"Le Sélectionneur Français" 2005 (56), **3-14**

CLONAGE POSITIONNEL DU GENE DE RESISTANCE LR10 CHEZ LE BLE TENDRE : STRATEGIES, ENSEIGNEMENTS ET PERSPECTIVES

MAP-BASED ISOLATION OF THE LEAF RUST DISEASE RESISTANCE GENE *LR10* FROM THE HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.) GENOME

Catherine FEUILLET,

UMR GDEC, INRA Site de Crouël 234 avenue du Brézet
63100 CLERMONT-FERRAND

1. INTRODUCTION

During the past decade, a number of disease resistance genes have been isolated from model plant species such as *Arabidopsis thaliana* and rice or from diploid crop plants such as tomato and barley (for recent reviews see 1, 2). In many cases, gene isolation was performed through map-based cloning. This requires the development of high-density genetic maps and the possibility to perform chromosome walking on large genomic fragments. Until recently, such positional cloning has been limited to small genomes and it remained very difficult in large (> 5000 Mb) and repetitive (>80%) genomes such as those of barley and wheat. *Mlo*, the first barley disease resistance gene isolated by map-based cloning (3) was identified through the use of a YAC library and the subsequent construction of a BAC library from the YAC clone spanning the resistance locus. More recently, the construction of a BAC library from the barley cultivar Morex (4) has greatly facilitated the map-based isolation of the powdery mildew resistance genes *Mla1* and *Mla6* (5, 6) as well as the stem rust resistance gene *Rpg1* (7). In wheat, BAC libraries have been constructed from the diploid species *Triticum monococcum* (8) and *Aegilops tauschii* (9) which genomes are related to the A and D genome of hexaploid wheat, respectively. The *T. monococcum* DV92 library has been used to isolate *VRN1*, a gene controlling vernalization response in *T. monococcum* (10) as well as a candidate gene for the *Q* gene which confers free-threshing character to domesticated wheat (11). Very recently, Huang *et al.* (12) have isolated the *Lr21* resistance gene introgressed on chromosome 1DS, using a cosmid library from the *Ae. tauschii Lr21* donor line.

Many genes of agronomic interest including more than 50 leaf rust (*Lr*) disease resistance genes have been characterized by genetic analysis in hexaploid wheat. The leaf rust resistance gene *Lr10* originates from hexaploid wheat and is located on chromosome 1AS (13). In the absence of BAC or cosmid libraries from a wheat variety containing *Lr10*, cloning of the complete *Lr10* resistance locus was not possible from hexaploid wheat. Therefore, we have developed a subgenome chromosome walking strategy in which genetic mapping was performed in a hexaploid wheat population segregating for the *Lr10* resistance and chromosome walking was done using BAC clones from the diploid *T. monococcum* DV92 (14). In one step of chromosome walking, a *T. monococcum* DV92 physical contig of 280 kb spanning the *Lr10* locus in hexaploid bread wheat was established. Sequencing of 211 kb

from this contig revealed the presence of two resistance gene analogs (RGAs), *rga1* and *rga2*, in a region that showed complete linkage to *Lr10* in *Triticum aestivum* (14, 15).
Here, two genes (*T10rga1* and *T10rga2-1A*) orthologous to the *T. monococcum* genes *rga1* and *rga2* were isolated from the *Lr10* donor line Thatcher*Lr10*. Haplotype studies in the wheat gene pool showed that these two genes are the best possible candidates for *Lr10*. Mutational analysis and stable transformation of the candidate genes demonstrated that *T10rga1* is the *Lr10* resistance gene. Our data show that map-based cloning of genes of agronomic interest is feasible from hexaploid wheat using a combination of subgenome chromosome walking and haplotype studies.

2. MATERIAL AND METHODS

1.1 Mutant screening.

3,000 seeds of the parental line Thatcher*Lr10* were treated with 0.35% Ethyl methanesulfonate (EMS). 52,000 M2 seedlings were then artificially infected with the leaf rust (*Puccinia triticina*) isolate (*AvrLr10*) 89-201 CBTB(TX) (13) using a large scale infection procedure modified from Schachermayr *et al.* (16). Thirty-three putative mutants were grown to the next generation and re-assessed for susceptibility by artificial infection. Five susceptible fertile mutants were crossed with the susceptible cv. Frisal and three of them (EMS_19, EMS_25 and EMS_31) resulted in susceptible F1 progeny suggesting that the mutation is in the *Lr10* gene.

