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# Rice–barley synteny and its application to saturation mapping of the barley Rpg1 region

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## ABSTRACT

**In order to facilitate the map-based cloning of the barley stem rust resistance gene *Rpg1*, we have demonstrated a high degree of synteny at a micro level between the telomeric regions of barley chromosome 1P and rice chromosome 6. We have also developed and applied a simple and efficient method for selecting useful probes from large insert genomic YAC and cosmid clones. The gene order within the most terminal 6.5 cM of barley chromosome 1P was compared with the most terminal 2.7 cM of rice chromosome 6. Nine rice probes, previously mapped in rice or isolated from YAC or cosmid clones from this region, were mapped in barley. All, except one, were in synteny with the rice gene order. The exception, probe Y617R, was duplicated in barley. One copy was located on a different chromosome and the other in a non-syntenic position on barley chromosome 1P. The barley probes from this region could not be mapped to rice, but two of them were inferred to be in a syntenic location based on their position on a rice YAC. This work demonstrates the utility of applying the results of genetic and physical mapping of the small genome cereal rice to map-based cloning of interesting genes from large genome relatives.**

## INTRODUCTION

Comparative genome mapping provides insights into the modes and dynamics of genome evolution. Although the conservation of genetic linkages during the course of evolution was described many years ago (1), the concept of genome synteny has been developing along with the molecular marker systems used in chromosome mapping. Restriction fragment length polymorphism (RFLP), the first class of genetic markers based on DNA sequence polymorphism, has proven invaluable in mapping human and other genomes. RFLP mapping, together with molecular cloning of rapidly increasing number of genes, set the stage for establishing syntenic relationships for a number of animal and plant species (2–4).

The levels of synteny vary greatly, depending mostly on evolutionary distances between the compared taxa. In plants, very good marker order conservation was observed between the closely related tomato and potato species (4,5) and among the members of the Triticeae tribe (6). The conservation of gene order is lower when more distantly related species are compared, even within the same family (7) or tribe (8,9). Genetic distances between markers were often similar for the species compared (10).

The comparisons reported to date involved markers distributed throughout the genome often with large genetic distances between them. This allowed detection of gross synteny or major rearrangements of chromosomes. Gross synteny between rice and two other cereals, maize (10) and wheat (11,12) has been reported. The high degree of synteny between barley and wheat (6) suggests that the synteny observed for rice–wheat is also relevant to rice–barley.

In order to apply the synteny relationships between the small genome cereal rice and large genome cereals such as barley for map-based cloning of genes, the conservation of gene order at sub-centiMorgan and sub-Mbase level needs to be established.

Our long-term goal is the molecular characterization of the telomeric region of barley chromosome 1P (S) and map-based cloning of the *Rpg1* gene located in this region. The *Rpg1* locus confers durable resistance to the stem rust pathogen *Puccinia graminis* f. sp. *tritici* (13). Extensive RFLP mapping in two barley crosses covered the most terminal 5 cM of chromosome 1P with a number of molecular markers (14). A very close distal marker (ABG704; 0.3 cM) was identified, but the closest proximal marker was still 1.5 cM from the target. One of our approaches to saturate this region with molecular markers is based on synteny with the small genome cereal rice.

The most comprehensive rice genetic map is 1575 cM distributed over 12 linkage groups (15). The rice haploid genome size is ~400 Mb (16), yielding an average of ~250 kb/cM. By comparison, the most comprehensive barley genetic map is 1245 cM (17) distributed over seven linkage groups. The barley haploid genome size is ~4900 Mb (16), yielding an average of ~4 Mb/cM. These comparisons clearly indicate the advantages of using the rice genome as a tool for map-based cloning of genes from barley and other large genome cereals as has been previously discussed (12). At least two criteria must be met to make this approach

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feasible. First, the synteny must hold over small genetic and physical distances and secondly, an efficient method for selecting probes from large insert clones that cross-hybridize between the two genomes must be developed. Here we report results that demonstrate the achievement of both criteria.

