

Genetic fine-mapping: examples in rice, sorghum, and maize

J. Bennetzen, J. Xu, A. Oliveira, and J. Li

*Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392
USA*

Introduction

Different genetic fine-mapping projects have specific problems and advantageous approaches. The reasons for these differences are associated with variations in the quality of the marker and mapping populations for specific species and traits, and differences in the degree to which the trait can be easily scored. Traits that are strongly affected by genetic background or the environment (low penetrance) or are multigenic in nature (quantitative trait loci, or QTL) are particularly difficult to fine map. We present three cases of simple traits that our lab has attempted to fine map, to serve as examples of how fine-mapping can be performed under different circumstances.

The *Lgs* region of rice

We found that several rice varieties do not make much or any striga germination stimulant by the plate assay, and that most of these varieties exhibited some field resistance to striga. (Newton, Oliveira and Bennetzen, unpub. obs.). In crosses between a high and a low germination stimulant producer, we found that low germination stimulant levels segregated as a single recessive gene. Unfortunately, we did not have much success in generation large progeny populations of rice under our greenhouse conditions and also had limited success with individual outcrosses. Hence, we needed to map this single gene trait in a small population, using fairly closely-related parents with low levels of polymorphism. Fortunately, rice has a very good set of positioned simple sequence repeats that can be mapped easily by using commercial primers. Using these markers, and only 46 F_2/F_3 progeny, we located the rice *lgs* gene to a region on rice chromosome 11. This region has previously been shown to have a syntenic relationship with linkage group J in sorghum that contains a gene that we and the Ejeta lab have mapped for sorghum's low germination stimulant production. Thus, we now

In: B.I.G. Haussmann, H.H. Geiger, D.E. Hess, C.T. Hash, and P. Bramel-Cox (eds.). 2000. Application of molecular markers in plant breeding. Training manual for a seminar held at IITA, Ibadan, Nigeria, from 16-17 August 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

intend to utilize the predicted colinearity of this region in sorghum and rice to fine map and isolate both the rice and sorghum genes, using the relative strengths of each species.

The *Pc* region of sorghum

In a collaboration with Dr. Larry Dunkle's lab at Purdue, we have begun studies to map and clone the *Pc* locus of sorghum. The *Pc* gene provides recessive resistance to the toxin encoded by *Periconia circinata*. The *Pc* gene had not been mapped, and the dominant susceptible allele is rare in sorghum varieties. We crossed *Pc/Pc* Colby sorghum to *pc/pc* Shanqui Red sorghum, hoping that their very different genetic backgrounds would provide a high level of DNA marker polymorphism. This cross was taken to the F₂, and then individual F₂ seed were selfed to create an F₃ population. F₃ families that were homozygous for either *pc* or *Pc* were identified by a seedling toxin resistance screen. All *pc/pc* individuals were used to make a pooled DNA preparation while all *Pc/Pc* individuals were separately pooled. Several hundred RAPD primers were then used to screen these two bulked segregant DNA pools, and a few bands were found that differentiated the two pools. These bands were then mapped, initially as RAPDs, between the individual F₃ families. Many of the markers did not show linkage to the *Pc* trait, but three did show good linkage. Unfortunately, these markers could not be mapped as RAPDs in any other cross, so they were cloned to use as RFLP markers. When used as RFLPs, each of them detected multiple bands in Southern analysis. A few of these bands were mapped in a standard sorghum mapping population that contained many RFLP markers. Several RFLP markers from the standard sorghum map were found to be near these new RAPD-derived RFLPs. When these standard RFLPs were tested on the Colby X Shanqui Red F₃ families, a few were found to be both polymorphic and linked. Unfortunately, this populations turns out to have a very low level of DNA marker polymorphism (at least near *Pc*), thus making it impossible to map most probes. Moreover, this appears to be a region of the sorghum genome that is mostly not colinear with the rice genome. Despite these problems, we have mapped *Pc* to a segment of sorghum linkage group F. Our closest RFLP marker, UIU101, is about 1 cM away. We have now used UIU101 as a probe to clone large DNA fragments from this region in a bacterial artificial chromosome (BAC) library. In most genic regions of sorghum, we expect that there will be about 100 kb to 300 kb per cM. The clones in our first step have covered an approximate 150 kb region. We can now use the ends of these UIU101-flanking BACs, that should cover 0.5 cM to 1.5 cM, in further RFLP mapping. Eventually, we will find a marker that has no detected recombination with *Pc*. This map-based cloning process is called chromosome walking. It provides both closer markers and, eventually, the cloned gene. Confirmation of a putative *Pc* clone could be difficult, although we do have some expectations with respect to the tissues in which it will be expressed, its molecular nature, and its instability. The chromosome walking process itself should be greatly accelerated

by the planned generation of a full-genome physical map by the Mullet lab at Texas A&M University within the next few months.

The *Rp1* region of maize

In the late 1980s, it was known that the disease resistance gene *Rp1* was found toward the distal end of the short arm of chromosome 10 in maize. *Rp1* provides largely dominant resistances to various races of the leaf rust fungus, *Puccinia sorghi*. We initiated our mapping studies in the early 1990s, using the proximal morphological marker oil yellow seedling (*oy1*) and known DNA markers from previous RFLP maps. Because different maize lines tend to have high levels of polymorphism, we found that most of our DNA markers could be mapped relative to both *Rp1* and *oy1*. Thus, by looking at only 100 backcross progeny, we were able to find and map three markers within 10 cM of *Rp1* in just a few months work (Hulbert and Bennetzen, *Mol. Gen. Genet.* 226:377-382, 1991). Two of these DNA markers were centromere-proximal, between *oy1* and *Rp1*, which are separated by about 10 cM. In order to more carefully map these proximal DNA markers, we analyzed the DNA of only those individuals that were recombined between *Rp1* and *oy1*, meaning that we needed to do Southern analysis on only one tenth of the progeny. This allowed us to show that one of the RFLPs was about 1 cM proximal to *Rp1* and the other about 6 cM proximal. These studies also demonstrated that different alleles of *Rp1* were spread across a region of over 3 cM, indicating that this was a complex locus with many *Rp* genes in it. *Rp1* was further fine-mapped by bulked-segregant analysis, isoline analysis, candidate gene mapping, and by cloning the gene.

TRANSPARENCIES

Fine-Mapping Examples in Rice, Sorghum and Maize

The *Lgs* region of rice

The *Pc* region of sorghum

The *Rp1* region of maize

The *Lgs* Region of Rice

Genetic basis of trait not known

General region not known

Small population size, low polymorphism level

Good SSR marker resources

Synten/colinearity with sorghum

The *Pc* Region of Sorghum

General region not known

Reasonable understanding of locus genetics and biology

Bulked segregant analysis with RAPDs
(most markers not useful, high labor)

A few RAPDS both successful in linkage and conversion to RFLPs

RAPD-derived RFLPs placed on standard sorghum map, indicated other possible RFLPs

Low polymorphism, no synteny/colinearity with rice

BAC clones isolated and mapped

Physical map should make fine-mapping and map-based cloning feasible

Confirm by expression, gene nature, unstable mutations, transformation

The *Rp1* Region of Maize

General region known, DNA and morphological markers available

Polymorphism common, near isolines available

Crude map on small progeny set

Use flanking morphological marker recombination to fine map on one side

Bulked segregant analysis and candidate genes to get more markers

Very large final mapping populations

Clone gene by transposon tagging
