCC[A/G]AAACCTCACCTCTTGCGTTCTTGTATAAGAGAGGTGTGATCGTGTTTTTCGGGAGAGCATTCAAACGACTGGATCTTTCTT
CAPS0071_AD0006_c40_85	S_A19_c40	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TTATTTTGTTTATGAANGNAAATGTTTTTGCTTATAGTGAAAGATGAATCTGAACAGTGTGTGATTGTTTCTCTTCCTCTCCCTA[G/T]GGCCTCTCCTCTTCCATCGACGAGAACACTTCCTCTCGCCTACCGGTTCTGAAGCCTTGTGCCACC
CAPS0071_AD0006_c42_224	S_A19_c42	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TTNATACAATGTTCAAAAATTGTTCTGTNTNTTAGAAAANNTTTATTCTGTTAANAAAGTGTACTT[A/C]AAAGAAATATTCACATGTTTTCAAAGAATATCTGTAAAANANAATTGTCTATGCAATGTAAATAAATGTTCCTGTA
CAPS0071_AD0006_c42_266	S_A19_c42	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	TTAANAAAGTGTACTTNAAAGAAATATTCACATGTTTTCAAAGAATATCTGTAAAANA[A/C]AATTGTCTATGCAATGTAAATAAATGTTCCTGTATTTTAAAACAAATGNCTATTTCNATTTNAAAATNTAAAACATGTGTT
CAPS0071_AD0006_c42_160	S_A19_c42	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	GCATGTGACATTTTAGAAAACAAATCCACCAATTCAAAAAAGATTTGTGACATTTTTAAATATGTT[C/T]ATACAATGTTCAAAAATTGTTCTGTNTNTTAGAAAANNTTTATTCTGTTAANAAAGTGTACTTNAAAGA
CAPS0071_AD0006_c42_25	S_A19_c42	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AAATTGTTTTTCAGACATTTTCAAA[A/G]AAATTGTATANAATGTAAAAACTAAATGTTTGCNTAATATAAGAAGTGTTTTGAA
CAPS0071_AD0006_c42_334	S_A19_c42	2HL	IBGSC et al. (2012)	HVVMRX83KHA0069A19	AATGTAAATAAATGTTCCTGTATTTTAAAACAAATGNCTATTTCNATTTNAAAAT[G/T]TAAAACATGTGTTTGGAAAATNTTATTGTGTATTCACAATAGTTTTCAAAACATATATTNTGGGAAAAAATGTACATCAT
CAPS0071_AD0006_c23_91	S_F09_c23	2HL	IBGSC et al. (2012)	HVVMRX83KHA0063F09	TGTTGTTATCACACCCTTAAAATGGCTATGCGACTGATAATGCAATANCACCAATAAAATGAAGTAATAACACCGTTGAAGTCAAGCTACT[A/G]GCATGGTGTTATTAATCTAATTTCACNGATAAAAATAAATTATTCCATAATTTCCTATTTA
CAPS0071_AD0006_c23_155	S_F09_c23	2HL	IBGSC et al. (2012)	HVVMRX83KHA0063F09	TGAAGTAATAACACCGTTGAAGTCAAGCTACTNGCATGGTGTTATTAATCTAATTTCACNGATAAAAATAAATTATTCCATAATTTCCTATTTAAC[A/G]AACTTAATTTAATGTTACTGGTTTGGTGGCATTTCCCA
CAPS0071_AD0006_c23_421	S_F09_c23	2HL	IBGSC et al. (2012)	HVVMRX83KHA0063F09	TCAGCTGAATTAGTTTTCCATTTTGTGCAAACAGGTACATTGATATCAACTCGACAATAATGTGACATTTTGGTGTTCTCCATGAA[A/G]CACTTTTTTAGAAGAGTGTTCAAGAGTGTAAGACTATTAGCTAGTCTGCCGGCAATGGCTATCTTTGCTGG
CAPS0071_AD0006_c23_271	S_F09_c23	2HL	IBGSC et al. (2012)	HVVMRX83KHA0063F09	TTCTTCATTTATAGTGCATGCCACAAGTTTGTAATATTCATAATTCACATAAGACCTTTTGGTGCGTCATC[A/G]TTATAATTCATAGAAACACTTTCTTGACATAATGAAGATGCAATTCTTTCTTGTTATTAACATTCAGCT
CAPS0071_AD0005_c43_260	S_C21_c43	2HL	IBGSC et al. (2012)	HVVMRX83KHA0124C21	AGGGCAGCATTACCCATAAAAGAAGTAAGAAAAAATATAGATGCNAATTATTGNNGTTGTGGAGATGCAACGACTTGGA[A/C]CAGGTCTTCGTCTACGGTAGCTCCAGTNGGTGGGGTCACCTCACATGATAGATGTCGAGGGAAATATCT
CAPS0071_AD0005_c54_122	S_C21_c54	2HL	IBGSC et al. (2012)	HVVMRX83KHA0124C21	CTAATGCTGATGAGCTGAAATTCATACANATGCGAGAAACATATTCAACCATTGAGGATTCAATCATTGGGAGGACCGA[A/C]GAGGAAAATAAAATATGGGTTTCTTTATCTGAGAACACAGTACAAGATCTCGTTGTCCTTGCTATGCACGGCA
CAPS0071_AD0005_c54_71	S_C21_c54	2HL	IBGSC et al. (2012)	HVVMRX83KHA0124C21	GGGATCACACATCAGTATCGGGAATTCATGTTAGTGCCGGGCACTAATGCTGATGAGCTGAAATTCATACA[A/G]ATGCGAGAAACATATTCAACCATTGAGGATTCAATCATTGGGAGGACCGANGAGGAAAATAAAATATGGG
CAPS0071_AD0005_c30_299	S_O23_c30	2HL	IBGSC et al. (2012)	HVVMRXALLHA0113O23	AAAAATGGAGTGAATCTCACGCATGAAATTATGTACCACCAAGAAAAAAA[A/T]TGGAATTTCTTGAATTTTTTTCGTATTTATTTTGATTTTTTTTACTCAGCTGGTGTGTAGATGAGGCCGATCTGTACTTG
CAPS0071_AD0005_c30_141	S_O23_c30	2HL	IBGSC et al. (2012)	HVVMRXALLHA0113O23	TGGGTGGTACATAATTTGATGCGTGAGATTCGCTCCAATTAGTGTATCATTTGAACATTTGAGCAGCTCT[C/T]GACAAAAAGGACAAATCGGATAAAAACAGTGCATGAATAGTAAACATTTTTATAGACCCCGAATTTTGTCTTTTTGCCGA

