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The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus

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We identify the wheat stem rust resistance gene *Sr50* (using physical mapping, mutation and complementation) as homologous to barley *Mla*, encoding a coiled-coil nucleotide-binding leucine-rich repeat (CC-NB-LRR) protein. We show that *Sr50* confers a unique resistance specificity different from *Sr31* and other genes on rye chromosome 1RS, and is effective against the broadly virulent Ug99 race lineage. Extensive haplotype diversity at the rye *Sr50* locus holds promise for mining effective resistance genes.

The fungal pathogen *Puccinia graminis* f. sp. *tritici* (Pgt) causes wheat stem rust disease and is a major threat to wheat production worldwide. Although the stem rust resistance gene *Sr31* present in wheat cultivars around the world contributed significantly to controlling the disease for more than 30 years[1], this ended with the emergence of the *Sr31*-virulent Pgt race Ug99 (TTKSK) in Uganda in 1999. The subsequent spread of Ug99 and derivative races to other parts of Africa and the Middle East[2] and the recent appearance of another widely virulent race in Germany and Ethiopia (http://rusttracker.cimmyt.org/?page_id=40) has highlighted the continuing need for new resistance sources to protect against this devastating pathogen.

Cereal rye (*Secale cereale*) has been the source of several widely used stem rust resistance (*Sr*) genes, including *Sr31*, which was introgressed into wheat as a translocation of the short arm of rye chromosome 1 (1RS) from the cultivar Petkus[3]. *Sr50* (previously known as *SrR*), also on 1RS, was introgressed from the rye cultivar Imperial[4,5], while a third 1RS gene known as SrR^{Amigo} came from rye cultivar Insave[6]. Although SrR^{Amigo} containing lines were reported to confer resistance to Ug99 races[7], the specificities of these rye 1RS genes were not clearly differentiated previously. We therefore tested the reactions of lines carrying these genes, as well as mutants of *Sr31* and *Sr50*, to various Pgt races (Supplementary Tables 1 and 2). Federation*4/Kavkaz, carrying *Sr31*, was resistant to North American races QFCSC and TPMKC and Australian race 98-1,2,3,5,6, whereas an *sr31* mutant in this genetic background was susceptible, confirming resistance was due to this single gene. *Sr50*-containing derivatives of Australian wheat variety Gabo were also resistant to these races and additionally to TTKSK, whereas an *sr50* mutant showed similar infection phenotypes to Gabo, including full susceptibility to 98-1,2,3,5,6. We also identified a mutant of this race virulent to *Sr50* (98-1,2,3,5,6 +*Sr50*) and found that the *Sr31* line, but not the *sr31* mutant, retained resistance to this strain (Supplementary Tables 1 and 3), thus clearly distinguishing these resistance specificities. SrR^{Amigo} was distinguished from *Sr31* and *Sr50* by its susceptibility to races TRTTF and TKKTP, while race QCMJC was virulent only for *Sr50* (Supplementary Table 2). Thus, *Sr50* encodes a different stem rust resistance specificity to other 1RS-derived genes.

We previously showed that the *Sr31* and *Sr50* loci are associated with a cluster of CC-NB-LRR genes[5,8] orthologous to the barley *Mla* powdery mildew resistance gene locus[9]. A probe derived from *Mla1* detected multiple hybridizing sequences at the *Sr50* locus and several *Sr50* mutants contained small deletions within this gene cluster[5]. To identify the deleted genes, we first screened a lambda phage genomic DNA library from an *Sr50* line and isolated clones corresponding to *Dra*I restriction fragments absent in the mutant, one of which encoded a near full-length intact candidate CC-NB-LRR gene (Supplementary Fig. 1). Next we screened a 1RS chromosome-specific bacterial artificial chromosome (BAC) library[10] and identified 175 BAC clones that were placed into six non-overlapping contigs by fingerprinting. Polymerase chain reaction (PCR) screening of BACs forming the combined minimum tiling paths of these contigs detected the candidate gene sequence in BAC p2D7 (Supplementary Fig. 2). Using BAC-end markers to screen the *sr50* mutant M2 (ref. 5), we identified three adjacent BAC clones, including p2D7, that spanned the deleted region (Fig. 1a). Sequencing these clones and the overlapping and adjacent BACs p2E7 and p2C7 identified seven related CC-NB-LRR genes, designated *ScRGA1-A* to *-G*, six of which occur in the deleted region (Fig. 1A and Supplementary Fig. 3).

