

Chapter 20

Stem Rust Resistance: Two Approaches

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Abstract Stem rust, caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*), is a destructive disease of wheat that has historically caused significant yield losses in much of the global wheat production area. Over the past 50 years, stem rust has been effectively controlled by deploying cultivars carrying stem rust resistance (*Sr*) genes. With the emergence of new *Pgt* races, namely Ug99 and its variants, there has been a reinvestment in stem rust research. This includes discovery, characterization, genetic mapping, and cloning of *Sr* genes. Here we investigated two such examples of genetic characterization and mapping of stem rust resistance. In the first example, a region on chromosome 6DS harbouring resistance to Ug99 was examined in several populations and from several sources. In the second example, a less typical genetic model of resistance was studied in which seedling resistance was activated by an independent locus exhibiting an apparent “nonsuppressing” effect. The knowledge gained by these and other lines of research will contribute to the goal of durable resistance to stem rust.

Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), is a disease of wheat that has the potential to cause devastating losses in grain yield. The last epidemic of stem rust in North America occurred from 1953 to 1955 (Peterson 1958). Since then, resistant cultivars have successfully controlled the disease.

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However, the appearance of *Pgt* race TTKSK, also known as Ug99, rendered most of the globally grown wheat cultivars vulnerable to stem rust (Singh et al. 2011; Fetch et al. 2012). The distinguishing characteristic of this race is its virulence to *Sr31*, a broadly deployed stem rust resistance (*Sr*) gene (Pretorius et al. 2000). Furthermore, Ug99 has continued to evolve and accumulate different virulence combinations (Jin et al. 2009; Park et al. 2011). This has led to renewed efforts to find and deploy new *Sr* genes (e.g. Njau et al. 2010). More generally, this threat from *Pgt* has stimulated investment in several aspects of wheat, *Pgt* and their interactions. This has also underscored the need to employ multiple strategies to achieving resistance and utilise diverse resistance. To meet these needs it is critical to understand the resistance present in breeding material and cultivars, understand the relationship between different sources of resistance (e.g. allelism, linkage, breadth of resistance, target in the pathogen etc.), and develop tools to implement responsible breeding strategies.

It is in the context of renewed and diverse investigation into the wheat stem rust host-pathogen system that this paper was presented. Two lines of research were presented: (1) comparative mapping and fine-mapping of a chromosome region conferring qualitative resistance and (2) genetic interactions that enhance stem rust resistance at the seedling stage and in field conditions across multiple environments. Here, the pertinent literature for these studies is reviewed and our preliminary findings are discussed.

Comparative Mapping of Ug99 Resistance on Chromosome 6DS

SrCad

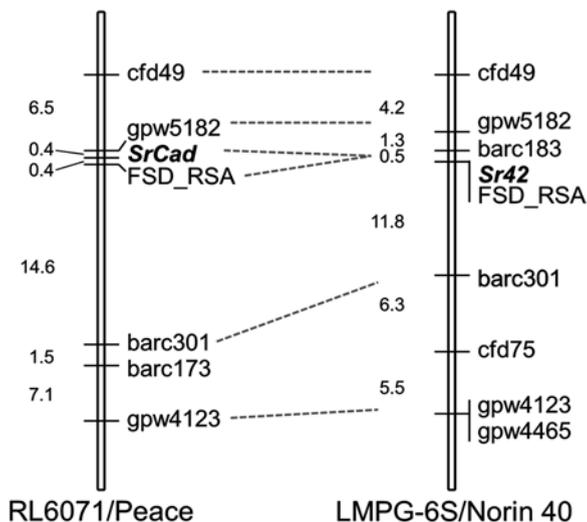
Most Canadian hexaploid cultivars are susceptible to *Pgt* races from the Ug99 race group and all of the cultivars that are grown on significant acreage are susceptible (Fetch et al. 2012). A screen of Canadian cultivars in Njoro, Kenya, where Ug99 races predominated, revealed that two hard red spring wheat cultivars, Peace and AC Cadillac, showed high levels of resistance. Peace and AC Cadillac are closely related cultivars and were believed to carry the same source of resistance to Ug99. Two populations were generated to study the inheritance and determine the chromosome location of the resistance (Hiebert et al. 2011). A doubled haploid (DH) population from the cross RL6071/Peace and an F₂ population from the cross LMPG-6S/AC Cadillac both showed that a single gene was responsible for the Ug99 resistance detected at the seedling stage. Mapping with SSR markers showed that the resistance in both populations mapped distally on chromosome 6DS in the same genetic interval. A gene for common bunt resistance, *Bt10*, has been mapped to a similar chromosome region (Menzies et al. 2006). DNA marker FSD_RSA,