1.2 PCR Amplification of the *T10rga1* and *T10rga2-1A* genes and RT-PCR.

Three overlapping fragments spanning the *T10rga1* gene were amplified by PCR on 50 ng of genomic DNA extracted from the three EMS mutants and from the hexaploid wheat line Canadian3842 using the following primer combinations: ThLr10_T (5'-CTGAGTGAGCATGAGCAAC –3') and ThLr10_P (5'-TGGAATTGAGACAGTACAC-3'), ThLr10_E (5'-AGCCCTAATATGGCAACC-3') and ThLr10_H (5'-TGTAGAACCGTGCCTTAC-3'), ThLr10_G (5'- GCTCTTCTAACGGGGATC-3') and ThLr10_J (5'- CATCTCTTGAAAGCTCC-3'). Four overlapping fragments spanning *T10rga2-1A* were amplified by PCR using the specific primer combinations: Rga2_F (5'-GATGGAGACGACGGTGCT-3') and Rga2_U (5'-CAACTGCTTGTGATCTGGT-3'), Rga2_V (5'-GAAGCCGGATTATAGTGTCA-3') and Rga2_W (5'-CTGCCCAGCTAAGTTCTTG-3'), Rga2_X (CAATTGTGATGAACTCCTCA-3') and Rga2_N (5'-AGGTGACAGATAGATTCAC-3'), Rga2_K (5'-CTTCTGCGAGTGCTGGAC-3') and Rga2_E (5'-TTCATAGCTCATTGCATC-3'). A second PCR amplification was performed for the regions where point mutations were identified to confirm that they did not result from *Taq* polymerase errors. RT-PCR reactions were performed on 3µg polyA$^+$ RNA using the primer combinations ThLr10_D (5'- GTCAAGATCCCGTATCAG-3')/ThLr10_H or ThLr10_G/ ThLr10_H for *T10rga1* and the specific primers Rga2_V/Rga2_W for *T10rga2-1A*.

2.3. Biolistic transformation of the susceptible wheat Bobwhite S 98 56.

A 4.4 kb genomic fragment containing the entire *T10rga1* coding region, 21 bp upstream sequence and 380 bp downstream sequence, was cloned under the control of the maize ubiquitin promoter using the strategy described in Clausen *et al.* (17) to generate the plasmid *pUbi_T10rga1*. *pUbi_T10rga2-1A* which contains a 3.5 kb *T10rga2-1A* full length cDNA with 65 bp upstream and 203 bp downstream of the coding sequence was generated using a

similar strategy. A total of 350 immature embryos of the Bobwhite accession SH 98 56 (18) were cotransformed with *pUBi_T10rga1*, *pUbi_T10rga2-1A* and a plasmid containing the selectable phosphomannose isomerase (*pmi*) marker (19) using the PDS-1000/He biolistic→ particle delivery System (BIO-RAD). Regeneration and selection of the transformed plants were performed as described in (18, 19). Eight independent T0 transgenic lines containing either the two transgenes or only one of them were obtained. T1 transgenic plants were artificially infected with the leaf rust isolate TCB/TD. This isolate is avirulent on *Lr10* and in contrast to the 89-201 CBTB(TX) isolate, it is virulent on Bobwhite SH 98 56. The number of *T10rga1* transgene integration events in the T1 transgenic plants was analyzed by Southern hybridization (13). Expression of the *T10rga1* transgene was analyzed by Northern analysis (13) with 20 μg of total RNA extracted from the T1 transgenic plants.

3. RESULTS

1.3 Isolation of two candidate genes for *Lr10* in hexaploid wheat.

Two resistance gene analogs, named *rga1* and *rga2*, have been previously identified in a genomic region of *T. monococcum* DV92 spanning the *Lr10* resistance locus in *T. aestivum* Thatcher*Lr10* (14, 15). To test whether these genes are the best candidate genes for *Lr10*, we have analyzed the *Lr10* locus at the molecular level in the wheat gene pool (25). Southern analysis of 113 wild and cultivated diploid and polyploid wheat lines has revealed the presence of two characteristic haplotypes on chromosome 1AS. Haplotype H1 is defined by the presence of the two full-length *rga1* and *rga2* genes whereas in haplotype H2, *rga1* is absent and only a truncated LRR domain of *rga2* can be detected (25) (Fig. 1A). In a survey of 56 hexaploid European wheat breeding lines, only 8 lines had the H1 haplotype indicating that the H2 haplotype is predominant in *T. aestivum* (Fig. 1B). Moreover, all the lines of the H2 haplotype showed identical hybridization patterns at the *Lr10* locus, *i.e.* they lack *rga1* and a single fragment hybridizes with the 3' end of the LRR domain of *rga2* on chromosome 1A (Fig. 1B). These data suggest that there is very little variability within the H1 and H2 haplotypes and that there are possibly no other haplotypes at the *Lr10* locus in the hexaploid wheat gene pool. Therefore, molecular analysis of these two haplotypes should allow the identification of the complete set of *Lr10* candidate genes.