Table A5: KASP markers that map to the *Rpst1*, *Rpst2*, and *Rpst3* genetic intervals.

Marker	Synonym	Chr.	Source identifier	Design Source	Population	Allele-1 forward primer	Allele-2 forward primer	Universal reverse primer
BOPA1_7023-448_120_F	K_2547604c	7H	morex_contig_2547604	Comadran et al. (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTGTGGCGATCATTTCACGAGA	GAAGGTGACCAAGTTCATGCTGTGGCGATCATTTCACGAGT	GCGCTGCCCTACCTCAAA
BOPA1_4991-1028_120_F	K_368884	7H	morex_contig_368884	Comadran et al. (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTCCTCGACCGACCGACGGATA	GAAGGTGACCAAGTTCATGCTCCTCGACCGACCGACGGATG	GGGTGACAACTTCCGGGG
SCRI_RS_237742_60_F	K_39540	7H	morex_contig_39540	Comadran et al. (2012)	AxR	GAAGGTCGGAGTCAACGGATTGCAGATCGTGGCGATGTAGC	GAAGGTGACCAAGTTCATGCTGCAGATCGTGGCGATGTAGT	TGCTACTGGTACACTGCCAC
BOPA1_4671-856_192_F	K_1574415	7H	morex_contig_1574415	Comadran et al. (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTTCTGCCATTTATCAACGACA	GAAGGTGACCAAGTTCATGCTTCTGCCATTTATCAACGACG	AAGTGCCCCCTTCTGTGC
SCRI_RS_185445_60_F	K_1560415b	7H	morex_contig_1560415	Comadran et al. (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTGTTGCAAAATGGAATGCCGA	GAAGGTGACCAAGTTCATGCTGTTGCAAAATGGAATGCCGG	AGCAGCCCAGTGCAATGG
SCRI_RS_134057_60_F	K_134057	7H	morex_contig_200698	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTGAAACCATCGGCAGCATGCA	GAAGGTGACCAAGTTCATGCTGAAACCATCGGCAGCATGCG	ACAGCCCTGGTTATCACTGAG
SCRI_RS_177876_60_F	K_177876	7H	morex_contig_157882	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCGCCGATCCTGCAGTTCGAA	GAAGGTGACCAAGTTCATGCTCGCCGATCCTGCAGTTCGAC	AAGCTAGACCACGGCGTG
BOPA1_4671-856_192_F	K_10687	7H	morex_contig_1574415	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTCTGCCATTTATCAACGACA	GAAGGTGACCAAGTTCATGCTTCTGCCATTTATCAACGACG	AAGTGCCCCCTTCTGTGC
BOPA2_12_30761_60_F	K_30761	7H	morex_contig_135384	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTACCTTGAGAATGAAGGAACA	GAAGGTGACCAAGTTCATGCTACCTTGAGAATGAAGGAACG	TGACCTCAATTTGGTTAGCCA
SCRI_RS_164623_36_F	K_164623	7H	morex_contig_46434	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTAGACTGGACAGAAACCCTTC	GAAGGTGACCAAGTTCATGCTAGACTGGACAGAAACCCTTT	ACTGTGAGTAACTGGCAACGT
BOPA1_13108-412_120_R	K_13108	7H	morex_contig_135384	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTAAGCTTGAATAAAGACTTGC	GAAGGTGACCAAGTTCATGCTAAGCTTGAATAAAGACTTGG	GCATACAGCGGCGCTTAC
SCRI_RS_155652_60_F	K_155652	7H	morex_contig_135731	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCATCAAGGACGTGGTGACCA	GAAGGTGACCAAGTTCATGCTCATCAAGGACGTGGTGACCC	CAGTCCCACCCGAAGCG
SCRI_RS_141470_60_F	K_141470	7H	morex_contig_1638869	Comadran et al. (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCAGAGTCCAGACCAGAGAAG	GAAGGTGACCAAGTTCATGCTCAGAGTCCAGACCAGAGAAT	ACGGCTCGAAGATCCCCA
contig_2547604_2254_R	K_2547604a	7H	morex_contig_2547604	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTAAGCAGGCTGAGATCTACCA	GAAGGTGACCAAGTTCATGCTAAGCAGGCTGAGATCTACCG	GGACAGAAACTCAGTGGCGA
contig_46411_4095_R	K_46411	7H	morex_contig_46411	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTAAAAGACGAAGCGAGCGGTA	GAAGGTGACCAAGTTCATGCTAAAAGACGAAGCGAGCGGTG	CCGGCCGGTCTCATTACC
contig_60221_1370_R	K_60221	7H	morex_contig_60221	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGACGGAGTCAGTCGTAGGAA	GAAGGTGACCAAGTTCATGCTGACGGAGTCAGTCGTAGGAG	GGTTGCACCACCCGCTTT
contig_1560299_1267_F	K_1560299a	7H	morex_contig_1560299	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTGGGAGCGCAGTTTACAGGGC	GAAGGTGACCAAGTTCATGCTGGGAGCGCAGTTTACAGGGT	TCCTCTTGGGCGTTTTGC
contig_2560853_129_F	K_2560853	7H	morex_contig_2560853	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCGTATGTATCCGCTGCTGCC	GAAGGTGACCAAGTTCATGCTCGTATGTATCCGCTGCTGCT	TCCTCCACTTGACCCGGT
contig_36837_3649_F	K_36837a	7H	morex_contig_36837	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTGTTGCGTTACCGTGGGAACG	GAAGGTGACCAAGTTCATGCTGTTGCGTTACCGTGGGAACT	TACATCACCCACGCAGCC
contig_368884_3456_196_R	K_368884	7H	morex_contig_368884	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCCTCGACCGACCGACGGATA	GAAGGTGACCAAGTTCATGCTCCTCGACCGACCGACGGATG	GGGTGACAACTTCCGGGG
contig_37728_1320_F	K_37728	7H	morex_contig_37728	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCGAGCTGAAGTTTCTGTGCC	GAAGGTGACCAAGTTCATGCTCGAGCTGAAGTTTCTGTGCT	GCTACGCGCTCAACTTCG
contig_1579877_1136_R	K_1579877	7H	morex_contig_1579877	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTACATCTCCAGCTCCTACCAA	GAAGGTGACCAAGTTCATGCTACATCTCCAGCTCCTACCAG	GGCGAGGACTCAAGAGCG
contig_175242_1402_R	K_175242a	7H	morex_contig_175242	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGGCTCGCCTTCTCGAACTCC	GAAGGTGACCAAGTTCATGCTGGCTCGCCTTCTCGAACTCT	CTTATCTCTGGCCGCCGG
contig_175242_1510_F	K_175242b	7H	morex_contig_175242	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTATGTTAACCAGGAGGAGGCA	GAAGGTGACCAAGTTCATGCTATGTTAACCAGGAGGAGGCG	TGTATGCAGAGGAGCGCG
contig_1574415_954_F	K_1574415	7H	morex_contig_1574415	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTCAGCCCACAAGCGTAATGGA	GAAGGTGACCAAGTTCATGCTCAGCCCACAAGCGTAATGGG	GTACGCCCCGACTTGGTG
morex_contig_58199_39_F	K_58199	7H	morex_contig_58199	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGAGAGCACATGCCCCCATTC	GAAGGTGACCAAGTTCATGCTGAGAGCACATGCCCCCATTT	TGGACAGGAAGTTGAAAGGAG
contig_361382_313_F	K_361382a	7H	morex_contig_361382	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTAACCAGTCTGGCTGCCTTCA	GAAGGTGACCAAGTTCATGCTAACCAGTCTGGCTGCCTTCC	TCGTGCTTGTCAAGGGCC
contig_361382_795_R	K_361382b	7H	morex_contig_361382	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTTGAGAAATATCCAACAACA	GAAGGTGACCAAGTTCATGCTTTGAGAAATATCCAACAACG	CCGAGATGCAACACAGAGGT
morex_contig_57421_936_F	K_57421	7H	morex_contig_57421	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTATCTCTGTCCTTCATCATGA	GAAGGTGACCAAGTTCATGCTATCTCTGTCCTTCATCATGG	ACCTAATGCTCGCTCCTACG
morex_contig_49978_1442_R	K_49978a	7H	morex_contig_49978	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTTTTGGTGAGGACGGGAGCC	GAAGGTGACCAAGTTCATGCTTTTTGGTGAGGACGGGAGCT	GGCCCTGTTCGTTTGGCA
morex_contig_49978_2243_R	K_49978b	7H	morex_contig_49978	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTTCCGAACGTTGGCAGGCTA	GAAGGTGACCAAGTTCATGCTTTCCGAACGTTGGCAGGCTG	AGCTGATGTGGCTCATACCC
morex_contig_49978_3011_F	K_49978c	7H	morex_contig_49978	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTAAGCAAAGCATCGCCTGACA	GAAGGTGACCAAGTTCATGCTAAGCAAAGCATCGCCTGACC	GGGCTTTGTGGTGGAGCT
morex_contig_49978_3638_F	K_49978d	7H	morex_contig_49978	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGGCGTTCCATTGTCCCATGC	GAAGGTGACCAAGTTCATGCTGGCGTTCCATTGTCCCATGG	GCATTGCCGGGTCTGGAA
morex_contig_37596_157_F	K_37596a	7H	morex_contig_37596	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTATTCCCGTGTGCTAGCTTTA	GAAGGTGACCAAGTTCATGCTATTCCCGTGTGCTAGCTTTG	TCTGAGAGAAAAGGGGCCG

