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Chromosomal localization and genomic organization of α-amylase genes in rice (*Oryza sativa* L.)

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Summary. Genes for α-amylase, alcohol dehydrogenase, and *Em*, an ABA-regulated gene expressed late in embryogenesis, were localized on rice chromosomes by the analysis of primary trisomics. The validity of the mapping approach was confirmed using *Adh-1* as a control. The *Adh-1* gene has previously been assigned to chromosome 11 using conventional techniques. In this study we confirm this assignment and report an additional locus for alcohol dehydrogenase (*Adh-2*) on chromosome 9. The α-amylase genes were located on chromosomes 1, 2, 6, 8, and 9 while the *Em* gene was mapped to chromosome 5. To facilitate trisomic analysis and correlation of cloned genes with bands observed on Southern blots, a nomenclature for the rice α-amylase genes has been proposed. In addition to mapping nine cloned α-amylase genes, we have identified two previously uncloned α-amylase genes as part of this study. Polymorphism for α-amylase genes belonging to each of the three subfamilies was observed between M202 and IR36. The maximum degree of polymorphism was found among genes belonging to the RAmy3 subfamily, which also has the most diverse group of genes.

Key words: Chromosomal mapping – Trisomic analysis – Molecular markers

Introduction

Alpha-amylases (EC3.2.1.1) are endoglycolytic enzymes that play an important role in cereal seed germination. They are primarily responsible for the hydrolysis of the starchy endosperm, producing the sugars needed to sustain the growth of the emerging seedling (for a review, see Fincher 1989). For this reason, expression of the α-amylase activity has been positively correlated to important agronomic traits such as germination rate, seedling vigor, yield, and cold tolerance (Williams and Peterson 1973).

In wheat and barley, Southern blot analysis of genomic DNA probed with α-amylase cDNA clones indicates that the isozymes of α-amylase are encoded by a multigene family (Lazarus et al. 1985; Khursheed and Rogers 1988). The barley α-amylase genes have been classified into two distinct subfamilies (Amy1 and Amy2), based on the isoelectric points of the isozymes encoded by them (the high pI and the low pI groups, respectively) (Khursheed and Rogers 1988). Using Southern blot analysis of wheat-barley addition lines, the high pI genes have been mapped to chromosome 6 and the low pI genes to chromosome 1 (Muthukrishnan et al. 1984). Following a similar classification scheme, the wheat α-amylase genes have been divided into three subfamilies. A similar analysis of nulli-tetrasomic wheat lines indicates that the Group I isozymes (pIs 6.3–7.5) are encoded by the α-*Amy1* class of homoeologous loci located on chromosome 6. The Group II isozymes (pIs 4.9–6.0) are encoded by the α-*Amy2* class of homoeologous loci, residing on chromosome 7 (Lazarus et al. 1985). The α-*Amy3* class is made up of a small group of genes expressed only in immature grains and has been mapped to chromosome 5 (Baulcombe et al. 1987).

In rice the α-amylases are also encoded by a multigene family. Thirty distinct genomic clones, representing eight genes, have been isolated and characterized from rice genomic libraries (Huang et al. 1990 b). These genes have been classified on the basis of cross-hybridization into five groups. Two additional genes, not present among

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these genomic clones, were identified as a result of this study.

Although nulli-tetrasomics and addition lines are not available in rice, mapping of genes to chromosomes has been accomplished using Southern blot analysis of genomic DNA from primary trisomics (McCouch et al. 1988). The rationale for this approach is based on the assumption that the critical trisomic (*AAA*), having three copies of the chromosome from which the restriction fragment originates, will exhibit gene dosage effects for that fragment. These effects can be detected as an increase in hybridization signal of the fragment of unknown location in Southern blots of DNA from the critical trisomic. Trisomics that are not critical (*AA*) will behave as disomics and will not show increased dosage of the restriction fragment in their DNA (Young et al. 1987).

We have employed a similar approach of utilizing inbred primary trisomics to localize known genetic sequences to their respective chromosomes. To determine whether an increased hybridization signal was due to true dosage effect or to interlane variations in DNA loading, another single-copy genomic fragment detected by a different probe was used as an internal standard in our study. Although any fragment can be used to make intralane comparisons, single-copy genomic sequences are preferable. Using this approach, two or more genes can be localized simultaneously if they reside on different chromosomes. No dosage effect is apparent if both genes are on the same chromosome.

In this paper, we mapped nine α-amylase genes to five different rice chromosomes and characterized the restriction site polymorphisms for these genes between M202 and IR36 varieties. In the process of mapping the α-amylase genes we determined the chromosomal location of the *Em* gene, an ABA-regulated gene expressed late in embryogenesis (Litts et al. 1987; Williamson and Quatrano 1988), and identified a second locus for rice alcohol dehydrogenase (*Adh-2*).

