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The coding sequence for rice seed catalase detects a locus different from that determined by isozyme analysis

N. Kishimoto, K. Higo, H. Higo, A. Saito

Department of Molecular Biology, National Institute of Agrobiological Resources, 1-2 Kannondai 2-chome, Tsukuba, Ibaraki 305, Japan

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Source of the probe

A cDNA library in λgt11 was constructed using poly $(A)^+$ RNA extracted from rice (indica type) immature seeds. A cDNA clone (C51) was isolated which included a 1.3-kbp insert containing a sequence that is similar to regions of the maize catalase cDNAs, though lacking the translation initiation codon and the adjacent region. The complete cDNA (OSCAT-A), 1,865 bp in length and containing the entire coding region, was later isolated and sequenced (Mori et al. 1992). The probe used in this study was prepared by amplifying the insert by the polymerase chain reaction using clone C51 as a template and two short oligonucleotides corresponding to the flanking regions of the vector as the primers. The DNA fragments to be used as probes were radiolabelled using a random oligonucleotide labelling kit (Amersham).

Plant analysis

Genomic DNA was extracted from the leaves of Kasalath (indica type) and FL134 (japonica type) cultivars of *Oryza sativa*, digested with *Bam*HI, *Bgl*II, *Eco*RV, or *Hin*dIII, subjected to electrophoresis, blotted to a nylon membrane, and hybridized to the probes described above, according to the method of Sambrook et al. (1989). All the filters were washed in 2 × SSC at 42 °C, and then subjected to autoradiography. F_2 seeds of the cross Kasalath × FL134 were produced, leaf tissue was harvested from 144 individual F_2 plants, and genomic DNA extracted.

The MAPL software program (Ukai et al. 1990) was used for genetic analysis. The recombination values were estimated by a maximum likelihood method (Allard 1956). The values which were significant at the 5% level in the chi-square test of independence were utilized for RFLP mapping. Within a linkage group, the putative order of the loci was determined by applying the metric multidimensional scaling method. Other details have been described by Saito et al. (1991).

Location and order of the probe and gene loci

The probe is a 1.3-kbp cDNA clone encoding a major portion of the rice catalase (OSCAT-A) expressed in immature seeds (Mori et al. 1992). The autoradiogram of Southern blots of DNAs from the Kasalath and FL134 cultivars revealed that *Bam*HI digestion produced polymorphic minor bands with sizes of 2.8 (in Kasalath) and 2.6 (in FL134) kbp, in addition to the major bands (4.5 kbp) which are common to both cultivars (Fig. 1).

The genomic DNAs of 144 individual F_2 plants of the cross Kasalath × FL134 were digested with *Bam*HI, subjected to electrophoresis, blotted to nylon membrane, and hybridized to the probes as described above. The autoradiogram data of this F_2 population were used for the linkage analysis. F_2 genotypes were classified according to the size of the minor band observed: type 1 (Kasalath type, 2.8-kbp band), type 2 (FL134 type, 2.6-kbp band), and type 3 (both bands) (Fig. 1). The map location of the catalase gene (locus name: *Cat-A1*) was determined to lie between two previously-known genomic RFLP markers on chromosome 3 (Saito et al. 1991), 2.1 cM from *XNpb-145*, and 1.4 cM from *XNpb-279* (Fig. 2).

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Correspondence to: A. Saito

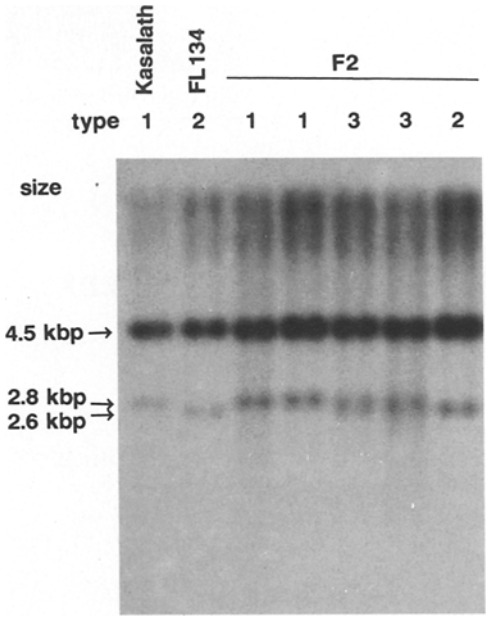

Fig. 1. Autoradiogram of *Bam*HI-digested genomic DNA of two parental lines (Kasalath and FL134) and several representative F_2 plants

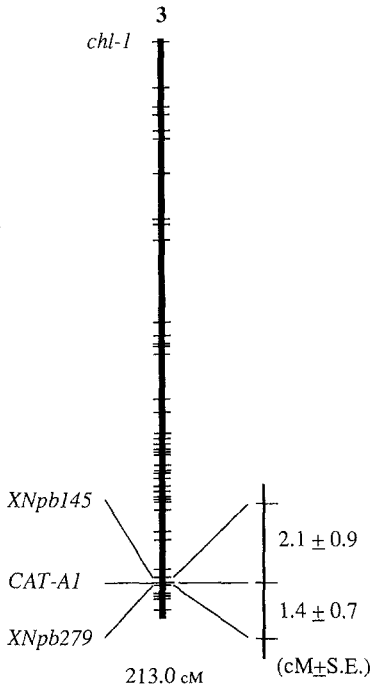

Fig. 2. Linkage map of rice chromosome 3 and the location of the catalase gene *Cat-A1*. Linkage relations and linear orders between the catalase gene and RFLP markers are shown at the right and the left of the *thick bar*, respectively. The total map distance of chromosome 3 (Saito et al. 1991) is shown at the bottom of the bar. Anonymous *horizontal bars* indicate other RFLP loci in the map; *chl-1* is a genetic marker that had previously been located in the map (Saito et al. 1991)

The chromosomal location of the *Cat-A1* locus differs from that of the previously-reported rice catalase locus (*Cat-1*) which has been located on chromosome 6 (chromosome nomenclature according to Khush 1990) by isozyme analysis (Wu et al. 1988; Ishikawa et al. 1989). It seems likely that the *Cat-1* gene encodes an isozyme different from that of the *Cat-A1* gene, although the possibility that the latter is a pseudogene cannot be ruled out. The former may correspond to the major 4.5-kbp band (Fig. 1), which we did not locate in the present work. In maize, genes for three catalase isozymes are located on two different chromosomes (Scandalios 1990).

Probe availability

Contact A. Saito.

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