morex_contig_37596_400_F	K_37596b	7H	morex_contig_37596	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGAGAAATAATGCTAGCCACG	GAAGGTGACCAAGTTCATGCTGAGAAATAATGCTAGCCACT	TACAGACCGTGTCGTCGC
contig_46434_3168_F	K_46434a	7H	morex_contig_46434	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTAGGATTTTCAGCATAACGTA	GAAGGTGACCAAGTTCATGCTAGGATTTTCAGCATAACGTG	CCACCAGTCGGTCACAGC
morex_contig_1559140_428_R	K_1559140	7H	morex_contig_1559140	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGGTTGGTGTTCGTCCTCCCA	GAAGGTGACCAAGTTCATGCTGGTTGGTGTTCGTCCTCCCC	GAGGGTCTCCAAGTTAGGCA
contig_110700_751_R	K_110700	7H	morex_contig_110700	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGGGTGATTGACTGCTTGCCA	GAAGGTGACCAAGTTCATGCTGGGTGATTGACTGCTTGCCG	CTGCAGTGTCACCCTGGG
*NLR-B*_428_R_s1	NLR-B	7H	barke_contig_1882436	IBGSC, (2012)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTGGTTGGTGTTCGTCCTCCCA	GAAGGTGACCAAGTTCATGCTGGTTGGTGTTCGTCCTCCCC	GAGGGTCTCCAAGTTAGGCA
contig_135731_1130_F	K_135731	7H	morex_contig_135731	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCAGGTTGCAAAACTGCACTC	GAAGGTGACCAAGTTCATGCTCAGGTTGCAAAACTGCACTG	AGTCCAAGCCTGCCTCAA
contig_1578420_3650_F	K_1578420	7H	morex_contig_1578420	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGCTTCTCTAGTTTCTAGCTA	GAAGGTGACCAAGTTCATGCTGCTTCTCTAGTTTCTAGCTG	CCAGGTTCAGCTTGCTGC
contig_46242_8203_R	K_46242	7H	morex_contig_46242	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTCTACTTGTATAATCAACTG	GAAGGTGACCAAGTTCATGCTTCTACTTGTATAATCAACTT	TGCCAGAATGGTGTCGGC
contig_274815_2790_R	K_274815	7H	morex_contig_274815	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGGCCAAGTTGCTCCAAAAGA	GAAGGTGACCAAGTTCATGCTGGCCAAGTTGCTCCAAAAGT	GCAATGGGCAGGGCGTTA
contig_47031_814_F	K_47031	7H	morex_contig_47031	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCCTCCTCCTGCTGCTCCTTA	GAAGGTGACCAAGTTCATGCTCCTCCTCCTGCTGCTCCTTG	CCTGGCGCGATCGATGAT
contig_1560415_5727_F	K_1560415a	7H	morex_contig_1560415	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTAATATCGTCAAGGGCTGGC	GAAGGTGACCAAGTTCATGCTTAATATCGTCAAGGGCTGGT	TCTCTACAGCTAGCCCCGA
contig_370395_1028_F	K_370395	7H	morex_contig_370395	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTTGTTCTCCCATGAGGTCCAC	GAAGGTGACCAAGTTCATGCTTGTTCTCCCATGAGGTCCAT	CCAAAGCCCCTTGCAGCT
contig_99933_5109_F	K_99933	7H	morex_contig_99933	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGTCACGACAGGAAATGCTAC	GAAGGTGACCAAGTTCATGCTGTCACGACAGGAAATGCTAG	CTGCATCCACCCAGCTCG
contig_1566503_747_R	K_1566503a	7H	morex_contig_1566503	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTCCGCAAACCACTCGCTATCC	GAAGGTGACCAAGTTCATGCTCCGCAAACCACTCGCTATCT	CTACTCTGGCCGCACGAG
contig_1579285_1990_R	K_1579285a	7H	morex_contig_1579285	IBGSC, (2012)	AxR	GAAGGTCGGAGTCAACGGATTGCTGCTACGTACCTGGTCAC	GAAGGTGACCAAGTTCATGCTGCTGCTACGTACCTGGTCAG	AGAAGGCACGGCAAGCAT
contig_175242_970_F	K_175242c	7H	morex_contig_175242	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTAGTACACCGATGAGCTTGCA	GAAGGTGACCAAGTTCATGCTAGTACACCGATGAGCTTGCG	CCTCGATTTGGCAAGCGC
contig_366867_2874_R	K_366867	7H	morex_contig_366867	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTATGGTAGGGGAGAAGGGCGA	GAAGGTGACCAAGTTCATGCTATGGTAGGGGAGAAGGGCGC	CGCCAAAGCAATCTCGGC
geneA_53_F	K_*NLR-Aa*	7H	barke_contig_54347	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTTGCTCTCATACTTGATTTC	GAAGGTGACCAAGTTCATGCTTTGCTCTCATACTTGATTTT	TGCCCGTGACAAAGCAGA
