Theor Appl Genet (2002) 105:364–368
DOI 10.1007/s00122-002-0919-z

V. Mohler · A. Klahr · G. Wenzel · G. Schwarz

A resistance gene analog useful for targeting disease resistance genes against different pathogens on group 1S chromosomes of barley, wheat and rye

Received: 8 August 2001 / Accepted: 10 December 2001 / Published online: 18 May 2002

Abstract Comparative sequence analysis of the resistance gene analog (RGA) marker locus aACT/CAA (originally found to be tightly linked to the multiallelic barley *Mla* cluster) from genomes of barley, wheat and rye revealed a high level of relatedness among one another and showed high similarity to a various number of NBS-LRR disease resistance proteins. Using the sequence-specific polymerase chain reaction (PCR), RGA marker aACT/CAA was mapped on group 1S chromosomes of the *Triticeae* and was associated with disease resistance loci. In barley and rye, the marker showed linkage to orthologous powdery mildew resistance genes *Mla1* and *Pm17*, respectively, while in wheat linkage with a QTL against fusarium head blight (FHB) disease was determined. The use of RGA clones for R gene mapping and their role in the expression of qualitative and quantitative resistance is discussed.

Keywords Disease resistance · Powdery mildew · Fusarium head blight · Resistance gene analogs

Introduction

PCR cloning of disease resistance gene analogs (RGAs) has been a promising approach to obtain disease resistance gene candidates. However, since disease resistance loci of the major class of cloned plant resistance (R) genes (NBS-LRR type) are commonly composed of arrays of resistance gene-like sequences harbouring the active gene copy (Simons et al. 1998; Meyers et al. 1999), the proof of gene function is exceedingly difficult. This has also been pointed out in a recent study of barley, in which sequence analysis of a contig across the *Mla* locus, conferring resistance against powdery mildew disease, detected three families of NBS-LRR R gene analogs with at least 11 members (Wei et al. 1999); thus, a tremendous effort had to be made to pinpoint one functional allele (Zhou et al. 2001).

With respect to the monophyletic origin of grass genomes and comparative genetic data, disease resistance loci at matchable map coordinates in barley, rye and wheat are functional orthologs. Therefore, the availability of RGAs provides entry points to study the incidence and conservation across related species, thus giving insight into the architecture of orthologous disease resistance loci.

The AFLP-derived STS marker aACT/CAA, displaying significant similarity to many NBS-LRR-type disease resistance protein sequences, shows very tight linkage to the multiallelic *Mla* locus located on the terminal segment of the short arm of chromosome 5(1H) of barley (Schwarz et al. 1999). In this paper, we report: (1) on sequence analysis and mapping of STS marker aACT/CAA in the genomes of barley, wheat and rye, and (2) on its linkage to known qualitative and quantitative disease resistance loci in the three respective genomes.

Communicated by R. Hagemann

V. Mohler (✉) · A. Klahr · G. Wenzel · G. Schwarz
Lehrstuhl für Pflanzenbau und Pflanzenzüchtung,
Department Pflanzenwissenschaften,
Wissenschaftszentrum Weihenstephan für Ernährung,
Landnutzung und Umwelt, Technische Universität München,
Alte Akademie 12, 85350 Freising-Weihenstephan, Germany
e-mail: mohler@wzw.tum.de
Tel.: +49-8161-713183, Fax: +49-8161-715173

Materials and methods

Plant material

PCR analysis was carried out on a sample of 11 common wheats including T1RS.1BL genotypes ‘Helios’ (*Pm8, Lr26*) and ‘Helami-105’ (*Pm17*), T1RS.1AL genotypes ‘Amigo’ (*Pm17*) and ‘100-85’ (*Pm17*), ‘Asosan’ (*Pm3a*), ‘Virest’ (*Pm22*), ‘Chiyacao’ (*Pm24*), ‘Chinese Spring’, ‘Caribo’, ‘Cansas’ and ‘Ritmo’ (*Pm2, Pm6*), two barley lines ‘P01’ (*Mla1*) and ‘1B-87’, ‘Insave’ rye, the *Pm17* donor line, and two oat cultivars ‘Kanota’ and ‘Mostyn’ (*Eg-3*). Known major resistance genes are given in brackets. Nulli-tetrasomics and di-telosomics of ‘Chinese Spring’ were used for the chromosomal assignment of STS marker aACT/CAA in wheat. Analysis of linkage was performed in bulked segregants and 67 F_3 families from the previously described ‘Helios × Hel-

ami-105' cross (Hsam et al. 2000) and 96 F_5 RILs from the winter wheat cross 'Cansas × Ritmo'.