With the exception of the variety Canadian3842 (Can3842), the H1 lines, including Thatcher*Lr10*, were resistant to leaf rust isolates avirulent on *Lr10* (*AvrLr10*) (data not shown). To identify all candidate genes possibly present at the *Lr10* locus in Thatcher*Lr10*, we have characterized the H1 and H2 haplotypes in more detail at the molecular level. Two BAC contigs were established at the *Lr10* locus in the tetraploid *T. durum* cv. Langdon (H1 haplotype) and in the *T. aestivum* cv. Renan (H2 haplotype) (unpublished data). Low-pass sequencing of the two BACs revealed the presence of the chromosome condensation factor (CCF), nodulin-like (NLL) and actin (ACT) genes which were already identified at the *Lr10* locus in *T. monococcum* DV92 (H1 haplotype) (15). It also confirmed the complete deletion of the *rga1* sequence and the presence of a truncated LRR domain of *rga2* in the Renan H2 haplotype (data not shown). These studies did not provide additional candidate genes to *rga1* and *rga2* in the physical interval between markers flanking *Lr10* in hexaploid wheat. Therefore, we conclude that *rga1* and *rga2* of haplotype H1 are the only promising candidate orthologs for the *T. aestivum* leaf rust resistance gene *Lr10*.

The *T. monococcum* r*ga1* and *rga2* genes were used as probes to isolate the orthologous genes *T10rga1* and *T10rga2* from a λ library of the resistant hexaploid wheat variety Thatcher*Lr10*.

The *T10rga1* gene (AY270157) has a length of 3,935 bp and contains one intron of 1,171 bp which, as in most cereal RGAs, is located in the NBS domain at the N-terminal end of the Kinase-2 motif (26). The gene encodes a Coiled-Coil (CC)- Nucleotide Binding Site (NBS)-Leucine Rich Repeat (LRR) (CNL) protein of 919 AA with 14 imperfect LRRs at the C-terminus (Fig. 2). The length of the CC, NBS, spacer and LRR domains as well as the type of amino acid residues contained in the different motives indicate that T10RGA1 is related to the CNL-C type of proteins (22). By Southern hybridization it was found that *T10rga1* is a single copy gene on chromosome 1AS in Th*Lr10*. For *rga2*, two homoeologous genes, *T10rga2-1A* and *T10rga2-1D*, were identified on chromosomes 1A and 1D, respectively. The *T10rga2-1A* gene (AY270159) is 4,756 bp long and has two introns of 944 and 310 bp. It encodes a CC-NBS-LRR protein of 1,169 AA that is unrelated to T10RGA1. Linkage analysis in a population of 3,120 F2 plants showed that *T10rga1* and *T10rga2-1A* are both completely linked to *Lr10*. RT-PCR analysis demonstrated that both genes are expressed in the resistant variety Thatcher*Lr10* (Fig. 5A, which is published as supporting information on the PNAS web site) and are not induced upon leaf rust infection (Fig. 5B).

3.2. Three independent mutations affecting *T10rga1* lead to susceptibility to leaf rust carrying *AvrLr10*.

Three independent *lr10* mutants (T10_EMS19, T10_EMS25 and T10_EMS31) were identified and characterized at the molecular level. The *T10rga1* and *T10rga2-1A* genes were amplified by PCR from the mutants and their sequence compared to the sequences of Thatcher*Lr10*. In all mutants, point mutations were detected in *T10rga1* but not *T10rga2-1A*. In *T10rga1_EMS19*, a C to T transition at position 151 resulted in a change of the last leucine residue in the putative coiled-coil domain into a phenylalanine residue (Fig. 2). In *T10rga1_EMS25*, a G to A transition at position 608 changed a glycine into an arginine residue in the third conserved glycine of the P-loop motif in the nucleotide binding site (Fig. 2). A similar transition was detected at position 3,461 in the LRR domain of the *T10rga1_EMS31* sequence. It introduced a stop codon instead of a tryptophan residue at the end of the 9th leucine rich repeat in the LRR domain (Fig. 2). Thus, three independent mutations affect the *T10rga1* gene at different positions and result in a loss of resistance against leaf rust, demonstrating that *T10rga1* is the *Lr10* gene. In addition, these data indicate that the last five LRR are required for *Lr10* function and underline the essential role of the leucine and glycine residues in the coiled-coil and P-loop motives, respectively.

3.3. Transgenic wheat plants overexpressing *T10rga1* show increased resistance to leaf rust avirulent on *Lr10*.

To test whether *T10rga1* is sufficient to confer rust resistance to wheat plants, plasmids containing the *T10rga1* (*pUbi_T10rga1*) and *T10rga2-1A* (*pUbi_T10rga2-1A*) genes under control of the maize ubiquitin promoter were cotransformed into the susceptible wheat accession Bobwhite SH 98 56 (18) which has the H2 haplotype. Artificial infection and molecular analysis performed on T1 progeny plants identified two families expressing resistance to leaf rust avirulent on *Lr10*. In both families (T14 and T17), cosegregation was found between the resistance phenotype and the *pUbi_T10rga1* transgene (Fig. 3A). *pUbi_T10rga2-1A* was present in all the *pUbi_T10rga1* transgenic plants of the T17 family but was not detected in plants of the T14 family. All resistant plants from the segregating T1 families expressed *pUbi_T10rga1* as shown by Northern analysis (Fig. 3B), confirming that *T10rga1* is the *Lr10* leaf rust resistance gene. Other T1 families, which all contained multiple transgene insertions and rearrangements, were susceptible suggesting gene silencing or incomplete transgene integration (data not shown). The level of expression in the transgenic resistant plants was 8 to 25 times higher than in Thatcher*Lr10* (Fig. 3B). Interestingly, in the