## MATERIALS AND METHODS

### Plant material

Four barley crosses, segregating for the *Rpg1* gene, used in this study were Steptoe × Morex (18, Devaux, unpublished), Harrington × TR306 (19), Harrington × Morex (unpublished) and SM89010×Q21861 (20). Morex, TR306 and Q21861 are resistant to the stem rust pathogen *P.graminis* f. sp. *tritici* race MCC (*Rpg1*), while Steptoe, Harrington and SM89010 are susceptible (*rpg1*). A total of 1100 doubled haploid (DH) lines, derived by *Hordeum bulbosum* (21) or by anther culture (22) methods, were used as segregating populations. The use of 'immortal' DH lines is advantageous since they permit repeated testing of the disease phenotype, thus insuring the high degree of accuracy essential for fine structure mapping. Markers mapped within 2 cM of *Rpg1* locus in one or more populations were also mapped with all 26 DH lines that have been identified as recombinants in the *Plc* to ABG077 region. An F2 population of 186 individuals derived from a cross of cultivars Nipponbare × Kasalath (15) was used for mapping in rice.

### RFLP techniques and linkage maps

All RFLP techniques and linkage map construction were as previously described for barley (18) and rice (15). For hybridization of heterologous probes the stringency conditions were reduced to 62°C and 1 × SSC.

### Large insert clones

Rice YAC library preparation and characterization has been reported (23). The rice cv. Nipponbare cosmid library was prepared in SuperCos1 (Stratagene) vector (Katayose, unpublished). DNA was isolated from yeast transformants carrying rice YAC clones as previously described (24,25). Cosmid DNA was isolated using the alkaline precipitation method (26). High molecular weight DNA from YAC clones was resolved by pulsed field gel electrophoresis (PFGE) using CHEF DR III system (BIO-RAD) and conditions recommended by the manufacturer. DNA from PFGE gels was depurinated by 20 min incubation in 0.25 N HCl prior to transfer to positively charged nylon membranes (DuPont or Boehringer Mannheim). Transfer, hybridization, washing and detection were as for RFLP techniques.

### Preparation of plasmid libraries

General molecular techniques were as described (26). DNA from the rice YAC clone Y617 was resolved using PFGE as described above. The ~490 kb YAC was excised from 1% agarose gel and electrophoretically transfered to LMT agarose. After agarase (New England Biolabs) digestion, the DNA was precipitated with ethanol, partly digested with *Mbo*I and cloned into a *Bam*HI digested Bluescript SK+ vector. A portion of the ligation reaction was used to transform *Escherichia coli* DH5α strain. Insert-containing clones were identified using blue/white selection.

DNA from cosmid Y617R10-1 (5 μg) was partly digested with *Taq*I and *Rsa*I. The restriction digest was separated in 1% LMT agarose and a 1–3 kb section of the gel excised. After agarase digestion and precipitation, restriction fragment ends were filled in using *Taq* polymerase (2.5 U) and dNTPs (0.2 mM) in a 50 μl reaction at 70°C for 20 min. The reaction was stopped by chlorophorm extraction, ethanol precipitated and ligated into linearized Bluescript SK+ containing 3′ dT overhangs (27). Transformation was as described for YAC subcloning.

### Selection of subclones crosshybridizing to barley

Restriction digests of subcloned DNA were transferred to membranes and hybridized with $^{32}$P labelled cDNA synthesized from a mixture of barley mRNA. The barley mRNA was isolated from immature embryo, immature inflorescence and Morex leaf tissues. Leaf tissue was from 3-week-old seedlings that had been inoculated with the *P.graminis* f. sp. *tritici* pathogen for 16 h prior to harvest and non-inoculated controls. Labelling was with AMV Reverse Transcriptase (Promega) using the conditions recommended by the manufacturer for first strand cDNA synthesis, modified by substituting random hexamers (Amersham) for oligodT and [α-$^{32}$P]dCTP (3000 Ci/mmol, New England Nuclear) for dCTP. Hybridization, washing and detection conditions were as described above. Positive clones were used for RFLP.

## RESULTS

### Linkage analysis

The plastocyanin precursor (*Plc*) locus co-segregates with the telomeric markers ABA301 and ABG312 and represents the most telomeric marker on barley chromosome 1P in the Steptoe × Morex map (17,18). The wheat marker W160, homologous to the barley plastocyanin probe (data not presented), maps to the most telomeric position of rice chromosome 6 (15). The *Wx* (*Glx*; ADP-glucose starch glycosyltransferase) locus maps 7.3 and 19.7 cM proximal to W160 (*Plc*) in rice and barley, respectively. These markers confirm the general synteny of the rice chromosome 6 and barley 1P chromosome telomeric regions and include our target, the barley stem rust resistance gene *Rpg1*. In order to determine gene microsynteny between rice and barley, we examined the barley and rice probes mapped in the barley *Plc* to MWG555A (5.1 cM) and rice W160 to C474 (2.7 cM) regions. The rice probes C474 and R560 were mapped in barley and confirmed the marker order conservation for this region (Fig. 1). The rice probes C1003B, Y4621L and R2869 did not hybridize well enough to be mapped in barley. It was not possible to map any of the barley probes in rice, although two of them (ABG077 and MWG036B) showed a weak hybridization signal to rice genomic DNA. Hybridization of these two probes to the YAC clones showed that they are located on the Y617 region that is not overlapped by Y4621 and in a syntentic position with barley (Fig. 1, Table 1).