geneA_53_R	K_*NLR-Ab*	7H	barke_contig_54347	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCGTGACAAAGCAGAAAAAGA	GAAGGTGACCAAGTTCATGCTCGTGACAAAGCAGAAAAAGG	TGCCGAGTTCCTCCCTAA
morex_contig_37596_2173_F	K_37596c	7H	morex_contig_37596	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCGCACTCTTTTACAGCGTCC	GAAGGTGACCAAGTTCATGCTCGCACTCTTTTACAGCGTCT	TGGACGCCAGACAGCTTC
morex_contig_37596_2211_F	K_37596d	7H	morex_contig_37596	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTCGCACTCTTTTACAGCGTCC	GAAGGTGACCAAGTTCATGCTCGCACTCTTTTACAGCGTCT	TGGACGCCAGACAGCTTC
morex_contig_37596_4090_F	K_37596e	7H	morex_contig_37596	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTAATTGGTCGGGATCAGGAC	GAAGGTGACCAAGTTCATGCTTAATTGGTCGGGATCAGGAT	GCCCAAAATGGTCCCGGT
morex_contig_37596_7613_F	K_37596f	7H	morex_contig_37596	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTGCTCCACGGCTCCGCTTCGC	GAAGGTGACCAAGTTCATGCTGCTCCACGGCTCCGCTTCGG	GAACTACCGCTCCGTCCG
contig_46434_2645_F	K_46434b	7H	morex_contig_46434	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTTCGTCCATTCAGCCTTCCA	GAAGGTGACCAAGTTCATGCTTTCGTCCATTCAGCCTTCCG	TGGCTGGTTCCCTCCTCA
contig_160371_969_F	K_160371	7H	morex_contig_160371	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTCACTGTGCCAGGGATTACA	GAAGGTGACCAAGTTCATGCTTCACTGTGCCAGGGATTACG	TCTTCTTCCTGCAGCAAAGGT
contig_140186_2474_R	K_140186	7H	morex_contig_140186	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTGCTTGACAAGATTACAGCGC	GAAGGTGACCAAGTTCATGCTGCTTGACAAGATTACAGCGT	GTCGTGGGCGTTTGCAAT
*NLR-C*_410_F	K_*NLR-C*a	7H	barke_contig_1788394	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTAGCATGTGCAAGAGGTTACC	GAAGGTGACCAAGTTCATGCTAGCATGTGCAAGAGGTTACT	GGTTCCCCGATTGCACCA
*NLR-C*_772_F	K_*NLR-C*b	7H	barke_contig_1788394	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTAAGGGACCTAAACAAGCTTA	GAAGGTGACCAAGTTCATGCTAAGGGACCTAAACAAGCTTC	CCGTTCCTTGGCACCTAGA
contig_40356_4069_F	K_40356	7H	morex_contig_40356	IBGSC, (2012)	SxGP	GAAGGTCGGAGTCAACGGATTTCTAGTCCATCCTGCCCTTG	GAAGGTGACCAAGTTCATGCTTCTAGTCCATCCTGCCCTTT	CGACCGAAGTTCAAGAGCG
U35_22699_p1_128_R	K_1560299b	7H	morex_contig_1560299	Moscou et al. (2011)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTTCCGGCTCGTCTAGAAAACC	GAAGGTGACCAAGTTCATGCTTCCGGCTCGTCTAGAAAACT	TCAGCAACTCCTCATCCGA
U35_22182_p2_347_F	K_1560415c	7H	morex_contig_1560415	Moscou et al. (2011)	AxR	GAAGGTCGGAGTCAACGGATTACAAGAGGTTTTGCGGGTGC	GAAGGTGACCAAGTTCATGCTACAAGAGGTTTTGCGGGTGT	CCAAATGGGTGCCATAATGCA
U35_3446_p1_481_R	K_1579285b	7H	morex_contig_1579285	Moscou et al. (2011)	AxR	GAAGGTCGGAGTCAACGGATTCTGCTTGCGGATCAATTCAC	GAAGGTGACCAAGTTCATGCTCTGCTTGCGGATCAATTCAT	GGACCTCCTCACATGGCC
U35_3415_p1_252_F	K_3415	7H	morex_contig_41454	Moscou et al. (2011)	SxGP	GAAGGTCGGAGTCAACGGATTACCCAGTGTTGAGCACCTCA	GAAGGTGACCAAGTTCATGCTACCCAGTGTTGAGCACCTCG	GCGACAGCACATGGGTCT
U35_19936_p1_407_F	K_19936	7H	morex_contig_275230	Moscou et al. (2011)	SxGP	GAAGGTCGGAGTCAACGGATTGCGCTAGACCATTCTTGTCA	GAAGGTGACCAAGTTCATGCTGCGCTAGACCATTCTTGTCG	ACCAGGGCAGTTTCACCA
U35_16617_p1_130_F	K_16617	7H	morex_contig_8932	Moscou et al. (2011)	SxGP	GAAGGTCGGAGTCAACGGATTCTGGGCGAGTAGACATACGA	GAAGGTGACCAAGTTCATGCTCTGGGCGAGTAGACATACGG	ACGGTCCCTTCAGCTGGT
U32_7023_p1_483_R	K_2547604d	7H	morex_contig_2547604	Muñoz-Amatriaín et al., (2011)	AxR	GAAGGTCGGAGTCAACGGATTGTCTCTGTTCTGAAGGGACC	GAAGGTGACCAAGTTCATGCTGTCTCTGTTCTGAAGGGACT	TGCCATGCTCTGATGGAAA