a dominant marker that was developed to select *Bt10* (Laroche et al. 2000), was added to the genetic maps of the Peace and AC Cadillac derived populations (Hiebert et al. 2011). FSD_RSA showed tight linkage to the *Sr* gene, which was given the temporary designation *SrCad*. Both AC Cadillac and Peace carry *Bt10* which was inherited from the breeding line BW553. In the Canadian Prairie Spring (CPS) class of hexaploid wheat, BW553 was a parent to several cultivars and used as a donor of *Bt10*. A survey of CPS cultivars revealed that all of the cultivars that carried the positive allele (the allele found in BW553, Peace and AC Cadillac) were also resistant to Ug99 while all other cultivars were susceptible (Hiebert et al. 2011). In the germplasm screened with FSD_RSA, the positive allele is rare and has been linked to Ug99 resistance in every instance to date (Hiebert et al. 2011; Ghazvini et al. 2012). Thus, it appears that the presence of FSD_RSA is indicative of *SrCad* though it would be too large an assumption to deem the marker diagnostic as recombinants between FSD_RSA and *SrCad* were recovered in mapping populations (Hiebert et al. 2011; Hiebert et al. unpublished data). Interestingly, the CPS cultivars that carry *SrCad* showed a lower level resistance to Ug99 in field nurseries in Njoro, Kenya compared to Peace and AC Cadillac. Both Peace and AC Cadillac carry *Lr34* while none of the CPS cultivars carrying *SrCad* carry *Lr34*. Hiebert et al. (2011) showed the combination of *SrCad* and *Lr34* improved the level of Ug99 resistance in field conditions.

Sr42

Given the map location of *SrCad*, there was interest in determining the relationship between *SrCad* and *Sr42*. *Sr42* was reported to be carried on chromosome 6DS however there were no published genetic maps. *Sr42* was found in Norin 40, a Japanese winter wheat (McIntosh et al. 1995). Using a DH population from the cross LMPG-6S/Norin 40, Ghazvini et al. (2012) mapped *Sr42* using SSR markers and FSD_RSA. *Sr42* mapped to the same genetic interval as *SrCad*. Norin 40, like Peace and AC Cadillac, carries the positive allele of FSD_RSA. Given that this marker allele is rare, it seems likely that Norin 40 and BW553, the donor of *SrCad* to all Canadian carriers, have a parent in common however no such parent could be traced. The sizes of the DH populations used to map *SrCad* (n=295; Hiebert et al. 2011) and *Sr42* (n=248; Ghazvini et al. 2012) yielded good map resolution and their respective map positions are not significantly different (Fig. 20.1). Both *SrCad* and *Sr42* conferred resistance to *Pgt* races Ug99 and RTQSC. While very limited, this does show an additional similarity between these resistances. New markers developed in ongoing fine-mapping experiments were unable to show differences in map position between *SrCad* and *Sr42* (unpublished data). It is likely that *SrCad* and *Sr42* are allelic and probably represent the same allele.

Fig. 20.1 Ug99 resistance on chromosome 6DS mapped in the RL6071/Peace (Hiebert et al. 2011) and LMPG-6S/Norin 40 (Ghazvini et al. 2012) DH populations. Genetic distances are in cM



Other Sr Genes on Chromosome 6DS that Confer Resistance to Ug99 Stem Rust

There have other *Sr* genes mapped to 6DS in a similar chromosome region as *SrCad* and *Sr42* that confer resistance to Ug99. One such example was reported by Olsen et al. (2013) where an *Sr* gene, temporarily designated *SrTA10187*, identified in *Aegilops tauschii* was transferred to common wheat and was mapped to chromosome 6DS. In that study, few DNA markers were mapped, however two of the three markers were also used to map *SrCad* (Hiebert et al. 2011). The map positions of *SrCad* and *SrTA10187* cannot be differentiated based on the data published to date. Recently, seedling resistance to Ug99 was mapped in five CIMMYT spring wheat cultivars and one US winter wheat cultivar (Lopez-Vera et al. 2014). In all six populations the resistance mapped to a similar region of chromosome 6DS however the map order varied slightly between the various maps. Whether the map differences are the result of experimental error such as misclassified progeny, or the detection of different genes is unknown presently. The authors postulate that two different genes were mapped, one that is near or allelic to *SrCad/Sr42* and one that is distal to these genes but closely linked. It was suggested that the resistance postulated to be allelic to *SrCad/Sr42* was *SrTmp* derived from the cultivar Triumph 64 and our preliminary map of *SrTmp* that would agree with this assertion. While Lopez-Vera et al. (2014) are basing their hypothesis on gene postulation experiments, we used a DH population from the cross LMPG-6S/Triumph 64 to generate a population to directly determine the map position of *SrTmp*. Using the LMPG-6S/Norin 40 and