Two ethyl-methanesulfonate-derived *sr50* mutants (M7 and M13) retained the *Mla*-related fragments[5]. When intercrossed, these lines produced no resistant progeny (Fig. 1b), indicating that they carried mutations in the same gene. Amplification and sequencing identified frameshift mutations in *ScRGA1-A* in each of the mutants (Fig. 1c), while *ScRGA1-B* to *-G* genes were unaltered. To confirm that *ScRGA1-A* confers *Sr50* resistance, two genomic DNA fragments of 7.9 and 9.7 kb containing this gene were separately transformed into the susceptible wheat cv. Fielder. Independent T0 transgenic lines containing either construct showed resistance to Pgt, while lines that lacked the transgene were susceptible (Fig. 2 and Supplementary Fig. 4). T1 progeny of four resistant transgenic lines segregated 3:1 for resistance to stem rust but were uniformly susceptible to *P. triticina* (causal agent of wheat leaf rust) and

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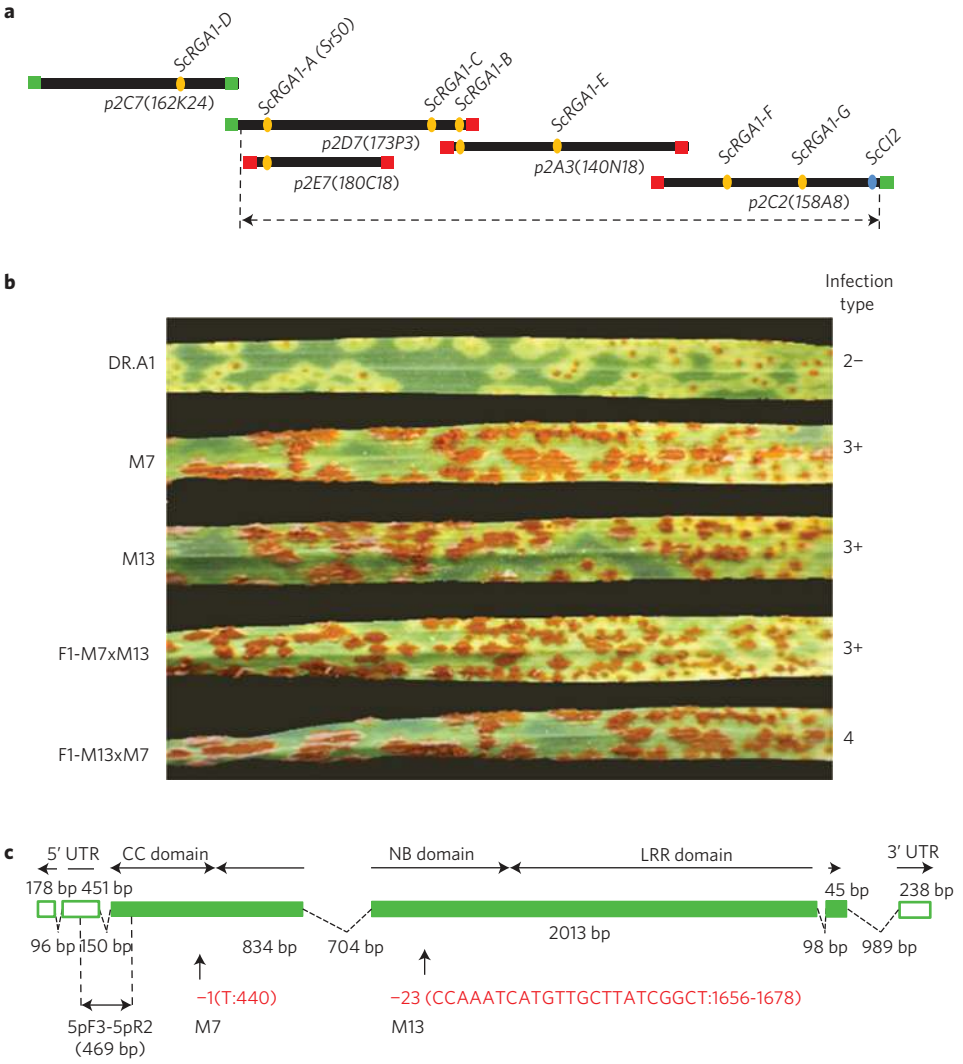