Materials and methods

Plant materials

Freeze-dried leaf material from the 12 primary trisomics used in this study was obtained from the International Rice Research Institute (IRRI), Los Banos, The Philippines. These trisomics were developed from the Indica rice variety IR36 (Khush et al. 1984). The cDNA clones used in this study were obtained from mRNA isolated from M202, a medium-grain, short-stature, and photoperiod non-sensitive rice variety (O'Neill et al. 1990). This variety was derived through the following crosses: 'IR-8'/'CS-M3'*2//'10-7'*2/3/'M-101'. Genomic clones were obtained from rice libraries constructed and characterized by Huang et al. (1990b).

DNA extraction and purification

DNA was extracted from each of the trisomic lines using the CTAB procedure (Saghai-Maroof et al. 1984). The precipitated DNA was spooled out, rinsed in 70% ethanol, and resuspended in 0.5 ml of TE buffer. The DNA was RNased (to 40 μg/ml) and extracted with 1 vol. of phenol/chloroform (1:1). The supernatant was extracted once with 1 vol. of chloroform/isoamyl alcohol (24:1) and the DNA was precipitated with 1/3 vol. of 10 *M* NH_4Ac and 2 vol. of 95% ethanol, resuspended in 0.5 ml of TE, and stored at 4 °C over chloroform.

Southern analysis

Samples containing 40 μg of DNA were digested with *Eco*RI (4 units/μg of DNA) for a period of 4–6 h under the conditions specified by the manufacturer. Ten micrograms aliquots of the *Eco*RI digest was further digested with *Bam*HI, *Hin*dIII, and *Kpn*I for double digests. Following digestion, the restriction fragments were separated by electrophoresis in 0.8% agarose gels. The DNA was transferred to a nylon (Nytran) membrane according to the procedure of Southern (1975).

A 1,555-bp *Xba*I fragment from the rice α-amylase cDNA clone pOS103 (O'Neill et al. 1990) was used to hybridize to *RAmy1* genes. A 2,300-bp *Sal*I fragment from the genomic clone λOSg6A was used to identify the RAmy2 subfamily (Huang et al. 1990b). The diversity of genes in the RAmy3 subfamily necessitated the use of several probes on high stringency blots to identify all these genes: (1) a cDNA clone, pOS137 (1,682-bp *Xba*I fragment), (2) a 2,000-bp *Eco*RI-*Hin*dIII fragment (gene *A*) from the genomic clone λOSg7D, (3) a 1,300-bp *Sph*I-*Sma*I fragment (gene *B*) from the genomic clone λOSg1, and (4) a 2,100-bp *Hin*dIII-*Xba*I fragment from the genomic clone λOSg7F. The probe for the rice *Em* gene was a 600-bp *Kpn*I-*Sal*I fragment from the rice genomic clone λOSg4B, which included 300 bp of the coding region plus 300 bp of the 5′ flanking sequence (J. Litts and R. Rodriguez, unpublished results). The rice *Adh-1* and *Adh-2* genes were detected by hybridization with the 710- and 880-bp *Pst*I fragments of maize *Adh-1*, cDNA clone pZML793 (Gerlach et al. 1982), kindly provided by M. M. Sachs.

Genomic blots were prehybridized (12–15 h) and hybridized (12–24 h) at 42 °C in a solution containing 5X SSPE, 5X Denhardt's (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA), 0.5% SDS, 200–400 μg/ml sheared, denatured salmon sperm DNA, and 50% formamide. The probes were labeled by the random hexamer primer method (Feinberg and Vogelstein 1983) to specific activities of 0.5 to 1×10^9 cpm/μg DNA. Hybridizations were carried out with 4 to 5×10^6 cpm/ml. The membranes were washed sequentially in 50% formamide, 5X SSPE, 0.2% SDS for 30 min at room temperature, in 0.2X SSPE, 0.2% SDS at 50 °C for 1 h, and then twice in the latter buffer at 65 °C for 2 h each. The low stringency blots were then exposed to X-ray film. High stringency blots received an additional wash in 0.1X SSPE, 0.1% SDS at 71 °C for 1 h. For the heterologous *Adh-1* probe, the stringency of washings was reduced by lowering the temperature of the final wash to 60 °C. The membranes were then exposed at −70 °C for 8 to 24 h to Kodak XAR-5 film with an intensifying screen. Autoradiographs were scanned on a Zeineh Soft Laser scanning densitometer (Biomed Instruments, Inc.) with computer data collection and analysis. The accuracy of the densitiometry was confirmed using a video image analysis system, DNA Vision, (Biological Vision, San Mateo/CA). Between three to six independent probings of three to six blots were used to obtain autoradiographs. The hybridization signals on these autoradiographs were quantified by scanning densitometry.

