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Chromosomal localization of four isozyme loci by trisomic analysis in rice (*Oryza sativa* L.)

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Summary. Four genes coding for isozymes in rice (*Oryza sativa* L.), were located to respective chromosomes through trisomic analysis. Twelve primary trisomics in IR36 background were crossed with 2 lines having contrasting alleles at four loci. For each gene, all 12 disomic and trisomic F_1 hybrids were screened for allele dosage effects. Either F_2 or BC1 populations of all cross combinations were assessed for gene segregation. Evidence from both sources indicated the following locations: *Pgi-1* on chromosome 4, *Sdh-1* on chromosome 6, *Est-8* on chromosome 7 and *Adh-1* on chromosome 11. The location of *Sdh-1* was further confirmed through the production of triallelic heterozygotes with trisomic 6.

Key words: Isozymes – Chromosomal location – Trisomic analysis – Gene dosage effects

Introduction

More than fourty polymorphic genes encoding isozymes have been reported in the *Sativa* group of Genus *Oryza* (Pai et al. 1973, 1975; Nakagahra 1978; Second and Trouslot 1980; Second 1982, 1985; Morishima and Sano 1984; Glaszmann et al. 1984; Glaszmann 1985; Sano and Barbier 1985. Linkage tests among these loci have been of little utility in determining their chromosomal locations. To date chromosomal location of only three genes, namely *Est-2, Amp-3,* and *Pgi-2,* have been determined and all were found to be on chromosome 3 (Nakagahra and Hayashi 1976; Sano and Morishima 1984; Sano and Barbier 1985). Availability of complete series of primary trisomics in the backgrounds of Indica (Khush et al. 1984) and Japonica (Iwata and Omura 1984) rices has provided new tools for locating isozyme loci to respective chromosomes.

We determined the chromosomal location of *Sdh-1, Adh-1, Est-8* and *Pgi-1* using the primary trisomics of indica rice.

Materials and methods

Materials used

Twelve primary trisomics, and two rice varieties were used in the study. The primary trisomics were produced at the International Rice Research Institute (IRRI) and are in the background of indica rice variety IR36 (Khush et al. 1984).

The diploid lines Mayin (Acc. 33364) and Chau, (Acc. 56036) were obtained from the International Rice Germplasm Center (IRGC) of IRRI. Both are Indica rices. As compared to IR36, Mayin carries contrasting alleles at *Sdh-1, Adh-1* and *Pgi-1* loci and Chau has contrasting alleles at *Sdh-1, Est-8* and *Pgi-1* loci (Table 1).

The 12 trisomics were crossed with the two varieties and 24 F1 populations were obtained. The trisomic F1 plants could be identified morphologically. They were subsequently selfed to obtain the F2 progenies. Since trisomics for chromosomes 1 and 4 are highly sterile, F2 populations from the

Table 1. The allelic constitution of IR36, Mayin and Chau at four loci under investigation

Materials	Acc. no.[a]	Alleles at loci			
		Sdh-1	*Adh-1*	*Est-8*	*Pgi-1*
IR36	30416	1 (4)[b]	1	1	1
Mayin	33364	2	3	1	2
Chau	56036	2	1	2	2

[a] Accession number in the world collection maintained at IRRI
[b] Trisomic plants heterozygous at *Sdh-1* were also used

hybrids of these trisomics could not be obtained. Therefore their F_1's were backcrossed to the test varieties.

Isozyme analysis

Isozyme analysis was performed with crude extracts of water soluble proteins prepared from various plant tissues. These were subjected to 6 h horizontal starch gel electrophoresis at 4 °C at pH 8.0 as described by Second (1982). Anodal slices were assayed for Shikimate dehydrogenase (SDH), Alcohol dehydrogenase (ADH), and Phosphoglucose isomerase (PGI). One cathodal slice was assayed for esterase (EST). The staining procedures were described by Second and Trouslot (1980) for ADH, PGI and EST and by Tanksley and Rick (1980) for SDH.

SDH is a monomeric enzyme encoded by *Sdh-1* gene in various parts of the shoots from the plumule stage until maturity. Homozygotes exhibit a single band and diploid heterozygotes exhibit two bands corresponding to the two alleles.

Enzymes encoded at loci *Adh-1* and *Est-8* are best observed in white shoot tissues. Plumules as well as the non-exerted part of developing leaves can be used. These enzymes are dimers produced by the association of two protomeric gene products. In homozygotes, a single type of protomer is available and a single "homodimeric" band is observed. In diploid heterozygotes, two allelic protomers are combined and produce three types of enzymes, two homodimers and one "heterodimer" of intermediate mobility. Three bands are observed.