U32_3345_p1_375_R	K_36837b	7H	morex_contig_36837	Muñoz-Amatriaín et al., (2011)	AxR	GAAGGTCGGAGTCAACGGATTTGTTGATCTGCTTCAGCTGC	GAAGGTGACCAAGTTCATGCTTGTTGATCTGCTTCAGCTGT	CTGGTGTGGTGTGTGGGT
U32_4671_p1_572_F	K_1574415	7H	morex_contig_1574415	Muñoz-Amatriaín et al., (2011)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTTTCTGTTAATTGCCGCTGTG	GAAGGTGACCAAGTTCATGCTTTCTGTTAATTGCCGCTGTT	CCCAACTCCTTGCATATCCGT
U32_7356_p1_71_F	K_1566503c	7H	morex_contig_1566503	Muñoz-Amatriaín et al., (2011)	AxR, SxGP	GAAGGTCGGAGTCAACGGATTCAGGTCATGTCCATGCCTAC	GAAGGTGACCAAGTTCATGCTCAGGTCATGTCCATGCCTAT	AGGAAGGAGGTTGGCCCA
Oz_43900_p1_398_R	K_2547604b	7H	morex_contig_2547604	Rice Synteny	AxR, SxGP	GAAGGTCGGAGTCAACGGATTTGACCGAGAGTACCATGCTC	GAAGGTGACCAAGTTCATGCTTGACCGAGAGTACCATGCTT	GGCAAAACACTCCATCGCC
OZ_43640_p1_174_F	K_1566503b	7H	morex_contig_1566503	Rice Synteny	AxR	GAAGGTCGGAGTCAACGGATTAAGGGAAACATGAGGCTGTG	GAAGGTGACCAAGTTCATGCTAAGGGAAACATGAGGCTGTT	ATTTTGATTGTGTGGCTCCAC
Oz_43350_p1_306_F	K_43350	7H	morex_contig_58586	Rice Synteny	SxGP	GAAGGTCGGAGTCAACGGATTCGTGGTGTTTGATGGCCTTA	GAAGGTGACCAAGTTCATGCTCGTGGTGTTTGATGGCCTTG	GCTGGGAGGTGAAGGTCG
1_0510_120_F	1_0510	4H	morex_contig_53987	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTCGGATAGGCCAAAATCAATC	GAAGGTGACCAAGTTCATGCTCGGATAGGCCAAAATCAATT	CGCGATCTCAAGCCGGAA
1_0751_120_F	1_0751	4H	morex_contig_46726	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTCATATGATGGAAGCACAACC	GAAGGTGACCAAGTTCATGCTCATATGATGGAAGCACAACT	TCTTCTCTCGGCCAGGCT
1_1398_77_F	1_1398	4H	morex_contig_1567222	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTAAATGTCTCACCAAGTGCTG	GAAGGTGACCAAGTTCATGCTAAATGTCTCACCAAGTGCTT	GGGATTGGTAAGGGAGGCAC
2_0384_120_F	2_0384	4H	morex_contig_6049	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTCGGGCAAACCTCCGACGTCA	GAAGGTGACCAAGTTCATGCTCGGGCAAACCTCCGACGTCG	CCGGAGGTTTGCCCATGT
2_0732_120_F	2_0732	4H	morex_contig_136881	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTACCCCTACAAAAATACCACA	GAAGGTGACCAAGTTCATGCTACCCCTACAAAAATACCACG	GCCCATCTTATTGCGCTGG
3_0718_60_R	3_0718	4H	morex_contig_5695	Muñoz-Amatriaín et al., (2011)	-	GAAGGTCGGAGTCAACGGATTCAACCTGCAGCCAGCTCTCA	GAAGGTGACCAAGTTCATGCTCAACCTGCAGCCAGCTCTCC	AGGCCGCATTGATCACCG
206D11_T7_p1_281_F	-	1H	morex_contig_64509	Wei et al. (1999)	-	GAAGGTCGGAGTCAACGGATTCAGGGGGTAGCCCTAAGCAC	GAAGGTGACCAAGTTCATGCTCAGGGGGTAGCCCTAAGCAG	CTCTCGTCGGCGTCTTCC