### Analysis of rice YAC clones

The overlapping YAC clones Y617 and Y4621 (23 and Kurata, unpublished), covering most of the distal region of rice chromosome 6, were chosen for analysis. The Y617 end clone Y617R co-segregates with W160 in the rice map, but Y617 and Y4621 failed to hybridize with *Plc* (W160) probe indicating that they did not extend to this region. Two additional YACs, Y3219 and

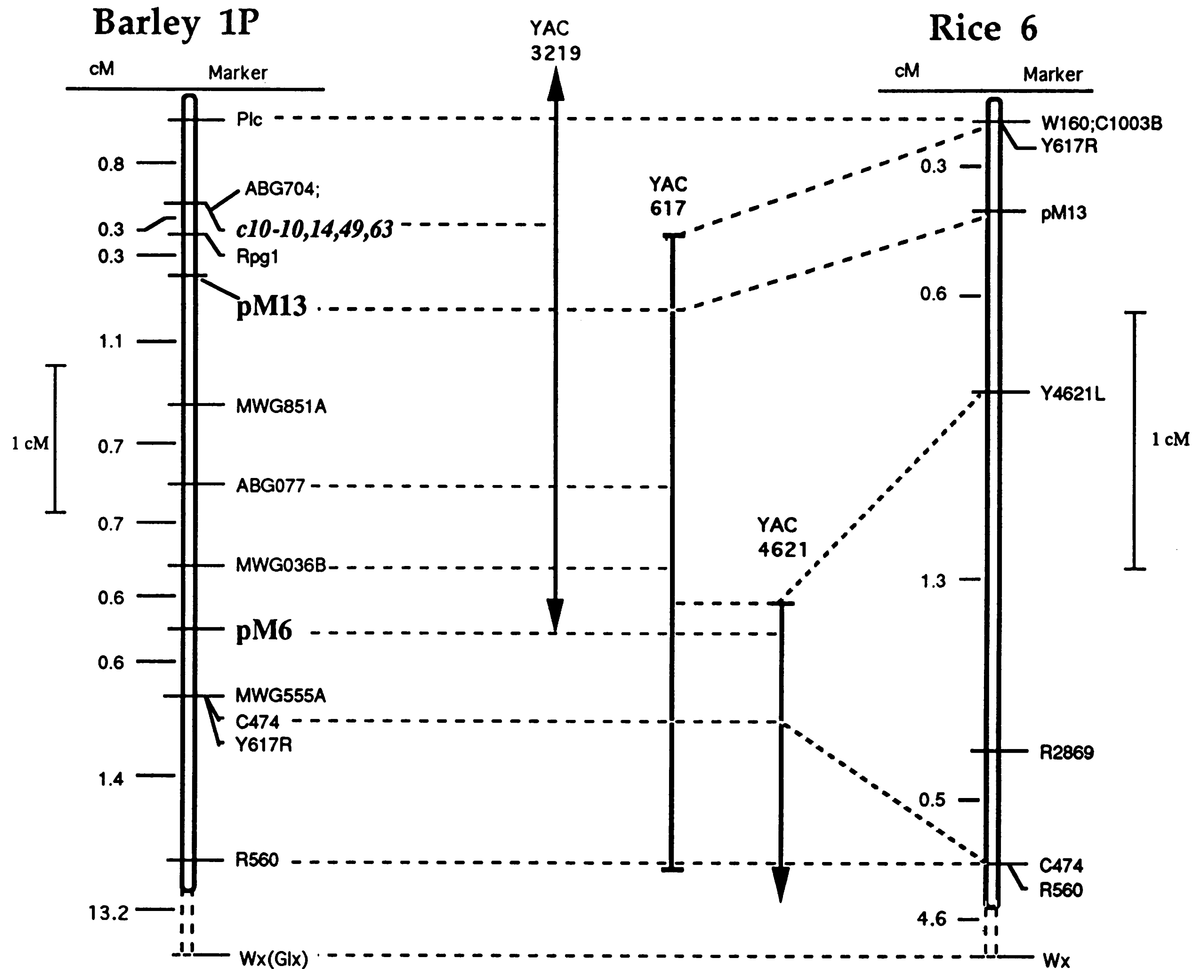


**Figure 1.** Synteny between barley chromosome 1P and rice chromosome 6 telomeric regions. Genetic distances from *Plc* (W160 in rice) to *Wx* are 19.7 cM for barley and 7.3 cM for rice, respectively. The *Wx* marker on both genetic maps is toward the centromere. Bars denoting 1 cM for each species are shown. Dashed lines (---) from markers crossing/connecting to symbols denoting YACs indicate a positive signal obtained for the YAC clone with that marker. The exact location of most of the markers on the YAC is not known except for YAC end clones Y4621L and Y617R. Arrows at the ends of a YAC indicate terminus not determined. Bold face markers on the barley map represent subclones of Y617 and italicized markers from the cosmid Y617R10-1. Most markers presented on the barley map were scored using a population of 1100 gametes with the following exceptions: *Plc*, MWG555A, MWG036B, pM6 and *Wx* (401 gametes); pM13 and Y617R (350 gametes); C474 and R560 (150 gametes). The rice map was constructed using 372 gametes from a cross of the cultivars Nipponbare × Kasalath. Linkage analyses were made as described in Materials and Methods.

Y3192, identified later, do hybridize with *Plc* (W160) and thus cover the *Rpg1* target region (Table 1).

## Generating probes from Y617

In order to generate probes for further microsynteny testing and tight linkage to the target gene *Rpg1*, we subcloned Y617 rice DNA into a plasmid vector. Of the ~60 subclones tested, 14 hybridized with a barley cDNA probe. These were tested for polymorphism with DNA from the barley cultivars used in the crosses being analyzed, cut with six restriction enzymes and rice DNA as a control. Many probes hybridized with a single fragment in rice and two or more fragments in barley. Two clones showed the opposite trend hybridizing with a few fragments in barley and several fragments or a smear in rice.