LMPG-6S/Triumph 64 DH populations we were able to show that *Sr42* and *SrTmp* differ in the breadth of resistance they confer. Thus, even if the two are allelic, they represent different allelic forms.

Conclusion

Resistance to Ug99-type stem rust races has been mapped to chromosome 6DS in a number of studies from a number of sources. Understanding the relationships between these genes is important for developing strategies to deploy these genes. If the genes are allelic it is prudent to determine which allele is the most relevant for a given growing region and the local *Pgt* population. If the genes are non-allelic it could be worthwhile to attempt to recover recombinants carrying gene combinations to generate a gene stack carried in a tight linkage group. The DNA markers developed from the fine-mapping of *SrCad* (unpublished) could be useful in delineating the *Sr* genes mapped to this chromosome region. To further characterize these genes phenotypically, near-isogenic lines (NILs) should be produced to allow an accurate comparison of their range of effectiveness against a panel of *Pgt* races.

Chromosome 7D: Carrier of a Suppressor and a Nonsuppressor

A Suppressor of Stem Rust Resistance

Canthatch is an old Canadian hard red spring wheat cultivar that is a derivative of Thatcher (McCallum and DePauw 2008). Canthatch is susceptible to several races of *Pgt* and was used in many genetic studies by Dr. Eric Kerber (retired, AAFC, Winnipeg, Canada). In an early experiment, the A and B genome component of Canthatch was isolated to generate an 'extracted tetraploid' which had the genome constitution of $2n=4x$, AABB (Kerber 1964). The extracted tetraploid, named Tetra Canthatch, lacked vigour, had low fertility, and was morphologically different from Canthatch and common tetraploids. Furthermore, Tetra Canthatch had seedling resistance to some *Pgt* races that were virulent to Canthatch. This implied that the D genome or a component of the D genome was suppressing *Sr* genes present on the A and/or B genomes in Canthatch. Using nullisomic and ditelosomic stocks in a Canthatch background, it was demonstrated that the suppression of *Sr* genes was caused by the long arm of chromosome 7D (Kerber and Green 1980). Induced mutants showed the same phenotype as Tetra Canthatch and Canthatch ditelo 7DS. Furthermore, the suppression was caused by a single gene on chromosome 7DL (Kerber 1991). Telocentric mapping showed that the suppressor locus was independent of the centromere.

Adult-Plant Resistance Genes Can Act as a Nonsuppressor

Lr34 is a well-known adult-plant resistance (APR) gene that confers quantitative resistance to all of three of the rust disease of wheat and is located on the short arm of chromosome 7D (Dyck 1987; Singh et al. 2012). In addition to conferring APR, *Lr34* was associated with nonsuppression of seedling stem rust resistance in a Thatcher background (Dyck 1987; Kerber and Aung 1999). Comparing Canthatch, Thatcher, Tetra Canthatch, Canthatch-nulli 7D and RL6058 (a Thatcher NIL carrying *Lr34*) with four races of *Pgt*, Dyck (1987) showed that (1) Canthatch and Thatcher were susceptible to all four races of *Pgt*, (2) Tetra Canthatch and Canthatch-nulli 7D were resistant to three of these races, (3) RL6058 was resistant to the same three races as Tetra Canthatch and Canthatch-nulli 7D, and (4) one race was virulent on all five genotypes. Thus, it appears that *Lr34* negates the effect of the suppressor locus and that that the stem rust resistance expressed in Thatcher-type wheats by either removing the suppressor or adding *Lr34* is race-specific. Figure 20.2 shows a conceptual drawing of the relationship between the suppressor locus and *Lr34*, although the actual mechanisms are not presently understood.