Statistical analysis

One-way analysis of variance and one-tailed student's *t*-test were employed to determine the location of *Em*, *Adh*, and various α-amylase genes.

Table 1. Nomenclature for rice α-amylase genes

Gene name	Genomic[a] clone	Hybridization[a] group
RAmy1A	λOSg2	1
RAmy1B	λOSg3A	1
RAmy1C	λOSgX[b]	1
RAmy2A	λOSg9C	4
RAmy3A	λOSg7D(geneA)	3
RAmy3B	λOSg1(geneB)	3
RAmy3C	λOSg1(geneC)	3
RAmy3D	λOSg1A	2
RAmy3E	λOSg7F	5
RAmy3F	λOSgY[b]	6

[a] See Huang et al. 1990a, b
[b] Gene for which genomic clone still unavailable

Results and discussion

Nomenclature for the rice genes

To facilitate the discussion of the Southern blots, a nomenclature is presented for the α-amylase genes of rice (Table 1). This nomenclature is based upon the degree of DNA sequence homology found among the α-amylase genes of rice, barley, and wheat (Huang et al. 1990a; Rodriguez et al. 1991). Although there is a consensus on nomenclature of wheat α-amylase genes, different systems of nomenclature exist in the literature for barley α-amylase genes. In barley, the nomenclature is based on the isoelectric points of the enzymes encoded by the genes. Thus, genes encoding low pI isozymes have been referred to as *Amy1* (Aoyagi et al. 1990), *Amy2* (Knox et al. 1987), and low pI gene family (Fincher 1989). The genes encoding high pI isozymes have been referred to as *Amy2, Amy1,* and high pI gene family by the same respective research groups. The rice RAmy1 and RAmy2 subfamilies consist of groups of genes corresponding to the Amy1 and Amy2 classes of barley (Knox et al. 1987) and wheat (Lazarus et al. 1985), while genes in the rice RAmy3 subfamily correspond to those in the Amy3 subfamily of wheat (Baulcombe et al. 1987).

Association of genes to Southern blot bands

In order to correlate the bands observed on Southern blots with particular cloned genes, representative clones from each subfamily were used to probe digests of rice genomic DNA. A composite of the results of these hybridizations is shown in Fig. 1. Each of the three panels of this figure displays the results obtained with M202 and IR36 DNA digested with a different combination of enzymes. Each pair of lanes in the panel depicts the results obtained from hybridization using different probes and stringency conditions. The first two lanes in each panel show the autoradiographic pattern produced by cDNA clone pOS137 (*RAmy3D*) at a stringency that allowed detection of all α-amylase genes. The same blot was stripped and hybridized with each of six probes representing the three subfamilies under high stringency conditions. These conditions are known to discriminate between the five α-amylase hybridization groups previously reported by Huang et al. (1990b). Arranging the data in this manner helps identify which band(s) in each digest corresponds to each probe. Restriction maps of the isolated clones (Huang et al. 1990b) enabled us to associate particular band(s) with a clone containing that frg-ment(s), even when a given probe hybridized to multiple fragments. In this way, individual genes could be associated with particular bands in each digest. Bands for which no clones were available were thus identified and assigned to the probe that produced the strongest hybridization signal for that band.

RAmy1 subfamily

Fragments containing the RAmy1 subfamily of genes were identified by probing with pOS103 under high stringency conditions. Three bands were found in each of the digests (Fig.1). This indicates that *RAmy1* contains three pOS103-like genes in both M202 and IR36. These bands and the genes contained on them were designated *RAmy1A*, *1B*, and *1C*. The molecular weights of the *RAmy1A* and *1B* bands were consistent with the restriction maps of λOSg2 and λOSg3A, respectively, in all three digests (Huang et al. 1990b). Gene *RAmy1C* has not yet been cloned. The enzymes used in our study detected no restriction site polymorphism between IR36 and M202 for genes *RAmy1A* and *RAmy1B,* however, *RAmy1C* was dimorphic with respect to an *Eco*RI site. The trisomic analyses were performed on the *Eco*RI bands of 11.5 kb (*RAmy1C*), 4.9 kb (*RAmy1B*), and 3.9 kb (*RAmy1A*).

RAmy2 subfamily

All digests produced a single band hybridizing to the *RAmy2* probe (λOSg6A) and were confirmed by independent analyses of the *RAmy2* gene in M202 (N. Huang, unpublished results). This band was the same size in M202 and IR36 DNA and indicates that the RAmy2 subfamily consists of a single gene, *RAmy2A,* contained on the genomic clone λOSg6A. *RAmy2A* is located on an 8.2-kb *Eco*RI fragment lacking *Bam*HI and *Kpn*I sites, but which is reduced to 5.1 kb by *Hin*dIII digestion. The 8.2-kb *Eco*RI fragment was used to map the *RAmy2A* gene on the trisomic blots.