Figure 1 | *Sr50* locus and mutants. a, Five BAC clones spanning the deletion (dotted line) in mutant M2 are shown with deleted end sequences indicated in red and those retained in green. Orange ovals represent *ScRGA1* gene family members, and a predicted chymotrypsin inhibitor gene (*ScCI2*) is shown in blue. **b**, Stem rust infection phenotypes 14 days after inoculation of Gabo 1DL.1RS-DR.A1, mutants M7 and M13, and their F1 progeny. See ref. 15 for a description of infection types. **c**, Structure of the *Sr50* (*ScRGA1-A*) gene including the coding region (solid green) and 5′ and 3′ untranslated regions (UTRs, open green boxes), and the position of the M7 and M13 mutations.

P. striiformis f. sp. *tritici* (causal agent of wheat stripe rust) (Supplementary Table 4), confirming that resistance is specific and not due to general enhancement of defence pathways. The combined mutation and complementation data identified *ScRGA1-A* as *Sr50*.

The *Mla* locus of barley is highly diverse, encoding more than 30 different resistance specificities[11] and homologues at this locus in wheat include the wheat powdery mildew resistance gene *TmMla1*[12] and the stem rust resistance gene *Sr33* (ref. 13). These genes occur in small clusters of about five paralogues in wheat and barley but this has expanded in rye to over 20 paralogues on 1RS ref. 5). Phylogenetic analysis of cereal Mla homologues placed the Sr50 protein in a clade with the other ScRGA1 sequences and the wheat-derived homologues TmMla1 and Sr33 (Supplementary Fig. 5). The *Sr33* resistance specificity was clearly distinguished from *Sr50* by its effectiveness against race 98-1,2,3,5,6 +*Sr50* (Supplementary Table 1).

We screened 114 geographically diverse rye accessions by PCR with primers flanking *Sr50* and could amplify related sequences from only 10 accessions (Supplementary Table 5 and Supplementary Fig. 6). In five accessions this gene was identical to *Sr50*, while three contained small substitutions and two were disrupted by sequence inversion (Supplementary Fig. 6). All these accessions except Dwarf Petkus R1 showed resistance to several races of stem rust, including the *Sr50*-virulent mutant (Supplementary Table 6), indicating the presence of additional *Sr* genes, which may be on other chromosomes. Despite the sequence similarity of the *Sr50* gene in most of these accessions, they contained diverse haplotypes of this complex locus (Supplementary Fig. 7), suggesting extensive recombination within this cluster in rye. Both *Sr31* and stripe rust resistance gene *Yr9* occur in the same 1RS region[8], and may also belong to this gene family. Thus this locus appears to be a hotspot for evolution of resistance specificities in cereals, including wheat, barley and rye, and the high diversity in rye shows promise for mining additional *Sr* genes.

Stem rust remains the major disease threat to wheat production because of its frequent evolution of new virulence and the devastation it causes, with near complete crop losses in severe epidemics. *Sr50* is effective against a broad range of worldwide Pgt races and is the first stem rust resistance gene cloned from the tertiary gene pool of wheat (that is, outside of wheat and its A, B and D genome progenitors). This advance will allow introgression of *Sr50* into new wheat varieties separately from deleterious rye