PCR and DNA sequence analyses

*Sse*8387I/*Mse*I AFLP and microsatellite analyses in wheat were performed according to Hartl et al. (1999) and Huang et al. (2000), respectively. Analysis of STS marker aACT/CAA was carried out using PCR conditions according to Schwarz et al. (1999). Amplicons were separated in 2% agarose for 2 h and 5 V/cm. After TA cloning of PCR products (pGEM-T Easy, Promega), DNA sequence analysis of plasmid inserts was performed from both ends on an ABI 377 platform (Applied Biosystems) using standard dye-terminator chemistry (Amersham-Pharmacia Biotech). Editing and aligning of DNA sequences were performed with Sequence Navigator software (Applied Biosystems).

Data-base analysis

Similarity searches were done using BLAST algorithms (Altschul et al. 1990). BLASTX (Gish and States 1993) was used to translate both strands from the query sequences into six reading frames and were compared to the swall protein data base on-line at the EMBL European Bioinformatics Institute server.

Marker nomenclature

Wheat microsatellites were designated gwm, followed by a probe number according to Röder et al. (1998). AFLP markers were designated according to the standard list for AFLP primer nomenclature deposited in the GrainGenes database by the Keygene company. Detected loci in wheat were marked with an 'X', the basic symbol for molecular-marker loci of unknown function in wheat.

Linkage analysis

Linkage groups were established with MAPMAKER/Exp version 3.0b (Lander et al. 1987) using the Kosambi mapping function (Kosambi 1944) and a LOD score of 3.0. QTL analysis was conducted using the composite interval mapping procedures of PLABQTL (Utz and Melchinger 1996). Charts of genetic linkage maps were produced with the computer package MapChart (Voorrips 2001).

Results

Sequence analysis of RGA aACT/CAA from wheat, rye and barley

PCR analysis of the RGA locus aACT/CAA in *Triticeae* genomes generated a single, 207-bp, amplification product in nine of the 11 wheats (while no product was obtained in the cultivars 'Helios' and 'Ritmo'), as well as in the two barley genotypes under consideration and 'Insave' rye. No amplification products were obtained from oat DNA. The nucleotide sequences of the cloned PCR products from the different cereals species were compared among each other, revealing a high level of relatedness (Fig. 1). The aACT/CAA products from wheat cultivars 'Chinese Spring', 'Caribo', 'Asosan' and 'Virest' were identical in sequence. Similarily, DNA sequences of aACT/CAA from 'Helami-105', 'Amigo' and '100-85', each carrying the resistance gene *Pm17* on their wheat-rye translocated chromosomes, were identical to that from 'Insave' rye, the donor of the translocated rye segment. This may prove the sequences of RGA aACT/CAA from the wheat-rye translocation lines to have originated from 'Insave' rye chromatin rather than from wheat chromosomes. The cloned 207-bp products from barley genotypes 'P01' and *H. spontaneum* '1B-87' were also identical in sequence. Overall, the level of identity of consensus sequences were for wheat vs rye, 99%, wheat vs barley, 93% and barley vs rye, 93%, suggesting the amplification of orthologous sequences across the *Triticeae* genomes.

A BLASTX search with RGA aACT/CAA from barley was conducted. The deduced amino-acid sequence