RAmy3 subfamily

This subfamily is both the most complex and the most divergent of the rice α-amylase subfamilies (Table 1). It

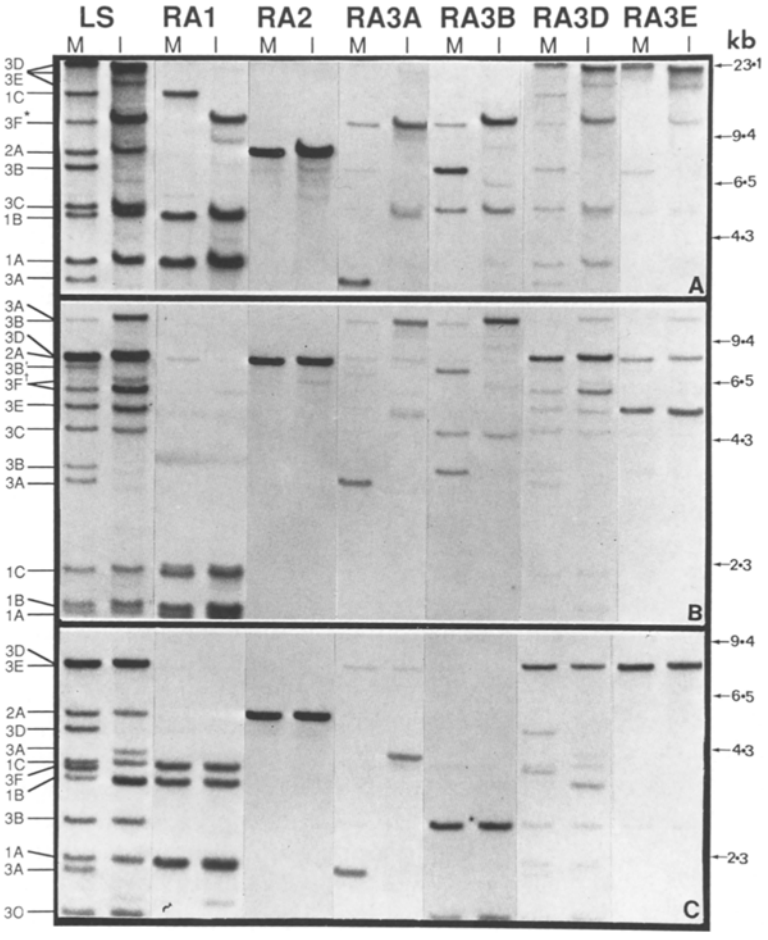

Fig. 1 A–C. Southern analysis of restriction-digested chromosomal DNA showing association of genes with bands. M202 and IR36 DNA was digested in sets with different restriction enzymes, run on agarose gel, blotted onto membranes, and probed with cDNA clone pOS137 at low stringency (LS). In each pair, M represents M202 DNA and I represents IR36 DNA. Panel **A** shows the restriction fragments released by an *Eco*RI digest. Panel **B** shows the fragments generated by the *Eco*RI/*Bam*HI digest. Panel **C** shows the fragments derived from the *Eco*RI/*Hin*dIII digest. The blot was stripped and reprobed with clones from each gene family (RAmy 1, 2, and 3) under group-specific hybridization conditions to identify the band(s) associated with each gene. * In IR36, *RAmy1C*, *RAmy3A*, and *3B* and a fragment from *RAmy3D* comigrate at this position. † In IR36 a fragment from *RAmy3D* comigrates at this position. Another fragment of slightly higher molecular weight that is not specifically picked up by any clone is also observed in IR36. It hybridizes weakly to pOS137 at low stringency and is likely to be analogous to *RAmy3F* of M202

consists of two groups of linked genes. *RAmy3A, 3B,* and *3C* have been characterized by Sutliff et al. (1991) while *RAmy3D* and *3E*, which belong to hybridization Groups 2 and 5, respectively, have been recently characterized by Huang et al. (1990a). The presence of a sixth gene (*RAmy3F*) was identified in this study but it has not yet been cloned. With the exception of *RAmy3B* and *3C*, which are about 95% identical at the protein level (Sutliff et al. 1991), the other genes in this subfamily are only 60–70% identical to each other (Huang et al. 1990a). Due to this diversity, four probes were used to detect all members of the RAmy3 subfamily.