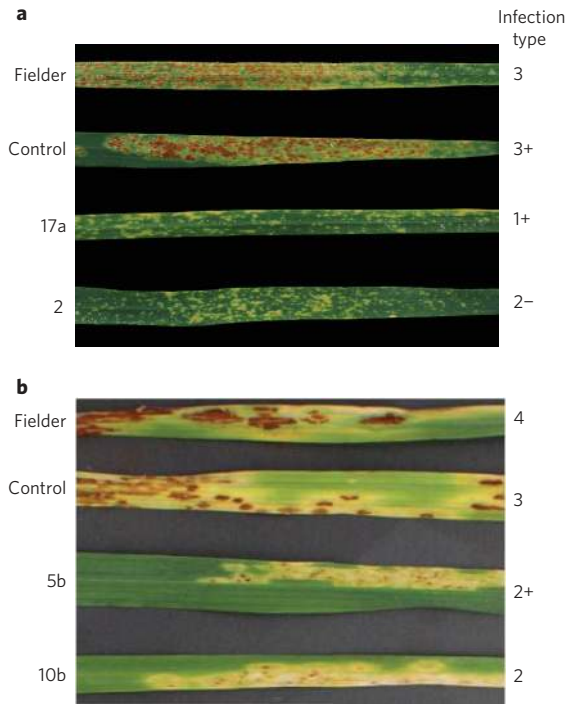

Figure 2 | *Sr50* transgenic lines express resistance to stem rust. Wheat cv. Fielder, two T0 *Sr50* transgenic lines and a non-transgenic control line were inoculated with Pgt race 98-1,2,3,5,6. **a**, pVecBar-*Sr50* lines 17a and 2. **b**, pVecNeo-*Sr50* lines 5b and 10b. See ref. 15 for a description of infection types.

sequences and pyramiding of this gene with other broadly effective *Sr* genes either through conventional breeding or by transgenic delivery to provide durable stem rust control. The isolation of a Pgt mutant virulent to *Sr50* also opens the way to identify the corresponding pathogen avirulence gene by genome sequencing[14].

Methods

Plant material and rust phenotyping. Wheat lines Gabo1BL.1RS and Gabo1DL.1RS-DR.A1 containing *Sr50* introgressions and their derived mutants M2, M7 and M13 have been described previously[5]. The Gabo1BL.1RS translocation was backcrossed into Federation five times to generate Federation*5/Gabo1BL.1RS-1-1 containing *Sr50* in the absence of Gabo background genes giving resistance to North American and African rust isolates. A mutant Pgt strain with virulence for *Sr50* (98-1,2,3,5,6 +*Sr50*; Plant Breeding Institute accession number 130176) was isolated from a single pustule observed after infection of an *Sr50* line with strain 98-1,2,3,5,6 (no. 781219). Infection of a set of differential wheat lines confirmed it was identical to the parental isolate but with virulence to *Sr50* (Supplementary Table 2). For rust infection assays, 1-week-old seedlings were inoculated with Pgt races 34-2,4,5,7,11, (no. 760785); 34-2,12,13, (no. 840552); 126-5,6,7,11, (no. 334), TTKSK (04KEN156/04, Ug99), TTKST (06KEN19v3, Ug99+*Sr24*), QFCSC (03ND76C), TPMKC (74MN1409), TRTTF (06YEM34-1), TKKTP (13GER16-1), QCMJC (07WA140-515) *P. triticina* race 104-2,3,(6),(7),11 (no. 890172) and *P. striiformis* f. sp. *tritici* race 110 E143 A+ (no. 861725). Infection types were scored as described in ref. 15.

DNA library construction and screening. Previously, we found that several *Sr50* mutants had lost two *Dra*I fragments of 10 and 12 kbp detected by hybridisation with the Mla-LRR probe B76 (ref. 5). Therefore, DraI digested genomic DNA fragments of 9–13 kb from Gabo1BL.1RS were purified by agarose gel electrophoresis, ligated to *BamH*I adaptors and cloned into EMBL3 λ-*BamH*I vector (Epicentre Technologies) and packaged using MaxPlax (Epicentre Technologies). A chromosome-specific BAC library made from Imperial rye chromosome 1RS flow-sorted from a wheat-rye ditelosomic addition line carrying *Sr50* in the background of wheat cv. Chinese Spring has been described previously[10]. Libraries were screened by hybridisation with probe B76 and positive clones were purified. BAC DNA was isolated using a modified alkaline lysis protocol[16] and aligned into six contigs of 2, 3, 11, 24, 71 and 72 clones based on high-information content BAC fingerprinting[17]. BAC clones were screened by PCR with primers (Sr50-5p-F3/R2) specific to the candidate gene. BAC end sequences were obtained by direct Sanger sequencing of clones and whole BACs were sequenced using the Roche 454 platform. Reads were assembled using Newbler v2.3, wheat repeat sequences (http://wheat.pw.usda.gov/ITMI/Repeats/blastrepeats3.html) masked and genes predicted using FGENESH (www.softberry.com) and GENSCAN (http://genes.mit.edu/GENSCAN.html). Nucleotide sequences of *ScRGA1-A* to *G* were submitted to GenBank (accession numbers KT725812 to KT725818).