PGI display similar zymograms whatever the stage and the tissue of the shoot. They are also dimers, but are encoded by two genes *Pgi-1* and *Pgi-2*, whose direct products are compatible. Double homozygotes exhibit three bands, corresponding to two homodimers and one interlocus heterodimer. Interlocus molecular hybridization makes heterozygous zymograms complex, with up to ten bands in diploid double heterozygotes. However, considering only the enzymes produced by association among allelic protomers encoded at locus *Pgi-1* allows zymogram interpretation as for *Adh-1* and *Est-8*.

Genetic analysis

The rationale of the trisomic analysis involves several independent sources of evidence: observation of allele dosage effects in F1; observation of distorted segregation in BC1 or F2, and; recovery of triallelic heterozygotes.

1 Allele dosages effects in F1. If the trisomic parent carries three copies of an allele (genotype aaa), its F1 progeny with a diploid marker line (genotype bb) will have two types of plants: (1) normal diploid plants, with one copy of each of the parental alleles (genotype ab), and (2) trisomic plants with two copies of the allele coming from the trisomic parent and one copy of the allele coming from the diploid parent (genotype aab). In backcross progenies, another type of heterozygote will be produced, with one copy of the allele from the trisomic parent and two copies of the allele from the diploid parent (genotype abb). In F2 progenies, an additional rare case will be that of tetrasomic plants when the extra chromosome was transmitted by both male and female gametes. Male transmission is quite frequent for trisomics 8 and 9 (14.3 and 27.3%, respectively) and rare, or absent for the other trisomics (Khush et al. 1984). Among tetrasomic plants, three heterozygous situations are possible with genotypes aaab, aabb and abbb. This possibility has to be kept in mind during the F2 analysis.

The search for allele dosage effects rests upon the assumption that dissimilary of allele doses in a plant results in unequal concentration of the allelic end products and, subsequently, unequal concentration of the various enzymes. Examples are given in Fig. 1.

2 Segregations in BC1 and F2. Double dose of the trisomics allele in F1 plants results in its higher frequency with gametes, and subsequently, in a "trisomic segregation" as compared to disomic segregation in 2n heterozygote.

In the segregating progenies, banding patterns are scored regardless of the intensity ratio of the bands and three types are distinguished, which show (1) only the bands that of the trisomic parent, (2) only the bands of the diploid parent and (3) bands of both parents, with or without the intermediate band depending on the enzyme examined. The segregation ratios were tested for a fit to a disomic or a trisomic ratio. While calculating a fit to a trisomic ratio, a 33% female transmission rate of the extra chromosome was assumed.

3 Triallelic heterozygotes. If three active alleles at a locus are known, it is possible to cross heterozygous trisomic plants with a diploid line carrying a third allele and produce "triallelic" heterozygotes. This would unequivocally identify the primary trisomic which carries three copies of the gene. Such test was carried out for *Sdh-1*.

Results

Sdh-1

The zymograms of the F1 plants of all trisomics except triplo-6 had two bands of similar intensity which characterized diploid heterozygotes. A few plants of the progeny of triplo-6 were characterized by a stronger intensity of the lower band, which corresponds to allele 1 transmitted by the trisomic (Fig. 2, sample 4). Visually, the intensity appears double to that of the other band. These zymograms corresponded to those expected in case of trisomy (Fig. 1).

The F2/BC1 segregations confirmed these results. For all trisomics except triplo-6, the segregation was

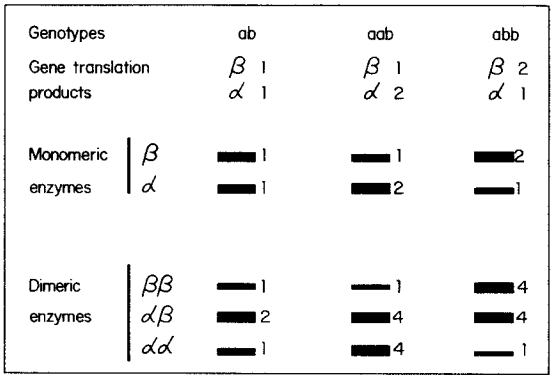

Fig. 1. Allele dosage effects of zymograms of trisomic heterozygous plants in the case of monomeric and dimeric enzymes

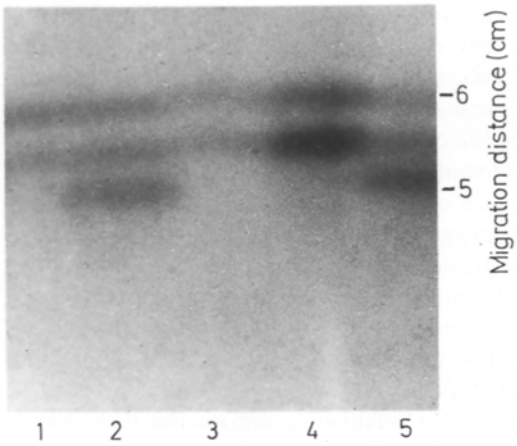

Fig. 2. SDH zymograms in F_1 progeny of triplo-6 *Sdh-1*^{114} × *Sdh-1*^{22}, with *Sdh-1*^{12} (samples 1 and 3), *Sdh-1*^{112} (sample 4) and *Sdh-1*^{124} (samples 2 and 5)