Another Thatcher NIL, RL6077, showed the same pattern of seedling stem rust resistance as RL6058 (Dyck 1987). RL6077 carries the gene *Lr67* which is similar to *Lr34* in its phenotype and its multiple-disease resistance (Hiebert et al. 2010). A preliminary study showed that *Lr67* was responsible for the expression of seedling stem rust resistance in RL6077 and appeared to act as a nonsuppressor like *Lr34* (Hiebert et al. 2012). Thus, two unique APR genes, which both confer multiple pest resistance, also appear to act as nonsuppressors of seedling stem rust resistance genes found in Thatcher-type wheats.

We presented preliminary data in three areas to (1) assess *Lr34* as a nonsuppressor, (2) preliminary map position of seedling stem rust resistance in Thatcher expressed in the presence of *Lr34*, and (3) preliminary map position of stem rust resistance in the field that is expressed in the presence of *Lr34*. Using mutants of *Lr34* in RL6058 (Spielmeyer et al. 2013), it appears that mutant lines that have lost *Lr34* activity

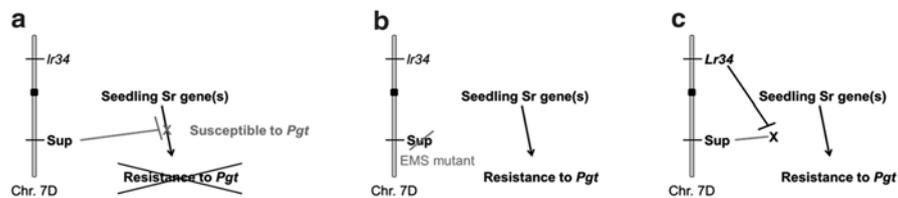


Fig. 20.2 A conceptual summary of the interaction of the suppressor (Sup) and *Lr34* on chromosome 7D. (a) The suppressor prevents the expression of seedling *Sr* genes from the A and/or B genomes of Thatcher-type wheats if the susceptible allele of *Lr34* is present. (b) Knocking out the suppressor by mutagenesis allows the expression of the otherwise suppressed *Sr* genes. (c) The resistant allele of *Lr34* appears to negate the effect of the suppressor and allows the expression of the *Sr* genes

have also lost the seedling stem rust resistance observed in the presence of a normal *Lr34* allele. Thus, we tentatively claim that it is *Lr34* that is responsible for the nonsuppressing character observed in RL6058 and not a gene tightly linked to *Lr34*. We developed two populations that are fixed for *Lr34* but segregate for Thatcher-derived seedling stem rust resistance and resistance in the field. Seedling resistance and field resistance were mapped as QTL in these preliminary analyses and chromosome 3B significantly contributed to both traits. These preliminary findings need to be confirmed and experiments are ongoing to do so.

Conclusions

One aspect of the relationship between the suppressor on 7DL and *Lr34* that is presently unknown is whether removing or inactivating the suppressor leads to the expression of the same *Sr* genes that are expressed in the presence of *Lr34*. Similarly, we do not know if *Lr67* and *Lr34* are interacting with the same genes. These lines of investigation are ongoing and should fill in the gaps in our knowledge of these interactions. From a practical standpoint, understanding these genetic interactions could be important for wheat breeders. It would be easier to retain *Sr* genes (i.e. the suppressed genes) in elite breeding material and add, for example, *Lr34* compared to the effort of introducing new or different *Sr* genes to enhance stem rust resistance in new cultivars. In Canada, Thatcher comprises a large component of coefficient of parentage for most of the widely grown hard red spring wheat cultivars (McCallum and DePauw 2008). Thus, identifying the genes in Thatcher that are expressed in the presence of *Lr34* is directly applicable.

Concluding Remarks

An array of strategies can be employed to achieve resistance to stem rust. There is not a “one size fits all” approach that must be followed. However, genetic resources for resistance to stem rust are valuable and implementing a strategy that prolongs the usefulness of *Sr* genes is the responsible approach. In order to accomplish this we must understand which genes are present in our germplasm, determine the relationships between genes and their breadths of effectiveness, develop tools for molecular breeding approaches, and unravel the genetic interactions that can lead us to more durable resistance. Here we examined two examples of the lines of research that can help geneticists, pathologists and breeders reach the goal of effective and durable resistance. With the recent investment in various aspects of wheat stem rust research more tools and resources are becoming available. It is now our responsibility to translate the advances made in the lab into rust resistant wheat cultivars in the field.

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