The level of polymorphism detected in barley was low. Only two probes, pM6 and pM13, could be mapped in barley and only one of these, pM13, was mapped in rice (Fig. 1). The pM13 probe provided further confirmation of the conservation of gene order between rice and barley in this region. More importantly, pM13 was only 0.3 cM proximal to *Rpg1* in barley and 0.3 cM proximal to the co-segregating markers W160, C1003B and Y617R in rice (Fig. 1). These data suggest that the rice genome region corresponding to the barley *Rpg1* region should be very close to the terminus of Y617, i.e. the Y617R locus.

## Analysis of Y617R

The Y617R probe is a single copy sequence in rice, but it detected two fragments in the barley genome. Both fragments were polymorphic and mapped. One copy mapped to chromosome 3M, between ABG389 and ABC161 on the barley SM map (17) and the other one co-segregated with MWG555A (Fig. 1). The

MWG555A location is 3.7 cM proximal to pM13 and out of synteny with the rice map.

**Table 1.** Presence or absence of probe sequences on four YAC clones covering the rice chromosome 6 telomeric region

| Probe | Rice YAC clones | | | |
|---|---|---|---|---|
| | Y3192 | Y3219 | Y617 | Y4621 |
| W160 | + | + | – | NT |
| C1003B | + | + | – | – |
| c10-10 | + | + | – | – |
| c10-14 | + | + | – | – |
| c10-49 | + | + | – | – |
| c10-63 | + | + | – | – |
| Y617R | + | + | + | – |
| pM13 | + | + | + | – |
| ABG077 | + | + | + | – |
| MWG036B | + | + | + | – |
| pM6 | NT | NT | + | + |
| C474 | NT | NT | + | + |
| R560 | NT | NT | + | + |

NT = not tested.

In order to generate more closely linked probes and to address the issue of non-syntenic location of the Y617R sequence, the Y617R probe was used to screen the rice cultivar Nipponbare cosmid library. Four positive clones were isolated covering ~50 kb. Neither *Plc* (W160) nor pM13 sequences were found to be associated with these clones, providing a minimal distance of 50 kb separating these loci.