Table A6: Primers used for assessment of *NLR-A* candidate gene.

Primer ID.	Application	Direction	PCR primer (5'- 3')
A02	BAC PCR screen	Forward	AGGCCGATCGATGTGCAG
A05	*NLR-A* marker	Forward	CGGCGTCATGCAAAAGGG
A08	BAC PCR screen	Reverse	ACCTGGTGACCTGGTAGCT
A11	Long PCR/ *NLR-A* marker	Reverse	GATTTCCCAGACCCGGCG
A12	Long PCR	Reverse	GCGTGCCTCTGGCTATGT
B01	Long PCR	Forward	GACAGGGTAGGAGCGGGA
B05	BAC PCR screen	Forward	CAGGGAAAGCATGCCCGA
B11	Long PCR/ BAC PCR screen	Reverse	CGAAGCATCGCCTGACCT
C03	Long PCR	Forward	TGCATTCCCGCACACACA
D01	Long PCR	Forward	AAAACCGTGCCTCTCGCA
H11	Long PCR	Reverse	GCGCCTGGAATTTGTTCGT

Table A7: ANOVA analysis of *Rpst2* and chromosome 3H QTL in the AxR $F_{2:3}$ population inoculated with *P. striiformis* f. sp. *tritici*.

Trait	Chr.	Marker	F-value	*p*-value (F-statistic)	AEE[1]	DEE	DEE / AEE	PVE[2]
Mean of $F_{2:3}$ families	3H	U32_7169	12.25	1.98E-05	0.44	-0.07	-0.17	17.26
Mean of $F_{2:3}$ families	7H	U32_7356_p1	15.41	1.76E-06	-0.44	-0.26	0.60	21.71
Median of $F_{2:3}$ families	3H	U32_7169	10.51	7.93E-05	0.41	-0.14	-0.33	15.12
Median of $F_{2:3}$ families	7H	U32_7356_p1	16.37	8.64E-07	-0.44	-0.36	0.81	23.56

[1]AEE: Allelic effect estimate, negative and positive values indicate resistance is contributed by the A and B alleles, respectively.
[2]PVE: Per cent of the phenotypic variation explained.