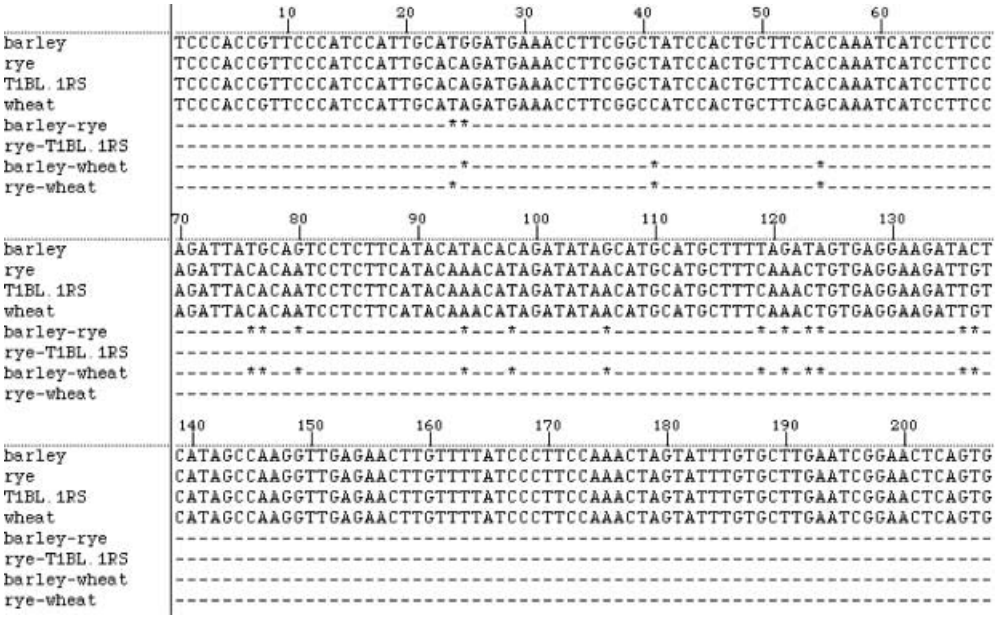

Fig. 1 Sequence alignment of RGA aACT/CAA from barley, rye and wheat. Single nucleotide polymorphisms are indicated by *asterisks*

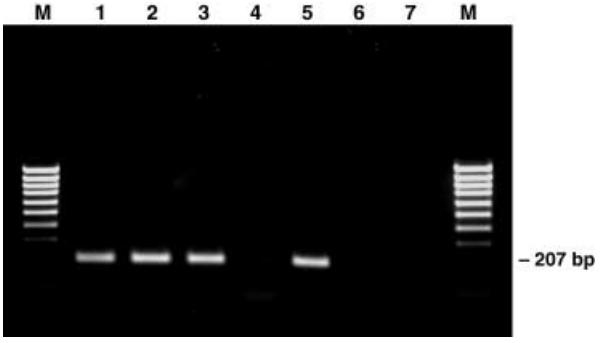

Fig. 2 Chromosomal and subchromosomal assignment of RGA aACT/CAA in wheat by means of nulli-tetrasomics and dit-elosomics of Chinese Spring. (*1*) Chinese Spring, (*2*) N1AT1B, (*3*) N1BT1D, (*4*) N1DT1B, (*5*) Dt1DS, (*6*) Dt1DL, (*7*) Control (H_2O), (*M*) 100-bp ladder

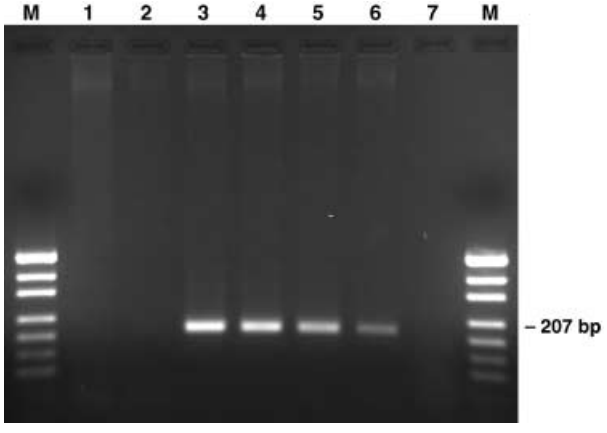

Fig. 3 Analysis of bulked segregants from the Helios (*Pm8*) × Helami-105 (*Pm17*) cross with RGA aACT/CAA. (*1*) Helios, (*2*) *Pm8* bulks, (*3*) *Pm17* bulks, (*4*) Helami-105, (*5*) Amigo (*Pm17*), (*6*) Insave rye, (*7*) Control (H_2O), (*M*) pUC19 DNA/*Msp*I

showed great similarity to the various number of R polypeptides, with highest scores for a barley NBS-LRR-type resistance protein (59% identity, 73% similarity; Leister et al. 1998) and the rice PI-TA protein (55%, 78%; Bryan et al. 2000). As supposed, the query sequence also had identities of 46% and similarities of 72% for both the barley MLA1 (Zhou et al. 2001) and MLA6 (Halterman et al. 2001) proteins.