plants overexpressing *T10rga1*, the infection type differed from the moderate resistance (small to middle size uredinias surrounded by chlorosis) conferred by the endogenous *Lr10* (Fig. 3C). The resistant transgenic plants either showed hypersensitive flecks or developed strong chlorosis and necrotic spots upon leaf rust infection and no uredinia were formed (Fig. 3C). These data suggest that overexpression of the *Lr10* gene enhances leaf rust resistance.

4. DISCUSSION

4.1. Map-based cloning in hexaploid wheat.

The construction of the first BAC library from the diploid wheat *T. monococcum* DV92 (8) has allowed the recent map-based isolation of *VRN1* directly from *T. monococcum* (10) and the identification of candidate gene orthologs for the hexaploid wheat genes *Lr10* (14) and *Q* (11) using subgenome map-based cloning. The *T. monococcum* DV92 line is not known to carry *Lr10*. Therefore, our work demonstrates that it is possible to isolate a gene from hexaploid bread wheat using available BAC resources from diploid relatives that do not necessarily contain the target gene. Very recently, the *Lr21* resistance gene, which originates from *Ae. tauschii,* has been isolated (12). In this case, map-based isolation of the gene was performed without chromosome walking as the RFLP probe KSUD14, which is part of the *Lr21* gene, could be used directly to screen a cosmid library from the *Ae. tauschii Lr21* donor line (12).

The subgenome map-based cloning of *Lr10* has succeeded because *T. monococcum* DV92 has the same haplotype (H1) as the target hexaploid wheat variety Thatcher*Lr10* and therefore colinearity between the two species was very high at the *Lr10* locus. A number of recent studies performed at the interspecific level (31-33) as well as between inbred lines (34) indicate that many rearrangements involving genes have occurred at orthologous loci during plant evolution. For this reason, the isolation of agronomically important genes from hexaploid wheat through subgenome map-based cloning strategies should be performed together with a detailed haplotype characterisation of the target locus in the wheat gene pool in order to identify the most appropriate genomic tools.

Our data suggest that the different wheat leaf rust resistance genes are not very closely related to each other. Consequently, each *Lr* gene must probably be isolated independently. High genome coverage BAC libraries have been very recently constructed from tetraploid (35) and hexaploid bread wheat (B. Chalhoub, personal communication). However, given the amount of clones required, *i.e.* ~1 million BAC clones for a 6 x time coverage of the hexaploid wheat genome, such libraries cannot be constructed for every wheat genotype of interest. In contrast, it should be possible to develop methods for rapidly constructing non-arrayed BAC libraries from a number of diploid wheat genotypes at low cost. We have shown here that libraries from diploid wheat relatives are efficient tools for the map-based isolation of genes from hexaploid wheat.

4.2. Overexpression of *Lr10* enhances resistance to leaf rust carrying *Avrlr10*.

In Thatcher*Lr10*, the *Lr10* gene provides only moderate resistance, is constitutively expressed and is not induced upon leaf rust infection. Plant disease resistance genes are only rarely induced by pathogen attack and are usually expressed at low levels (2) suggesting that too high expression might have negative effects. This is supported by the finding that in the *A. thaliana* epigenetic variant *bal1* which overexpresses an NBS-LRR gene, developmental abnormalities such as late flowering, dwarfing and altered floral structures were described (43). Interestingly, we have observed that some resistant transgenic plants overexpressing

Lr10 are smaller and have less tillers compared to non transgenic plants (unpublished data). Possible correlations between the level of *T10rga1* expression and developmental alterations will be analysed in the next generations. The overexpression of *Lr10* resulted in enhanced resistance with a complete prevention of rust sporulation compared to Thatcher*Lr10*. In addition, a new necrotic phenotype which has not been described before for *Lr10* (30) has been observed in some transgenic resistant plants. Similar to transgenic barley plants expressing the stem rust resistance gene *Rpg1* (44), it was not possible to strictly correlate the level of *Lr10* transgene expression with the resistance types observed in the transgenic plants. Analysis in the next generation will provide additional information about the genotype of the T1 plants and will allow the comparison of rust fungal growth in the two resistant types. Thus, our data suggest that overexpression of a disease resistance gene can improve resistance. This has also been shown in the cases of the *Pto* and *Prf* genes in tomato (45, 46) and more recently for the *Rpg1* resistance gene in barley (44).