Table A8: Annotation of Abed Binder 12 BAC clone #4931-1 11E harbouring *NLR-A.*

Name	Length (bp)
LTR/Copia BARE1_HV	3,826
DNA/CMC-EnSpm	4,601
DNA/CMC-EnSpm	536
DNA/CMC-EnSpm	1,229
DNA/CMC-EnSpm	5,270
DNA/CMC-EnSpm	1,098
Low complexity A-rich	94
DNA/CMC-EnSpm	2,396
DNA/CMC-EnSpm	1,111
Unknown REP1_SB	52
LTR/Copia IKEROS_HV	5,817
Satellite TREP106	253
LINE/L1 LINE1-56_SBi	669
LINE/L1 LINE1-55_SBi	213
DNA/TcMar-Stowaway ICARUS_TM	88
NLR-A	3,723
DNA-8-1_TA	138
Low complexity	89
Low_complexity	34
LTR/Gypsy SUKKULA1_HV-LTR	753
LTR/Copia WIS2_TM-int	1,230
LTR/Copia BARE-2_HV_LTR	1,356
LINE/L1 LINE1-61_SBi	98
LTR/Copia BARE1_HV	8,113
LTR/Copia BARE1_HV	8,945
LINE/L1 LINE1-20_SBi	87
LINE/L1 LINE1-47_SBi	86
DNA/CMC-EnSpm	49
DNA/TcMar-Stowaway	145
DNA/PIF-Harbinger HARB-5_SBi	911
Satellite TREP106	531
Satellite TREP106	535
Satellite TREP106	107
NLR-D	2,700
LTR/Copia Copia-8_PD-I	52
Low_complexity GA-rich	49
LINE/L1 L1_TD	325
LINE/L1 L1_TD	976
LTR/Copia BARE-2_HV_LTR	1,811
LTR/Copia BARE-2_HV	8,586
LTR/Copia BARE-2_HV_LTR	1,811
LTR/Gypsy Gypsy-12_TA-LTR solo	795
DNA IR12_TM	17
MLOC.19985.1	1,212
LTR/Copia WIS2_TM-LTR	1,753
LTR/Copia Angela	8,804

LTR/Copia WIS2_TM-LTR	1,762
LTR/Copia Copia1_HV-int	3,877
LTR/Copia Copia1_HV-LTR solo	325
DNA/PIF-Harbinger	250
MLOC_41646.1	296
DNA/CMC-EnSpm EnSpm-N3_TA	489
DNA/CMC-EnSpm EnSpm-21_SBi	133
LTR/Copia Copia19-ZM_I-int	212
MLOC_8985.1	462
DNA/CMC-EnSpm	263
DEIMOS	275
DNA/TcMar-Stowaway	258
NLR-E	3,936

Table A9: Amino acid and DNA similarity between full length *NLR-A*, *NLR-D*, and *NLR-E*.

		Whole length (aa) % similarity				
		NLR-A	**NLR-B**	**NLR-C**	**NLR-D**	**NLR-E**
Whole length (gDNA) % similarity	**NLR-A**		-	35	58	63
	NLR-B	-		-	-	-
	NLR-C	49	-		33	37
	NLR-D	67	-	49		67
	NLR-E	70	-	50	73	

Table A10: Amino acid similarity for NBS and LRR domains of *NLR-A*, *NLR-D*, and *NLR-E*.

		NBS domain (aa) % similarity				
		NLR-A	**NLR-B**	**NLR-C**	**NLR-D**	**NLR-E**
LRR domain (aa) % similarity	**NLR-A**		-	49	75	80
	NLR-B	-		-	-	-
	NLR-C	38	-		44	48
	NLR-D	59	-	41		86
	NLR-E	57	-	49	63	

Table A11: Accessions of barley used for inoculation with *P. striiformis* f. sp. *hordei* in chapter 6.

Accession	Gene	McNeal	Reaction Type[1]
HOR 1428	*RpsHOR1428-1, RpsHOR1428-2*	1	R
Abed Binder 12	*rps2*	1	R
HOR 2926	*rps1.a, rpsHOR2926*	1	R
HOR 3209	*rpsHOR3209-1, rpsHOR3209-2*	2	R
I 5	*rps3, rpsI5*	2	R
Grannenlose Zweizeilige	*rpsGZ*	2	R
Prisma		2	R
mlo11 BC Ingrid	*rpsGZ*	2	R
1000 resistant	*rpsGZ*	2	R
1010 susceptible	*rpsGZ*	2	R
West China		2	R
Hiproly	*rpsHi1, rpsHi2*	3	R
Mazurka	*rps1.c*	3	R
Benton		3	R
L94		3	R
Cebada Capa		4	I
mlo5 ror2 A44		4	I
Barke		4	I
Black Hull-less		5	I
Golden Promise		5	I
Varunda	*rpsVa1, rpsVa2*	5	I
Trumpf	*rpsTr1, rpsTr2*	5	I
Apex		5	I
Haisa		5	I
Pallas		5	I
Pallas		5	I
CI 16137		5	I
CI 16154		5	I
CI 16155		5	I
Multan		5	I
Steptoe		6	I
Zephyr		6	I
20007		6	I
G-88		6	I
Abyssinian 14	*rpsA14-1, RpsA14-2*	6	I
Bigo	*rps1.b*	6	I
Q21861		6	I
Carlsberg II		6	I
Riso 5678 (Carlsberg II)		6	I
Pallas		6	I
CI 16138		6	I
CI 16140		6	I
CI 16151		6	I
CI 16156		6	I
Ingrid		6	I
mlo3 BC Ingrid		6	I