Mapping of RGA aACT/CAA across the barley, wheat and rye genomes

As reported earlier (Schwarz et al. 1999), RGA marker locus aACT/CAA is tightly linked to the barley powdery mildew resistance gene *Mla1* (see Fig. 4A), showing two recombinants out of 972 F_2 individuals. To locate RGA marker aACT/CAA in the genome of common wheat, 21 nulli-tetrasomics of 'Chinese Spring' were employed for PCR analysis. The aACT/CAA fragment was lacking only in line N1DT1B, indicating allocation to chromosome 1D. Subsequent analysis with di-telosomic lines of 'Chinese Spring' refined the marker-locus position to the short arm of 1D, traced back to amplification in Dt1DS (Fig. 2). Segregation analysis was done in 96 F_5 RILs from the wheat cross 'Cansas × Ritmo', which was designed to localize QTLs conferring resistance against fusarium head blight (FHB) disease. The mapping data positioned *XaACT/CAA* 13.0 cM away from SSR marker locus *Xgwm106* (see Fig. 4B). By means of single-marker regression, this marker locus was estimated to explain 12% of the phenotypic variance for FHB resistance. Composite interval mapping with PlabQTL across four environments revealed a LOD of 3.2 for this QTL, explaining 14.5% of the phenotypic variance (data unpublished).

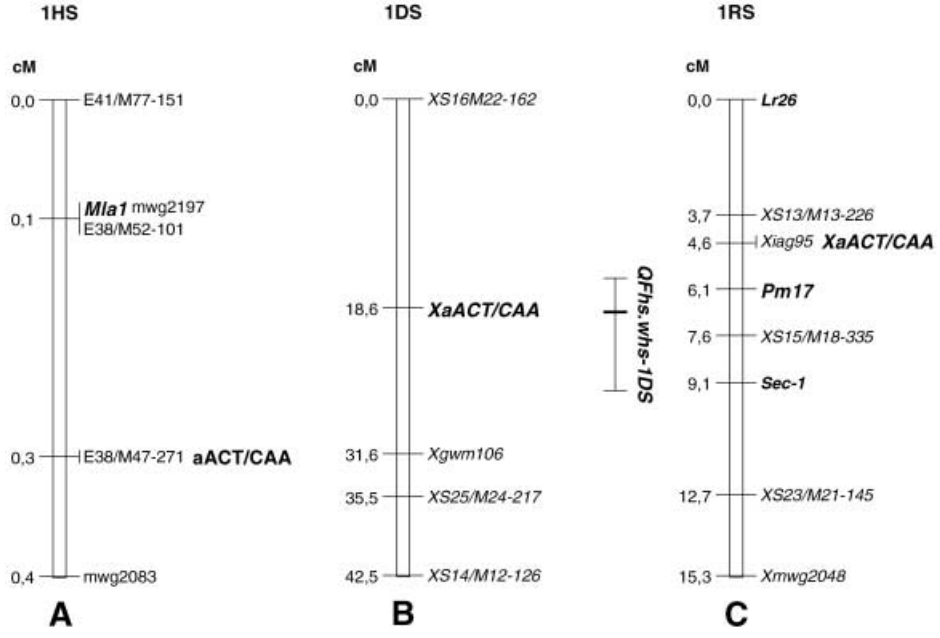

Fig. 4 Map positions of RGA aACT/CAA in the genomes of (**A**) barley (Schwarz et al. 1999), (**B**) wheat (The QTL position, given as horizonal line, was determined when the LOD score reached its maximum; a support interval with a LOD fall-off of 1.0 is also indicated.) and (**C**) rye

Bulked segregants and 67 F_3 families from a cross between the T1RS.1BL wheat-rye translocation genotypes 'Helios', carrying resistance genes *Pm8* and *Lr26* on 1RS, and 'Helami-105', with *Pm17* on 1RS, were used for genetic analysis of aACT/CAA in rye chromatin. Exclusively the bulked segregants for the *Pm17* phenotype and the *Pm17*-carrying wheats 'Helami-105' and 'Amigo' showed the specific PCR fragment which was also produced in Insave rye, the donor of the *Pm17* allele (Fig. 3). Subsequent segregation analysis detected one recombination to the *Pm17* resistance locus, corresponding to a map distance of 1.5 cM (Fig. 4C).