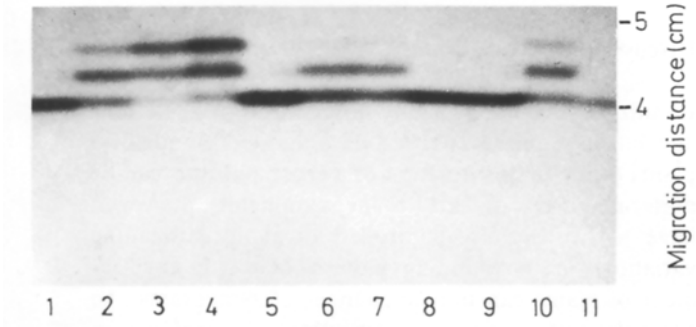

Fig. 3. ADH zymograms in F_2 progeny of triplo-11 with *Adh-1*^{11} or *Adh-1*^{111} (samples 1, 5, 8, 9 and 11), *Adh-1*^{13} (samples 2 and 10), *Adh-1*^{113} (samples 6 and 7) and *Adh-1*^{133} (samples 3 and 4)

disomic (Table 2). The F_2 segregation for triplo-6 was significantly different from disomic but agreed with the trisomic ratio. Moreover, it was possible to observe allele dosage combinations such as *Sdh-1*^{122} which was different from the trisomic F_1.

The third line of evidence was the occurrence of triplo-6 plant which was heterozygous for three alleles (*Sdh-1*^{124}) (Fig. 2, samples 2 and 5). This resulted from the cross of a rare plant of triplo-6 (*Sdh-1*^{114}) with disomic tester line (*Sdh-1*^{22}). These results clearly show that *Sdh-1* is located on chromosome 6.

Adh-1

The F_1 progenies of all trisomics except those of triplo-11 produced the typical diploid heterozygote zymogram with the two parental bands of similar intensity and a band of intermediate mobility and higher intensity (Fig. 3). In the case of triplo-11 progeny, some plants exhibited a three banded zymogram with the lower two bands of a similar intensity but stronger than that of the higher band (Fig. 3). This corresponds to the expected trisomic zymogram.

The F2 or BC1 segregations confirmed these results. All trisomic progenies except those of triplo-11 were characterized by a typical Mendelian segregation. However in case of triplo-11, the segregation was significantly distorted and agreed with the expected trisomic segregation (Table 2, Fig. 3). In case of triplo-11, converse trisomic heterozygote zymograms were observed (Fig. 3).

Thus *Adh-1* is located on chromosome 11.

Table 2. Segregation at four loci coding for isozymes in progenies of homozygous primary trisomics (allele T) crossed with homozygous diploid marker lines (allele D); three classes are considered, corresponding to plants carrying *i* allele T only, *ii* allele D only and *iii* alleles T and D; segregations which differ at 5% significance level from Mendelian segregations are underlined; those conforme to trisomic segregations are in boxes

Primary trisomic	Progeny	Locus			
		Sdh-1	*Adh-1*	*Est-8*	*Pgi-1*
1	BC	0:47:54	0:45:54	0:43:57	0:44:55
2	F_2	32:29:62	32:29:62	24:30:62	25:25:50
3	F_2	31:28:62	35:30:65	30:26:62	37:45:78
4	BC	0:82:96	0:86:102	0:67:81	0:37:103
5	F_2	20:18:39	24:22:49	29:29:64	25:14:46
6	F_2	78:22:103	36:32:69	29:25:54	79:57:135
7	F_2	31:25:51	31:30:64	33: 5:72	25:22:60
8	F_2	20:21:42	24:20:49	26:25:54	29:23:68
9	F_2	28:28:64	38:30:64	53:132:177	65:55:111
10	F_2	28:30:62	39:36:76	26:30:60	21:24:48
11	F_2	20:21:46	59:20:98	34:35:73	38:33:79
12	F_2	29:28:48	35:28:56	32:29:67	21:32:49

[a] Mendelian segregations are 0:1:1 for BC and 1:1:2 for F_2
[b] Trisomic segregations are 0:2:7 for BC and 10:2:15 for F_2, assuming a 33% female transmission rate of the extra chromosome

Est-8

Variation in the zymograms of F1 plants was observed only in the case of triplo-7. In the triplo-7 F_1 progenies, in addition to the usual three-banded balanced diploid zymogram (Fig. 4), another zymogram was observed in which the lower two bands had similar intensity but were more intense than the higher band (Fig. 4). This corresponds to the expected trisomic zymogram.