The *RAmy3A* gene was detected by hybridization with a fragment from λOSg7D. This probe hybridized strongly with a single band in each lane. The probe also hybridized to a lesser extent to several bands from other members of the RAmy3 subfamily (Fig. 1). Polymorphism between IR36 and M202 was detected by this probe. Except for the *Eco*RI/*Bam*HI digest, the *RAmy3B* probe from λOSg1 hybridized most intensely to a single band in each of the digests and with lesser intensity to another band corresponding to *RAmy3C*. The *RAmy3B* probe detected polymorphism between M202 and IR36 as well as within the M202 variety. This polymorphism was evident by the fact that the *RAmy3A* and *3B* probes, which hybridize to different fragments in M202 *Eco*RI and *Eco*RI/*Bam*HI digests, hybridized to an identical 11.5-kb fragment in the same digests of IR36 (Fig. 1, panels A and B). This indicates the presence of *Eco*RI and *Bam*HI sites between the *RAmy3A* and *3B* genes in M202 that are not present in IR36. The additional *Eco*RI site in M202 is located 3.5 kb from the *RAmy3A* end of the 11.5-kb *Eco*RI fragment in IR36 (Huang et al. 1990b). Likewise, the *Bam*HI site is present at approximately 3.5 kb from the *RAmy3B* end of the 11.5-kb *Eco*RI fragment of IR36 (Huang et al. 1990b). Within M202, the *RAmy3B* probe detects two bands of 7.5 kb (3B′) and 3.5 kb (3B) with equal intensity in the *Eco*RI/*Bam*HI digest (Fig. 1, panel B). We believe that these bands represent a polymorphism for a *Bam*HI site in the vicinity of the *RAmy3B* gene. We have not yet determined whether the two bands of equal intensity detected by *RAmy3B* are due to duplication or heterozygosity. The 11.5-kb *Eco*RI fragment from IR36 containing

RAmy3A and *3B* was analyzed on trisomic DNA Southern blots of both *Eco*RI and *Eco*RI/*Bam*HI digests for mapping these genes.

The *RAmy3C* gene is easily observed as a less intense 5.4-kb band on blots hybridized with the *RAmy3B* probe, because of a very high degree of homology between these two genes (Fig. 1, panel B). No heterogeneity was found in the restriction sites surrounding *RAmy3C* with the enzymes used in this study. The 5.4-kb *Eco*RI fragment was mapped by trisomic analysis using both *Eco*RI and *Eco*RI/*Bam*HI digests.

The *RAmy3D* gene was analyzed by hybridization to the pOS137 cDNA, which is identical to the exons of λOSg1A (Huang et al. 1990a). The probe hybridized to a single predominant band in all digests of M202, indicating the presence of a single *RAmy3D* gene. Except for the *Eco*RI/*Bam*HI digest, the probe for *RAmy3D* gene hybridized to a single fragment in IR36 DNA as well. Two fragments of 8 kb and 6 kb could be observed in the *Eco*RI/*Bam*HI digests of IR36. These two fragments could either be due to the presence of an internal *Bam*HI site in the *RAmy3D* gene in IR36 or to the presence of two genes analogous to *RAmy3D* gene of M202. In addition to the polymorphism for the *Bam*HI site, the *RAmy3D* genes of IR36 and M202 were also polymorphic for an *Eco*RI site (Fig. 1, panel A). The 9-kb *Eco*RI/*Hin*dIII band was analyzed in the trisomic DNA to assign *RAmy3D*.

Gene *RAmy3E* was identified by hybridization with a fragment of λOSg7F. The *Eco*RI, *Eco*RI/*Hin*dIII (Fig. 1, panels A and C), and *Eco*RI/*Kpn*I bands (data not shown) detected by the λOSg7F probe were identical in size to those detected by the *RAmy3D* probe (λOSg1A). This is consistent with recent work showing that genomic clones for *RAmy3D* and *3E* are tightly linked (Huang et al. 1990a). The 9-kb *Eco*RI/*Hin*dIII fragment was analyzed in order to assign the chromosomal location of *RAmy3E* gene.

When probed with pOS137 cDNA probe at low stringency, an unidentified band was observed in all digests of M202 DNA (Fig. 1, panels A–C). This band, however, does not hybridize with any other α-amylase probes at high stringency. A similar band of 6.5 kb was observed in IR36 DNA digested with *Eco*RI/*Bam*HI, but is absent from all other digests of IR36 DNA. This suggests the presence of a sixth member of the RAmy3 subfamily in M202. This gene has been given the tentative designation *RAmy3F*. Like the *RAmy1C* gene, a genomic or cDNA clone for *RAmy3F* has not yet been isolated. Since we have been unable to clearly relate the band containing *RAmy3F* to a unique IR36 band at high stringency, this putative α-amylase gene could not be mapped.

The data presented in Fig. 1 establishes the relationship between specific α-amylase probes and band(s) observed on Southern blots. The associations between bands and specific genomic clones are summarized in Table 1. The results of these studies indicate that the α-amylase multigene family comprises a minimum of 10 genes, two of which have yet to be cloned.