Discussion

Using sequence-specific PCR, we could show that RGA locus aACT/CAA is highly conserved both at the sequence level and in its location on corresponding chromosome arms among the *Triticeae* members barley, wheat and rye. Specifications of comparable map positions were not feasible due to the lack of polymorphisms between common markers, such as RFLP markers iag95 and mwg2048. Conservation is diminished among various tribes of the family *Poaceae* and may be the reason for the failure of RGA amplification in oat DNA since oat belongs to the tribe *Aveneae*. This conforms with the investigation of Leister et al. (1998) in which hybridisation-based analyses of RGA loci frequently failed proof of the conservation of map location among barley, rice and foxtail millet.

Due to the conserved chromosomal location of RGA aACT/CAA, and its high similarity at the nucleotide level among members of the *Triticeae*, we could demonstrate its applicability for genetic mapping of disease resistance loci across the genomes of closely related species. While, in barley and rye, RGA marker aACT/CAA mapped in the vicinity of the orthologous powdery mildew resistance genes *Mla1* (1HS) and *Pm17* (1RS), respectively, in wheat, it was associated with a QTL on chromosome 1DS for FHB resistance. Similarily, an RGA clone, already known to reveal a co-segregating marker for stripe rust resistance gene *Yr10* located 5 cM to the *Gli-B1* locus of wheat (Frick et al. 1998), detected markers co-segregating with leaf rust resistance genes *Lr21* and *Lr40* in the corresponding region of 1DS (Spielmeyer et al. 2000). With the availibility of high-throughput SNP (single nucleotide polymorphism) detection techniques such as DHPLC (Oefner and Underhill 1998) and minisequencing (Ahmadian et al. 2000), RGA clones become more and more interesting as molecular markers. RGAs are primarily located close to resistance genes and are often members of local multigene families (Lübberstedt et al. 2002). Due to their clustered organisation the identification of the active gene copy remains a major challenge, but a high probability is given to find an informative SNP associated with the resistance phenotype. Costs of marker development may be also reduced since there is a chance of RGA markers to be used across the genomes of the *Triticeae*.

RGA marker aACT/CAA is located in the major gene-rich region ("1S0.8 region") of the *Triticeae* homoeologous group-1S chromosomes, which are known to contain many qualitatively inherited disease resistance genes such as powdery mildew (*Mla*, *Pm17*), leaf rust and yellow rust resistance genes (Sandhu et al. 2001). Co-localization of major resistance genes and QTLs against pathogen defence have been reported (Backes et al. 1996; Zimnoch-Guzowska et al. 2000); therefore, one could expect the presence of disease resistance QTLs in this genomic region. Consequently, we have allocated a disease resistance QTL (in particular the FHB resistance QTL) to this gene-rich region. The occurence of linkage relationships among major resistance genes and disease resistance QTLs might be explained by the hypothesis of Ellingboe (1976) that the genetic basis of quantitative resistance correlates with 'defeated' genes for race-specific defence reactions. In rice, Li et al. (1999) demonstrated that a major resistance gene against *Xanthomonas oryzae* pv *oryzae* lost its dominance but was still acting at a reduced level as a QTL. In general, the architecture of disease resistance loci as families of linked genes might be the source to generate new and useful resistance specificities by inter- and intra-genic crossovers following mispairing (Hulbert 1998). Therefore, sequencing of such clusters is recommendable for the differentiation of R genes, disease resistance QTLs and RGAs. We have demonstrated that RGA aACT/CAA is associated with different host/pathogen relationships; accordingly, there may be common factors fundamental to both qualitative and quantitative disease resistance pathways.

Clusters or common roots of resistances affect combination breeding and gene pyramiding in a positive manner. Even crosses with wild species or primitive cultivars as donors of resistance, make the simultaneous incorporation of additional genes with untested resistance properties by linkage-drag probable. Thus, knowledge of gene associations will increase the efficiency in combination and hybrid breeding.

Acknowledgements We are grateful to Dr. G. Zimmermann, Bayerische Landesanstalt für Bodenkultur und Pflanzenbau (Freising), for providing seeds from the 'Cansas × Ritmo' cross and conducting the field trials.

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