PCR amplification. All primers used for PCR are listed in Supplementary Table 7. Amplified sequences were compared for nucleotide variations using CLUSTAL (http://www.ebi.ac.uk/Tools/sequence.html). Two predicted introns in *Sr50* were confirmed by PCR with reverse transcription and 5′ and 3′ untranslated regions (UTRs) were detected by rapid amplification of cDNA ends as described[12].

Wheat transformation. The pVecBarSr50 construct contained a 7.5 kbp fragment including 2.4 kbp upstream and 1.4 kbp downstream regions amplified from Gabo1DL.1RS genomic DNA using primers Sr50-F1/R1 followed by nested primers Sr50-F2/R2 with PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies). The pVecNeoSr50 construct contained a 9.8 kbp NotI fragment from BAC clone p2E7 including 4.2 kbp upstream and 1.9 kbp downstream regions. The respective binary vectors pVecNeo and pVecBarII are derivatives of pWBvec8 (ref. 18), in which the 35S hygromycin gene was replaced by a 35S NPTII gene from pCMneoSTL2 (ref. 19) or the bialaphos resistance gene (bar). Wheat cv. Fielder was transformed using the *Agrobacterium tumefaciens* strain GV3101 (pMP90) as described[20]. T0 and T1 transformants were tested for the presence of transgenes by DNA gel blot hybridization as described[5].

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References

1. Ellis, J. G., Lagudah, E. S., Spielmeyer, W. & Dodds, P. N. *Front. Plant Sci.* **5,** 641 (2014).
2. Singh, R. P. *et al. Annu. Rev. Phytopathol.* **49,** 465–481 (2011).
3. Zeller, F. J. in *Proc. 4th International Wheat Genetics Symposium* (eds Sears, E. R. & Sears, L. M. S.) 209–221 (Univ. Missouri, 1973).
4. Shepherd, K. W. in *Proc. 4th International Wheat Genetics Symposium* (eds Sears, E. R. & Sears, L. M. S.) 745–760 (Univ. Missouri, 1973).
5. Mago, R., Spielmeyer, W., Lawrence, G. J., Ellis, J. G. & Pryor, A. *Genome* **47,** 112–121 (2004).
6. Zeller, F. J. & Fuchs, E. Z. *Z PflZücht* **90,** 285–296 (1983).
7. Jin, Y. & Singh, R. P. *Plant Dis.* **90,** 476–480 (2006).
8. Mago, R. *et al. Theor. Appl. Genet.* **112,** 41–50 (2005).
9. Wei, F. *et al. Genetics* **153,** 1929–1948 (1999).
10. Šimková, H. *et al. BMC Genomics* **9,** 237 (2008).
11. Seeholzer, S. *et al. Mol. Plant Microbe Int.* **23,** 497–509 (2010).
12. Jordan, T. *et al. Plant J.* **65,** 610–621 (2011).
13. Periyannan, S. *et al. Science* **341,** 786–788 (2013).
14. Upadhyaya, N. *et al. Front. Plant Sci.* **5,** 759 (2015).
15. McIntosh, R. A., Wellings, C. R. & Park, R. F. *Wheat Rusts: An Atlas of Resistance Genes* (CSIRO Publishing, 1995).
16. Sinnett, D., Richer, C. & Baccichet, A. *BioTechniques* **24,** 752–754 (1998).
17. Luo, M.-C. *et al. Genomics* **82,** 378–389 (2003).
18. Wang, M.-B., Matthews, P. R., Upadhyaya, N. M. & Waterhouse, P. M. *Acta Hortic.* **461,** 401–405 (1998).
19. Maas, C. *et al. Mol. Breeding* **3,** 15–28 (1997).
20. Richardson, T., Thistleton, J., Higgins, T. J., Howitt, C. & Ayliffe, M. *Plant Cell Tis. Org. Culture* **119,** 647–659 (2014).

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Author contributions

R.M., J.E., P.D. and E.L. conceived and planned the work. R.M., P.Z., S.V., H.S., U.B., M.-C.L., M.R., H.K., S.P., J.K., Y.J., M.A., H.S.B., R.F.P., R.McI., J.D. and H.B. performed the experiments and analysed the data. R.M. and P.D. wrote the manuscript and all co-authors commented on the draft.

Additional information

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Competing interests

The authors declare no competing financial interests.