Chromosomal assignments

Previous studies indicated substantial linkage among the rice α-amylase genes. Therefore, we sought an internal standard that was single copy and unlinked to any of the α-amylase genes for measuring dosage effects. The *Em* gene proved to be a suitable candidate because only a single band was obtained by hybridizing the *Em* probe to *Eco*RI digests of M202 DNA (Fig. 2B). Since all other restriction digests used in this study also showed a single band (data not shown), we concluded that *Em* was a single-copy gene in rice. Furthermore, subsequent multiple comparisons of various α-amylase fragments to the *Em* fragment consistently indicated that *Em* was located on chromosome 5.

Using the *Em* gene as an internal standard for measuring gene dosage, we localized the α-amylase genes to their respective chromosomes based on dosage effects observed in primary trisomic lines. Total genomic DNA from each line was digested with *Eco*RI and *Eco*RI/*Hin*dIII and subjected to Southern blot analysis. Blots were hybridized with two probes simultaneously: *Em* and an α-amylase probe from each of the five hybridization groups under group-specific conditions (Fig. 3). Scanning densitometry was used to measure the intensities of

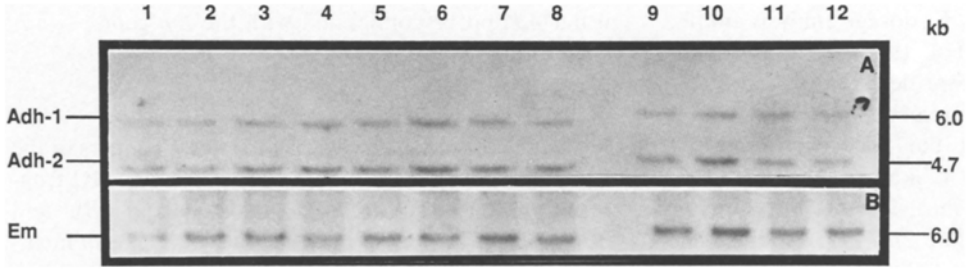

Fig. 2A and B. Positive control and internal standard. The *Adh-1* probe hybridized strongly to two bands in all 12 trisomics, and *Em* hybridized to a single band in all trisomics digested with *Eco*RI. * The *RAmy1C* band shows a slightly higher migration in trisomics 9–12 as compared to trisomics 1–8, because the DNA was run on two different gels and blotted on to one piece of membrane. The relative position of all bands in all the panel is, however, the same

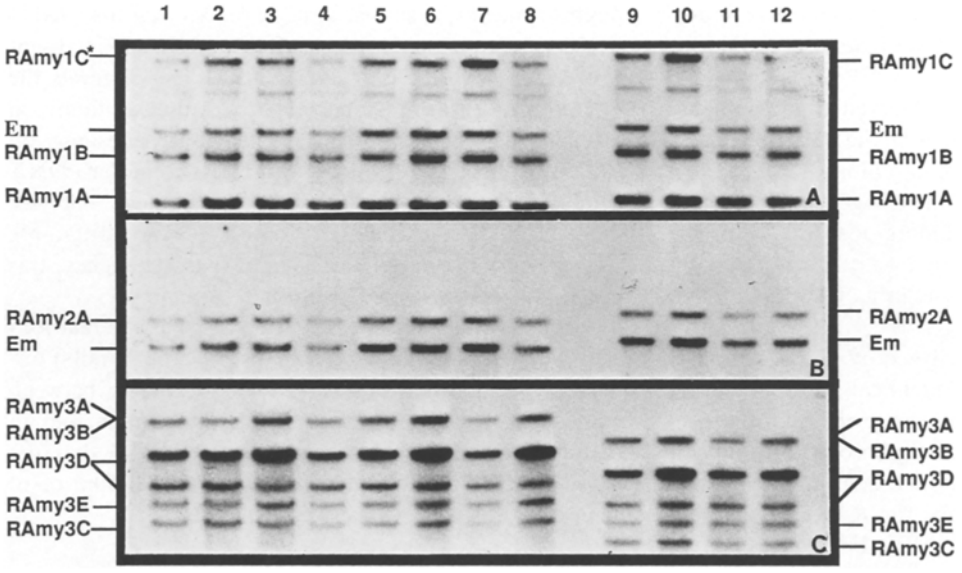

Fig. 3. Southern analysis of trisomic DNA. Trisomic DNA, digested with *Eco*RI and *Eco*RI/*Bam*HI, was run on agarose gels and blotted on membranes. The membranes were probed with representative clones from each of the gene families under group-specific hybridization conditions

α-amylase and *Em* hybridization signals in each lane. Ratios of intensities of α-amylase to *Em* were calculated, and multiple scans were averaged to obtain the mean intralane ratio of hybridization intensity for each pair of genes (i.e., a specific α-amylase gene and *Em* gene; Table 3). One-way analysis of variance indicated that there were significant differences among trisomic ratios for each α-amylase gene. Trisomic 5 consistently showed the lowest ratio with respect to all α-amylase probes used. One-tailed student's *t*-test for each α-amylase gene indicated that one of the trisomics showed a ratio that was significantly larger than the average ratio of all other trisomics (excluding trisomic 5). This trisomic was considered to be the critical trisomic indicating the location of a particular α-amylase gene.

