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Linkage localization of the starch branching enzyme I (Q-enzyme I) gene in rice

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Source of probe and primers

Clone QEI (QEI-40, D10752), which contains a full-length cDNA encoding the starch branching enzyme I, or Q-enzyme I, was isolated from a cDNA library of developing rice endosperm in λ gt11(Nakamura and Yamanouchi 1992). Based on the sequence of this clone, we synthesized four oligonucleotides which were used as primers for the polymerase chain reaction amplification (Table 1). Using these synthesized primers and λ gt11 primers (Takara), three kinds of PCR products were prepared as probes: namely, a full-length cDNA (QEI-40-1), the open reading frame region (QEI-40-2), and the 3′ untranslated region which is specific for rice QEI (QEI-40-3). These three probes were employed for RFLP analysis and linkage mapping.

RFLP analysis, linkage analysis and the location of QEI

The plants used for mapping of the QEI gene were the two rice parental varieties, Nipponbare, a *japonica* variety, and Kasalath, an *indica* variety, and their F_2 progenies which consisted of 186 plants.

The level of polymorphism of each probe (QEI-40-1,2,3) was determined in the two parental varieties using eight restriction enzymes: *Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII, *Apa*I, *Dra*I, *Eco*RI and *Kpn*I (Fig. 1). Southern hybridizations were carried out with the ECL direct nucleic acid labelling and detection system (Amersham). In hybridization with QEI-40-1 and QEI-40-2, one-to-three hybridized bands were detected with all eight restriction enzymes (Fig. 1, A and B). The QEI-40-1 probe showed polymorphic bands with *Bam*HI, *Hin*dIII and *Apa*I. The QEI-40-2 probe also showed polymorphism with *Bam*HI and *Hin*dIII. In the case of the QEI-40-3 (3′-untranslated region) probe, a single band was detected in each lane with seven restriction enzymes, but not with *Apa*I (Fig. 1, C). This indicates that the gene encoding QEI is present in a single copy in the genomic DNA of rice based on the minimum number of hybridizing bands per genome with eight kinds of restriction enzyme. The DNA polymorphism with probe QEI-40-3 was recognized with two restriction enzymes, *Bam*HI and *Apa*I. A single and polymorphic band was detected with *Bam*HI.

The segregation of F_2 progenies was mainly scored for the presence of the 9.5-kb fragment (Nipponbare genotype), the 12-kb fragment (Kasalath genotype), or both fragments (heterozygote), with *Bam*HI. The heteroygote was clearly distinguishable and the presence of a fragment was codominant. In addition, the RFLP bands of QEI-40-3 with *Apa*I and of QEI-40-2 with *Hin*dIII were also examined. The linkage relationships were analysed with the

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Table 1 Oligonucleotide sequences used as primers

Probe	Primer name	Primer sequence (5′–3′)	Amplified region
QEI-40-1	λ gt forward[a] λ gt 11 reverse[a]	GGTGGCGACGACTCCTGGAGCCCG TTGACACCAGACCAACTGGTAATG	Full-length
QEI-40-2	Primer 1 (#40) Primer 2 (#40)	CAGTTGATGGTGCCACAATA CACTGACGTCTGCATTTATC	Open reading frame
QEI-40-3	Primer 3 (#40) Primer 4 (#40)	CGCGTTGATGAAGATCGTGA GAACAGCTGTTTTATTCTGG	3′-Untranslated

[a] λ gt11 primer set (Takara)