In a near future, additional wheat disease resistance genes will be isolated using similar strategies as in this work. Their characterisation will provide a better understanding of the molecular basis of disease resistance in wheat and allow in the long term, the development of genomics-guided transgenes strategies (47) such as the combination of resistance specificities, the overexpression of resistance genes as well as the use of the cloned genes as "perfect" markers for molecular breeding. These strategies should ultimately lead to improved resistance of wheat against fungal diseases.

5. REFERENCES

1. Dangl, J. L. & Jones, J. D. G. (2001) *Nature* **411,** 826-833.
2. Hulbert, S. H., Webb, C. A., Smith, S. M. & Sun, Q. (2001) *Annu. Rev. Phytopathol.* **39,** 285-312.
3. Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., vanDaelen, R., vanderLee, T., Diergaarde, P., Groenendijk, J., *et al.* (1997) *Cell* **88,** 695-705.
4. Yu, Y., Tomkins, J. P., Waugh, R., Frisch, D. A., Kudrna, D., Kleinhofs, A., Brueggeman, R. S., Muehlbauer, G. J., Wise, R. P. & Wing, R. A. (2000) *Theor. Appl. Genet.* **101,** 1093-1099.
5. Halterman, D., Zhou, F. S., Wei, F. S., Wise, R. P. & Schulze-Lefert, P. (2001) *Plant J.* **25,** 335-348.
6. Zhou, F. S., Kurth, J. C., Wei, F. S., Elliott, C., Vale, G., Yahiaoui, N., Keller, B., Somerville, S., Wise, R. & Schulze-Lefert, P. (2001) *Plant Cell* **13,** 337-350.
7. Brueggeman, R., Rostoks, N., Kudrna, D., Kilian, A., Han, F., Chen, J., Druka, A., Steffenson, B. & Kleinhofs, A. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 9328-9333.
8. Lijavetzky, D., Muzzi, G., Wicker, T., Keller, B., Wing, R. & Dubcovsky, J. (1999) *Genome* **42,** 1176-1182.
9. Moullet, O., Zhang, H. B. & Lagudah, E. S. (1999) *Theor. Appl. Genet.* **99,** 305-313.
10. Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T. & Dubcovsky, J. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 6263-6268.
11. Faris, J. D., Fellers, J. P., Brooks, S. A. & Gill, B. S. (2003) *Genetics* **164,** 311-321.

12. Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N. & Gill, B. S. (2003) *Genetics* **164,** 655-664.
13. Feuillet, C., Schachermayr, G. & Keller, B. (1997) *Plant J.* **11,** 45-52.
14. Stein, N., Feuillet, C., Wicker, T., Schlagenhauf, E. & Keller, B. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 13436-13441.
15. Wicker, T., Stein, N., Albar, L., Feuillet, C., Schlagenhauf, E. & Keller, B. (2001) *Plant J.* **26,** 307-316.
16. Schachermayr, G. M., Messmer, M. M., Feuillet, C., Winzeler, H., Winzeler, M. & Keller, B. (1995) *Theor. Appl. Genet.* **90,** 982-990.
17. Clausen, M., Krauter, R., Schachermayr, G., Potrykus, I. & Sautter, C. (2000) *Nat. Biotechnol.* **18,** 446-449.
18. Pellegrineschi, A., Noguera, L. M., Skovmand, B., Brito, R. M., Velazquez, L., Salgado, M. M., Hernandez, R., Warburton, M. & Hoisington, D. (2002) *Genome* **45,** 421-430.
19. Wright, M., Dawson, J., Dunder, E., Suttie, J., Reed, J., Kramer, C., Chang, Y., Novitzky, R., Wang, H. & Artim-Moore, L. (2001) *Plant Cell Reports* **20,** 429-436.
20. Li, W. H. (1993) *J. Mol. Evol.* **36,** 96-99.
21. Kimura, M. (1980) *J. Mol. Evol.* **16,** 111-120.
22. Meyers, B. C., Kozik, A., Griego, A., Kuang, H. & Michelmore, R. W. (2003) *Plant Cell* **15,** 809-834.
23. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) *Nucleic Acids Res.* **25,** 4876-4882.
24. Felsenstein, J. (1985) *Evolution* **39,** 783-791.
25. Scherrer, B., Keller, B. & Feuillet, C. (2002) *Funct. Integr. Genomics* **2,** 40-50.
26. Bai, J. F., Pennill, L. A., Ning, J. C., Lee, S. W., Ramalingam, J., Webb, C. A., Zhao, B. Y., Sun, Q., Nelson, J. C., Leach, J. E., *et al.* (2002) *Genome Res.* **12,** 1871-1884.
27. Bergelson, J., Kreitman, M., Stahl, E. A. & Tian, D. (2001) *Science* **292,** 2281-2285.
28. Spielmeyer, W., Sharp, P. J. & Lagudah, E. S. (2003) *Crop Sci.* **43,** 333-336.
29. Song, R., Llaca, V. & Messing, J. (2002) *Genome Res.* **12,** 1549-1555.
30. McIntosh, R. A., Wellings, C. R. & Park, R. F. (1995) in *Wheat Rusts: an Atlas of Resistance Genes*, eds. McIntosh, R. A., Wellings, C. R. & Park, R. F. (CSIRO, Melbourne; Kluwer, Dordrecht).
31. Bennetzen, J. L. & Ramakrishna, W. (2002) *Plant Mol. Biol.* **48,** 821-827.
32. Brunner, S., Keller, B. & Feuillet, C. (2003) *Genetics* **164**, 673-683.
33. Wicker, T., Yahiaoui, N., Guyot, R., Schlagenhauf, E., Liu, Z.-D., Dubcovsky, J. & Keller, B. (2003) *Plant Cell* **15,** 1186-1197.
34. Fu, H. H. & Dooner, H. K. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 9573-9578.
35. Cenci, A., Chantret, N., Xy, K., Gu, Y., Anderson, O. D., Fahima, T., Distelfeld, A. & Dubcovsky, J. (2003) *Theor. Appl. Genet.* **In press**.
36. Luck, J. E., Lawrence, G. J., Dodds, P. N., Shepherd, K. W. & Ellis, J. G. (2000) *Plant Cell* **12,** 1367-1377.
37. Anderson, P. A., Lawrence, G. J., Morrish, B. C., Ayliffe, M. A., Finnegan, E. J. & Ellis, J. G. (1997) *Plant Cell* **9,** 641-651.
38. Dinesh-Kumar, S. P., Tham, W. H. & Baker, B. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 14789-14794.
39. Tornero, P., Chao, R. A., Luthin, W. N., Goff, S. A. & Dangl, J. L. (2002) *Plant Cell* **14,** 435-450.
40. Jin, Y., Cui, G. H., Steffenson, B. J. & Franckowiak, J. D. (1996) *Phytopathology* **86,** 887-890.