To verify whether or not a change in the copy number of a gene in a particular trisomic could be detected as an increase in intensity of hybridization signal from that trisomic, a probe for maize *Adh-1* (Gerlach et al. 1982) was hybridized to *Eco*RI-digested genomic DNA from the 12 primary trisomics. Rice *Adh-1* was previously localized on chromosome 11 by F_1 dosage analysis and F_2 segregation data (Ranjhan et al. 1988). Two restriction fragments of 6 and 4.7 kb were detected by the *Adh-1* probe (Fig. 2A). Intralane comparisons of the intensities of each band revealed that the 6-kb band had the strongest signal relative to the 4.7-kb band in trisomic 11, while the 4.75-kb band was most intense in trisomic 9 relative to the 6-kb band (Table 2). This indicated that rice has two loci for alcohol dehydrogenase, one on chromosome 11 and the other on chromosome 9. These results validate the accuracy of this approach and are consistent with a recent report that describes two different

Table 2. Relative ratios of Adh restriction fragments[a]

Trisomic	
T-1	1.66
T-2	2.55
T-3	2.05
T-4	1.45
T-5	1.72
T-6	1.61
T-7	1.64
T-8	2.62
T-9	4.18*
T-10	2.27
T-11	1.02*
T-12	1.55

[a] Ratio of 4.7-kb fragment to 6-kb fragment
* Significant at the 1% level

Adh genes in rice (Yong and Wu 1989). We could not use *Em* as an internal standard for assigning *Adh* genes to chromosomes, because one of the fragments hybridizing to the *Adh* probe comigrated with the *Em* gene.

RAmy1A, 1B, and 1C

The results of this analysis indicated that members of the RAmy1 subfamily were contained on three *Eco*RI fragments of 3.9 kb (*RAmy1A*), 4.9 kb (*RAmy1B*), and 11.5 kb (*RAmy1C*). The ratio of the hybridization intensity of the *RAmy1A* restriction fragment to the *Em* band was highest for trisomic 2 and lowest for trisomic 5 (Table 3, column 1). Ratios for the *RAmy1B* and *RAmy1C* band were highest for trisomics 1 and 2, respectively, and lowest

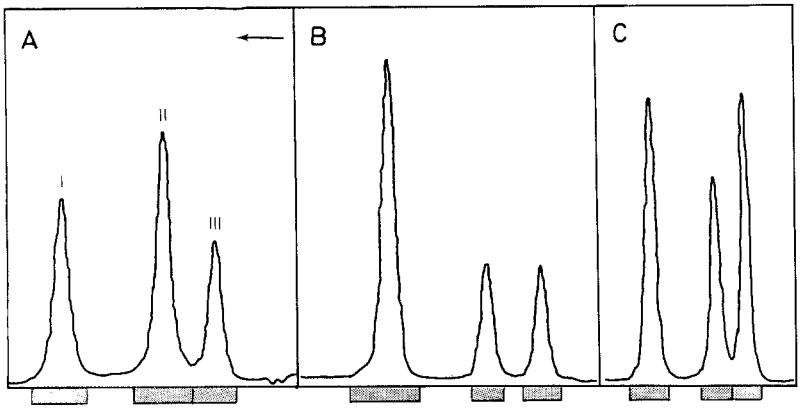

Fig. 4 A–C. Densitometer scans of the autoradiographs. In each panel, I depicts the peak for *RAmy1A*, II depicts the peak for *RAmy1B*, and III depicts the peak for the internal standard *Em*. **A** Scans of autoradiograph of trisomic 1 lane showing the highest peak for *RAmy1B*. **B** Scans of autoradiograph of trisomic 2 lane showing the highest peak for *RAmy1A*. **C** Scans of autoradiograph of trisomic 5 lane showing the high peak for *Em*