The cosmid Y617Rcos10-1 was subcloned into a plasmid vector as previously described for Y617. Over 120 clones were generated with an average insert size of 1.5 kb. Labelled first strand cDNA was used to identify probes cross-hybridizing with barley. Positive probes were tested for polymorphism with the barley parent cultivar DNA. Four probes (c10-10, c10-14, c10-49 and c10-63) were identified as very polymorphic among the parents being used in this study. These probes identified from one to several bands in barley and rice. Interestingly, c10-49 and c10-63 generated a pattern of bands that was identical among the genotypes resistant to stem rust (*Rpg1*), but different among the susceptible (*rpg1*) ones. All four probes detected loci that co-segregated with ABG704 and mapped just 0.3 cM (three crossovers in a population of 1100 gametes) from the target region (Fig. 1). These data indicate that only a small fragment around Y617R has moved to the new location and most of the barley sequences are in synteny with the rice marker order.

## DISCUSSION

Data presented here demonstrate a very high degree of synteny at the sub-cM level between barley chromosome 1P and rice chromosome 6 telomeric regions. Both species have similar marker order and orientation with respect to the telomere. Genetic distances were only slightly different. One marker, Y617R, was found to be an exception to this general rule. This sequence was duplicated in barley and one copy had moved to a different chromosome while the other one had relocated to a more proximal position within barley chromosome 1P. Sequencing of Y617R clone (data not presented) did not reveal any obvious features that could account for this mobility. Detailed analysis showed that the out-of-synteny region is probably small, although its exact borders remain to be determined. The telomeric location of the region we have analyzed may contribute to the rearrangement of Y617R sequences. The highly recombinogenic nature of telomeres and tendency toward amplification/deletion processes is well established in yeast (28–30) and malaria parasites (31). A high degree of recombination between satelite-like telomeric repeats was found in numerous species (reviewed in 32) pointing to non-homologous recombination as an important mechanism for genome evolution at sub-terminal regions of chromosomes (33).

Another important aspect of our work is the demonstration that subclones representing expressed genes mapping to the target region can be easily obtained from large insert genomic clones. We reasoned that expressed sequences would provide the best source of probes between two distantly related species. Generating small insert plasmid libraries from YACs or cosmids and probing with barley cDNA resulted in selection of rice probes that hybridized strongly to barley genomic DNA. Probes that were polymorphic mapped to the region covered by the large insert clone. Several methods have been previously described for selection of expressed sequences carried by large insert genomic clones (reviewed in 34). Exon trapping (35) does not directly rely on the availability of representative cDNA libraries. However, the size of the genomic insert suitable for this method limits its feasibility. In contrast, cDNA enrichment methods (34,36,37) can be applied to YACs or even YAC contigs. The enrichment scheme utilizing biotin–streptavidin magnetic bead technology (34) seems particularly powerful. Although the use of magnetic beads offers several thousand-fold enrichment for cDNAs from YAC clones (or cosmid contigs) its effectiveness is determined by the representativeness of the cDNA library used (34). The representation of cDNA sub-libraries created by this method may also be affected by the PCR process, especially with several rounds of enrichment. The procedure we have described here is simple, rapid and did not generate any false positives in the sample tested. It may be a method of choice, particularly when access to cDNA libraries from a large variety of tissues is limited.

The clone Y617 covers the rice map from Y617R to C474, a distance of 2.7 cM. The physical distance per cM for this region of the rice genome is ~180 kb, based on the Y617 size of 490 kb. This agrees well with the average distance of 250 kb/cM and range of 120–1000 kb/cM previously reported (23). The co-segregating markers W160 and Y617R, are, however, ≥ 50 kb apart. Since the rice map is constructed based on 186 F2 plants or 372 chromosomes, co-segregation suggests a genetic distance of <0.3 cM on average. Based on the above calculations, 0.3 cM would be ~60 kb.

Since Y617R is out of synteny with rice it cannot be used for the barley comparison. However, the barley genetic distance from pM13 to C474 is 3.7 cM. The comparable distance in rice is 2.4 cM. Unfortunately, we do not have data about the physical distance spanned by these markers in barley, therfore we cannot compare the amount of DNA 'between genes' in barley versus the amount between their homologs in rice. Based on the overall barley genome size and linkage map, an average value of 4 Mb/cM can be calculated. Even if this is a gross overestimate, the value of using rice to identify and map expressed sequences for the purpose of

map-based cloning of genes from barley, or any large genome cereal species, is clearly apparent.

In conclusion, we want to stress that the barley 1P and rice chromosome 6 telomeric region showed very good synteny. This, together with the ability to efficiently select markers suitable for mapping and tightly linked to the target region, should provide encouragement for the use of rice as a resource species for positional cloning of important genes from large genome cereals.

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