mlo4 BC Ingrid		6	I
mlo5 BC Ingrid		6	I
Algerian		6	I
Franger		6	I
Hanna		6	I
Psaknon		6	I
Long Glumes		6	I
Rupee		6	I
Spiti		6	I
H602		6	I
Astrix	*Rps4, rpsAst*	7	S
Honan wang ta mai		7	S
Emir	*rpsEm1, rpsEm2*	7	S
Riso 6018 (Carlsberg II)		7	S
Riso 7085 (Carlsberg II)		7	S
Riso 7372 (Carlsberg II)		7	S
Diamant		7	S
Pallas		7	S
CI 16139		7	S
CI 16143		7	S
CI 16144		7	S
CI 16146		7	S
Durani		7	S
Duplex		7	S
Haruna Nijo		7	S
Morex		8	S
Spontaneum I		8	S
55		8	S
32		8	S
Cambrinus	*Rps4*	8	S
Heils Franken	*Rps4, rpsHF*	8	S
Akashinriki		8	S
B5540		8	S
Foster		8	S
Vada		8	S
SusPtrit		8	S
Mutante 66 (Haisa)		8	S
Probstdorfer Vollkorn		8	S
H 3502 (Probstdorfer Vollkorn)		8	S
Malteria Heda		8	S
MC-20 (Malteria Heda)		8	S
S Z 5139B (Diamant)		8	S
Manchuria		8	S
CI 16141		8	S
CI 16142		8	S
CI 16145		8	S
CI 16147		8	S
CI 16148		8	S

CI 16149		8	S
CI 16152		8	S
CI 16153		8	S
Kwan		8	S
Nigrate		8	S
Bowman		8	S
HOR 3401		8	S
Trebi		8	S
Russell		9	S
22		9	S
Clipper		9	S
Stauffers Obersulzer	*rpsSO-1, RpsSO-2*	9	S
CI 16150		9	S

[1]R: resistant, I: Intermediate, S: Susceptible.

Table A12: Phenotypic scores of 21 key recombinant lines defining the *rps2* locus.

No. from Fig. 21	**Recom ID.**	**McNeal scores**																	**Mean**	**Classification[1]**
		Set 1 (03/03/2014)								Set (10/03/2014)										
1	AD_0040-2B1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	R
2	AD_0040-10B1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	R
3	AD_0040-14A3	1	1	1	1	0	1	1	1	1	2	1	2	1	2	2	1	-	1	R
4	AD_0040-28B5	4	6	5	4	3	3	1	3	1	7	8	2	8	8	1	1	-	4	H
5	AD_0040-14C5	0	5	1	6	4	0	0	6	3	2	1	1	1	3	8	1	-	3	H
6	AD_0040-34A4	0	4	4	5	4	6	4	3	2	6	3	4	4	5	6	4	-	4	H
7	AD_0040-6C3	3	3	5	3	8	8	1	1	4	5	3	6	3	8	1	6	-	4	H
8	AD_0040-13B5	4	2	6	4	4	6	6	8	5	4	8	5	5	1	1	5	-	5	H
9	AD_0040-25C5	8	5	5	2	4	5	4	3	8	3	2	3	3	4	6	4	-	4	H
10	AD_0040-9C4	4	6	8	6	5	-	-	-	5	2	8	6	4	2	5	-	-	5	H
11	AD_0040-22B4	3	0	0	4	5	0	3	4	5	6	7	1	6	6	5	6	-	4	H
12	AD_0040-7A1	0	4	3	5	0	3	4	4	6	4	3	3	2	2	3	3	-	3	H
13	AD_0040-2D1	8	4	1	3	2	4	3	4	8	4	5	4	4	1	9	8	-	5	H
14	AD_0040-12A5	4	4	6	3	5	5	4	1	1	2	3	3	4	4	0	1	-	3	H
15	AD_0040-10D3	6	5	6	4	5	5	5	4	5	5	5	5	6	5	5	5	3	5	H
16	AD_0040-8C4	4	5	6	5	4	6	6	6	4	4	4	4	4	8	4	4	-	5	H
17	AD_0040-1B3[2]	5	5	6	5	6	6	5	6	3	3	4	4	2	3	2	3	-	4	S
18	AD_0040-28B2	8	8	8	6	6	6	8	8	8	8	8	8	8	8	8	8	-	8	S
19	AD_0040-33B4	8	8	6	4	4	8	8	8	8	8	8	8	8	8	6	6	-	7	S
20	AD_0040-13C1[2]	5	6	6	6	5	5	6	8	3	3	4	4	0	3	3	3	-	4	S
21	AD_0040-34B2	8	8	8	8	8	8	8	8	6	6	8	8	8	8	8	8	-	8	S

[1]R: Resistant, H: Segregating, S: Susceptible.
[2]Families need to be re-tested due to poor inoculations giving low phenotypic scores.

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