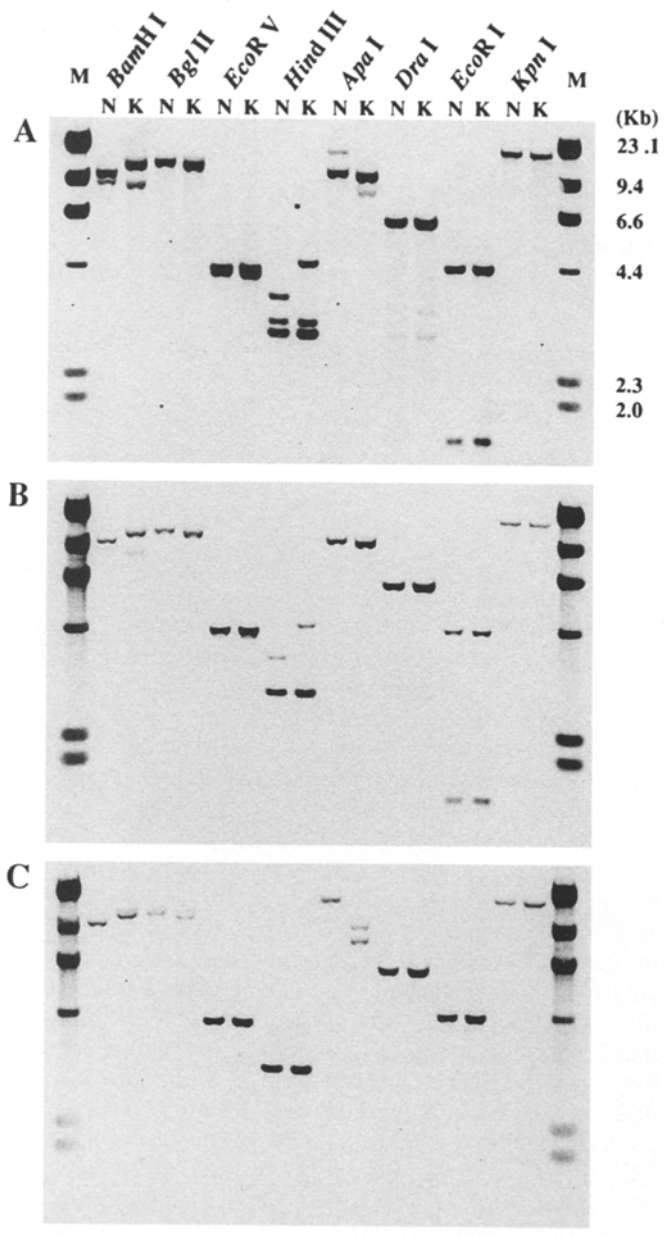

Fig. 1A–C Southern hybridization of probes QE1-40 to genomic DNA from the two parental varieties, Nipponbare (*N*) and Kasalath (*K*). Probes: **A** (QEI-40-1, full-length cDNA), **B** (QEI-40-2, ORF) and **C** (QEI-40-3, 3′UT region) Molecular-weight marker (*M*: λ DNA *Hin*dIII digest) on the right

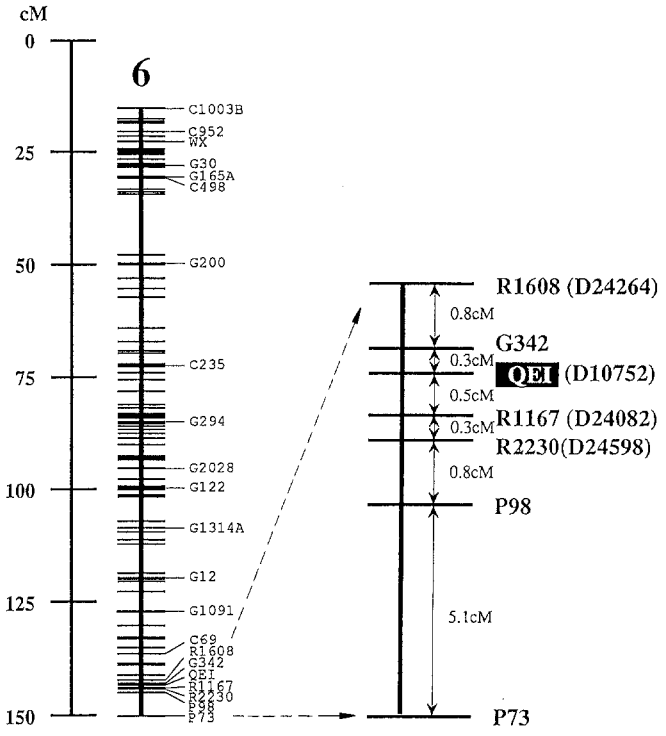

Fig. 2 Linkage localization and map distance of QEI on chromosome 6. The different markers are represented as follows: *G*XXX (random genomic clones), *R*XXX (cDNA clones derived from root library) and *P*XX (RAPD markers). The *D* number has already been submitted to the DNA DataBank of Japan (DDBJ)

software MAPMAKER (Lander et al. 1987) and the map position of QEI was determined on the existing high-density RFLP map of rice (Nagamura et al. 1993).

The results of linkage analysis indicated the same locus for the QEI-40-2 probe with the open reading frame and the QEI-40-3 probe with the 3′ UT region. Their probes were mapped on chromosome 6 with very tight linkage to the R1167 and G342 DNA markers and a map distance of 0.5 centimorgans (cM) and 0.3 cM, respectively (Fig. 2). It is generally known that the 3′UT region in expressed genes has a unique sequence. Therefore, it is concluded that the rice endosperm QEI gene is a single copy in the rice genome and was located in the region near the end of rice chromosome 6.

We are now examining the location of QEII, the other Q-enzyme isozyme in rice (Yamanouchi and Nakamura 1992), in an attempt to analyze the map location of branching enzyme II (QEII) relative to that of QEI.

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References

Lander ES, Green P, Abrahamson J, Baarlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Nakamura Y, Yamanouchi H (1992) Nucleotide sequence of a DNA encoding starch branching enzyme, or Q-enzyme I, from rice endosperm. Plant Physiol 99:1265–1266

Nagamura Y, Yamamoto K, Harushima Y, Wu J, Antonio BA, Sue N, Shomura A, Lin SY, Miyamoto Y, Toyama T, Kirihara T, Shimizu T, Wang ZX, Tamura Y, Ashikawa I, Yano M, Kurata N, Minobe Y (1993) A high-density STS and EST linkage map of rice. Rice Genome 2:3

Yamanouchi H, Nakamura Y (1992) Organ specificity of isoforms of starch branching enzyme (Q-enzyme) in rice. Plant Cell Physiol 33:985–991