Table 3. Ratios of *RAmy 1, 2,* and *3* and *3* α-amylase genes to *Em* gene

Trisomic	*RAmy1*			*RAmy2*	*RAmy3*		
	A	B	C	A	A and B	C	D and E
T-1	1.95	1.67*	0.74	2.07	0.98	1.00	1.71
T-2	3.19*	1.15	1.61**	2.34	0.86	1.00	1.63
T-3	1.75	1.20	0.84	2.59	0.88	1.00	1.75
T-4	1.41	0.98	0.78	2.00	0.95	1.05	1.86
T-5	0.85**	0.64**	0.49**	1.50*	0.63**	0.40**	1.15*
T-6	1.62	0.96	1.07	3.68**	0.95	1.00	1.69
T-7	1.92	1.05	1.08	1.87	0.85	0.80	1.78
T-8	1.66	1.14	1.05	2.43	0.85	0.95	2.84*
T-9	2.02	0.97	1.02	2.82	1.28**	2.25**	1.91
T-10	1.95	1.15	1.14	2.78	1.09	0.92	1.82
T-11	1.70	0.97	0.82	2.08	0.82	0.92	1.60
T-12	1.46	0.99	0.75	2.12	0.79	0.88	1.84

*,** Significant at the 5% and 1% levels, respectively

for trisomic 5. Figure 4 displays the scans obtained for trisomic 1 (panel A), trisomic 2 (panel B), and trisomic 5 (panel C). In each panel the first peak is for the *RAmy1A* fragment, the second peak is for *RAmy1B*, and the third peak is for *Em*. In trisomic 5, the *Em* peak is highest as compared to the other two peaks, while in trisomics 2 and 1 the peaks for *RAmy1A* and *RAmy1B* are higher, respectively. This indicates that *RAmy1A* and *1C* were located on chromosome 2, *RAmy1B* on chromosome 1, and the *Em* gene on chromosome 5.

RAmy2A

A single gene belonging to the RAmy2 subfamily was present on an 8.2-kb *Eco*RI fragment. Table 3 (column 5) shows that the probe for this group exhibited dosage effect in trisomic 6. Therefore, *RAmy2A* was localized on chromosome 6. The ratio for trisomic 5 was lowest, as expected, indicative of the location of *Em* gene.

RAmy3A, 3B, 3C, 3D, and 3E

The *RAmy3A* and *3B* genes were found as a single 11.5-kb *Eco*RI fragment, and *RAmy3C* was contained on a 5.4-kb *Eco*RI fragment. Ratios for both bands relative to *Em* were highest in trisomic 9 and lowest in trisomic 5 (Table 3, columns 6 and 7). These results indicate that the α-amylase genes are linked on chromosome 9, and supported the conclusion that *Em* is on chromosome 5.

RAmy3D and *3E* were difficult to resolve and localize on the basis of single *Eco*RI digests because they were contained on an *Eco*RI fragment greater than 23 kb. However, an *Eco*RI/*Hin*dIII digest of primary trisomic DNA separated these genes on smaller fragments. In *Eco*RI/*Hin*dIII digests, the ratio for the 9-kb *RAmy3D* and *3E* bands to *Em* was highest for trisomic 8 and lowest for trisomic 5 (Table 3, columns 8). This indicates that *RAmy3D* and *3E* genes are present on chromosome 8, which is consistent with studies indicating that these two genes are closely linked (Huang et al. 1990a). When *RAmy3A, 3B, 3C, 3D,* and *3E* were localized with each other as internal standards, the assignments were the same (data not shown). The locations of nine α-amylase genes on their respective chromosomes are summarized in Fig. 5.

The experimental approach outlined in this report has proven to be an effective and economical means of

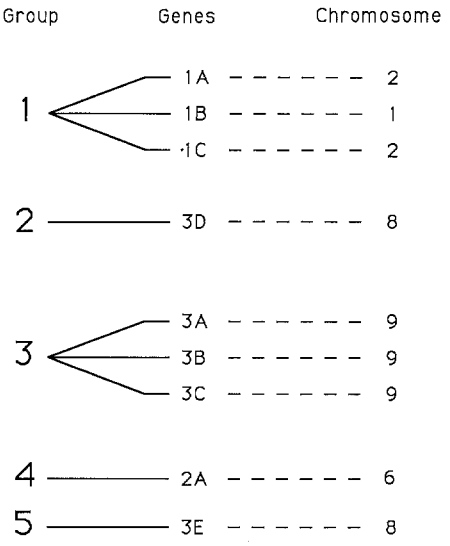

Fig. 5. Summary of chromosomal locations for the rice α-amylase genes. 'Group' refers to the hybridization groups identified by Huang et al. (1990b), while 'gene' refers to the *RAmy* gene designations adopted for this analysis

mapping several cloned genes and restriction fragments on the chromosomes of rice. This approach should be useful to those involved in plant genome mapping and sequencing projects. A similar approach has already been used for constructing RFLP maps of tomato (Vallejos et al. 1986), maize, and rice genomes (McCouch et al. 1988). Our results for the chromosomal locations of rice α-amylase genes have been confirmed by linkage analysis of F_2 segregation data by S. D. Tanksley (personal communication).

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