41. Leister, D., Kurth, J., Laurie, D. A., Yano, M., Sasaki, T., Devos, K., Graner, A. & Schulze-Lefert, P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 370-375.
42. Pan, Q., Liu, Y.-S., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D. & Fluhr, R. (2000) *Genetics* **155,** 309-322.
43. Stokes, T. L., Kunkel, B. N. & Richards, E. J. (2002) *Genes Dev.* **16,** 171-182.
44. Horvath, H., Rostoks, N., Brueggeman, R., Steffenson, B., von Wettstein, D. & Kleinhofs, A. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 364-369.
45. Oldroyd, G. E. D. & Staskawicz, B. J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 10300-10305.
46. Tang, X. Y., Xie, M. T., Kim, Y. J., Zhou, J. M., Klessig, D. F. & Martin, G. B. (1999) *Plant Cell* **11,** 15-29.
47. Strauss, S. H. (2003) *Science* **300,** 61-62.

FIGURES

Figure 1 Two haplotypes defined by the presence or absence of two candidates for the *Lr10* resistance gene on chromosome 1AS are present in the hexaploid wheat gene pool. **A.** Schematic representation of the H1 and H2 haplotypes at the *Lr10* locus. The candidate genes *rga1* and *rga2* are present on chromosome 1AS in lines with the H1 haplotype (e.g. Thatcher*Lr10*) but not in lines with the H2 haplotype (e.g. Frisal). **B.** The H2 haplotype is predominant and is very conserved in the wheat gene pool. Southern hybridisation with *Hin*dIII (top panel) and *Dra*I (bottom panel)-digested genomic DNA isolated from a subset of 56 hexaploid European wheat breeding lines. Hybridisations were performed with *rga1* (top panel) and the LRR domain of *rga2* (bottom panel) as probes. Lines with an H1 haplotype are underlined. The fragments corresponding to *T10rga2-1A* on chromosome 1AS and *T10rga2-1D* on chromosome 1DS (25) are indicated with arrowheads.

Figure 2 Amino acid sequence of the *Lr10* gene. The Coiled-Coil (CC), Nucleotide Binding Site (NBS), spacer and Leucine Rich Repeat (LRR) domains are indicated. Amino acids belonging to characteristic motives in each domain are in bold letters. In the NBS domain they are in the following order: P-loop, RNBS-A, Kinase2, RNBS-B, RNBS-C, GLPL, RNBS-D, MHDV. The four domains which have been used in the Ka/Ks analysis are separated from each other by a empty line. The spacer sequence is indicated in italics whereas the aliphatic (a) residues in the consensus (xxaxaxx) region of the LRR domain are boxed in yellow. The amino acid residues which are modified in the three EMS mutant genes are highlighted with red boxes with the number of the mutant above them.

Figure 3 Transgenic wheat seedlings overexpressing *T10rga1* show enhanced resistance to leaf rust. **A.** Southern hybridisation of *Hin*dIII-digested genomic DNA extracted from Bobwhite SH 98 56 (S56), Thatcher*Lr10* (Th10) and 17 transgenic T1 plants of the T17 family (T17A_x) with *T10rga1* as a probe. The arrowhead indicates the 5.6 kb fragment which is expected from a *Hin*dIII digest of the *pUBI_T10rga1* construct. **B.** Northern blot of total RNA extracted from the same seedlings hybridised with *T10rga1* as a probe. The same blot was hybridised with the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as a control. The relative intensity of the hybridisation signals in the transgenics vs. wild type plants was estimated with the Cyclone Gene Array System (Packard Bioscience). Transgenic plants showing chlorotic hypersensitive resistance reaction (*i.e.* T17A_2) are in blue letters, those with a necrotic phenotype (*i.e.* T17A_3) are in red letters whereas susceptible T1 plants are in black. **C.** Phenotypes of transgenic plants overexpressing *Lr10* compared to the resistant Thacher*Lr10* and the susceptible Bobwhite SH 98 56, 10 days after artificial infection with the leaf rust isolate TCB/TD *AvrLr10*.

FIGURE 1

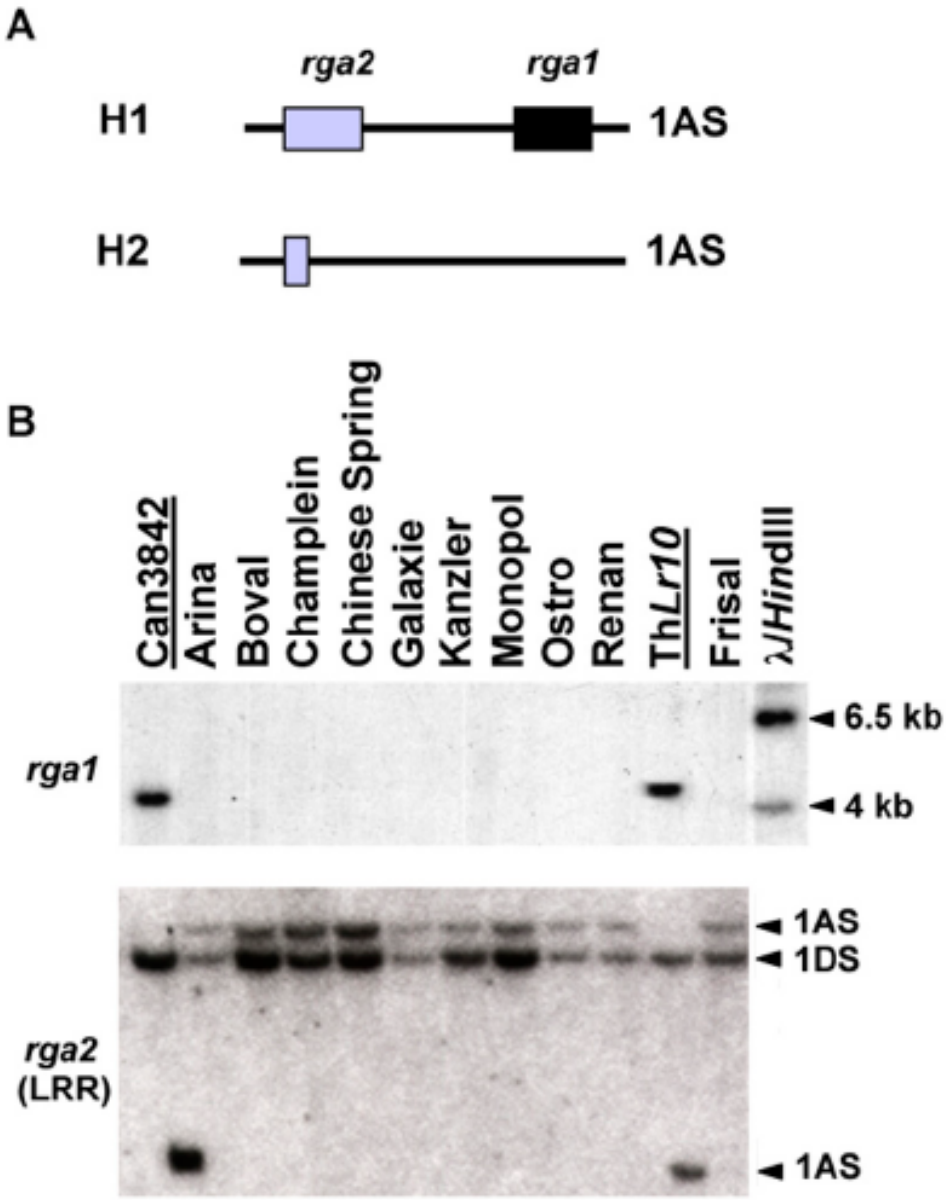
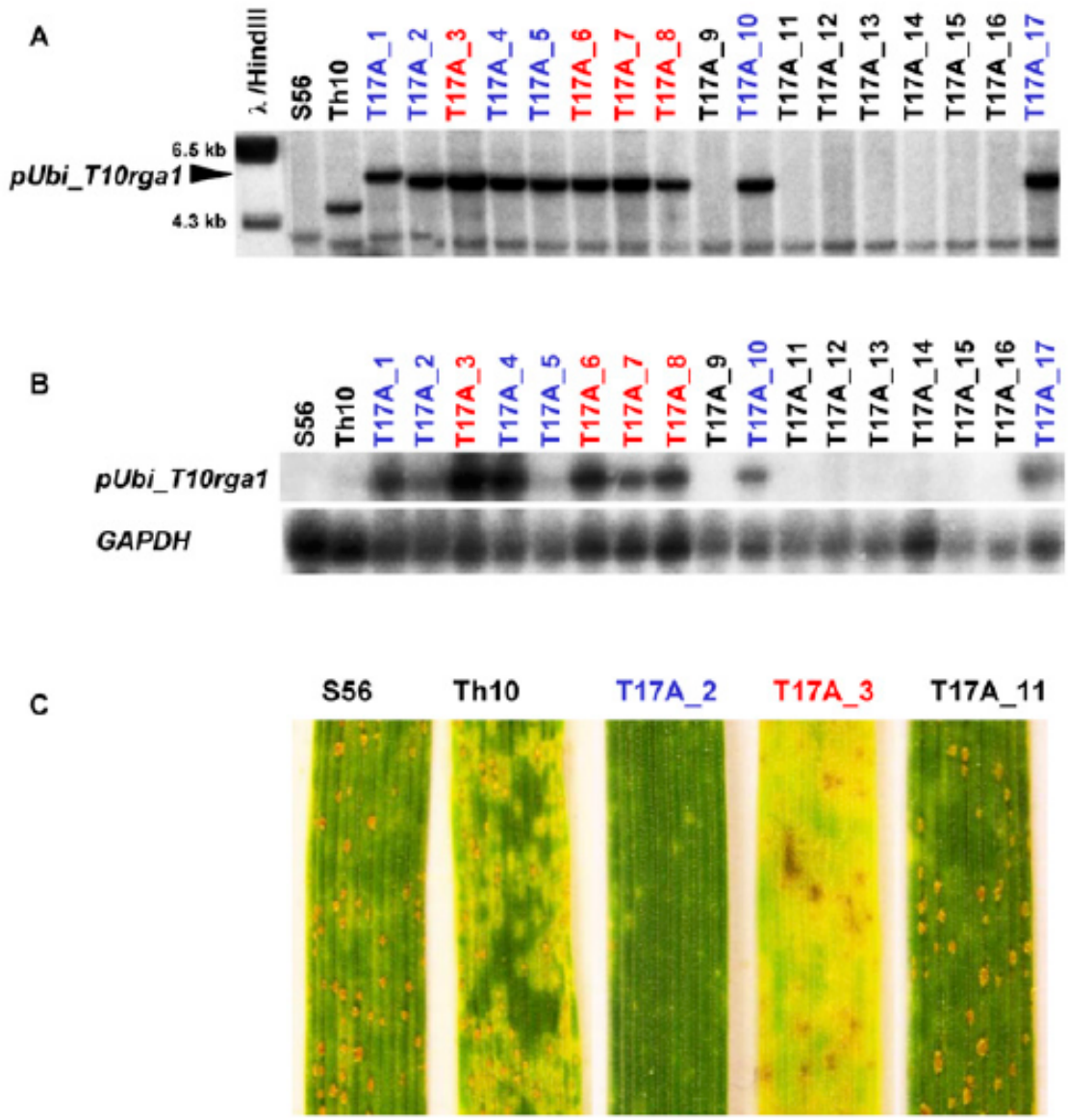

A
rga2
rga1
H1
1AS
H2
1AS
B
Can3842
Arina
Boval
Champlein
Chinese Spring
Galaxie
Kanzler
Monopol
Ostro
Renan
ThLr10
Frisal
λ/HindIII
rga1
6.5 kb
4 kb
1AS
1DS
rga2
(LRR)
1AS

FIGURE 2

FIGURE 3


A
λ /HindIII
S56
Th10
T17A_1
T17A_2
T17A_3
T17A_4
T17A_5
T17A_6
T17A_7
T17A_8
T17A_9
T17A_10
T17A_11
T17A_12
T17A_13
T17A_14
T17A_15
T17A_16
T17A_17
6.5 kb
pUbi_T10rga1
4.3 kb
B
S56
Th10
T17A_1
T17A_2
T17A_3
T17A_4
T17A_5
T17A_6
T17A_7
T17A_8
T17A_9
T17A_10
T17A_11
T17A_12
T17A_13
T17A_14
T17A_15
T17A_16
T17A_17
pUbi_T10rga1
GAPDH
C
S56
Th10
T17A_2
T17A_3
T17A_11