All F2 or BC1 progenies except those of *triplo-7* and *triplo-9* showed disomic segregation (Table 2). The F2 population of triplo-7 segregated in trisomic fashion. In the case of F2 population of triplo-9 the distortion was due to an excess of homozygotes for the

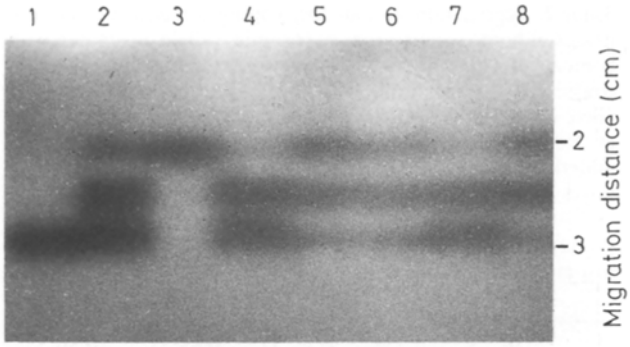

Fig. 4. EST-8 zymograms in F_2 progeny of triplo-7, with *Est-8*^{11} or *Est-8*^{111} (sample 1), *Est-8*^{22} or *Est-8*^{222} (sample 3), *Est-8*^{12} (samples 5 and 6), *Est-8*^{112} (samples 2, 4, and 7) and *Est-8*^{122} (sample 8)

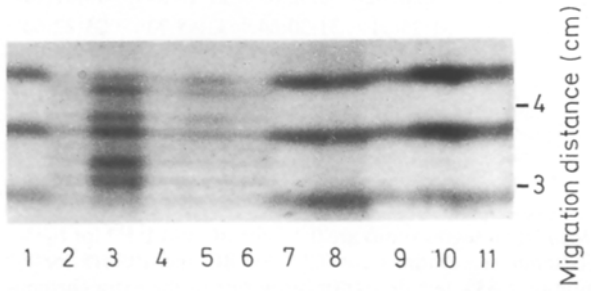

Fig. 5. PGI zymograms showing various dosage effects in double homozygotes and double heterozygotes at *Pgi-1* and *Pgi-2:* IR36 (*Pgi-1*^{11}, *Pgi-2*^{22}; samples 1, 7, 9, and 11); triplo-4 (*Pgi-1*^{111}, *Pgi-2*^{22} sample 8); triplo-3 (*Pgi-1*^{11}, *Pgi-2*^{222}; sample 10); IR36/Mayin (*Pgi-1*^{12}, *Pgi-2*^{12}; samples 2, 4 and 6); triplo-4/Mayin//Mayin (*Pgi-1*^{122}, *Pgi-2*^{12}; sample 3); triplo-3/Mayin (*Pgi-1*^{12}, *Pgi-2*^{122} sample 5)

allele contributed by the trisomic parent at the expense of the homozygotes for the allele contributed by the diploid parent. The distorted segregation in case of triplo-9 was not due to trisomy. Besides, some plants in the F2 of triplo-7 displayed allele dosage effects in both directions, (Fig. 4) thus confirming the F1 results. These results show that *Est-8* is located on chromosome 7.

Pgi-1

The F1 progenies of all trisomics except triplo-4 displayed a single zymogram. Some plants in the F1 progenies of triplo-4 produced the zymograms expected for a trisomic. Among the enzymes made of allelic protomers encoded by *Pgi-1*, the two slower migrating ones exhibited similar levels of activity, which was higher than the faster migrating one.

Distorted segregation was observed in the BC1 of triplo-4 only (Table 2). The BC1 population was comprised of about three times more heterozygotes than homozygotes, which corresponded to the pattern expected in case of trisomy. Some BC1 plants exhibited converse dosage effect with the two higher bands being more intense than the lower one (Fig. 5).

During the course of the PGI analysis, allele dosage effect was found at *Pgi-2* locus in the progeny of triplo-3 (Fig. 5) which confirms the location of this gene established by linkage analysis.

The particular feature of PGI being encoded by two genes with interlocus dimerisation provided an opportunity to test gene dosage effects in homozygous trisomics. Figure 5 shows zymograms of triplo-3 and triplo-4 separated by zymograms of IR36. In triplo-4, the lower band is relatively more intense than that in IR36. In triplo-3, the higher band is more intense than that in IR36. This confirms the locations of the two genes and indicates the possibility to identify triplo-3 and triplo-4 by PGI electrophoresis.

Discussion and conclusions

Localizing isozyme genes by observation of allele dosage effect requires analysis of a limited number of plants of the first generation of a cross and thus can be performed very quickly. Some parameters, however, have to be carefully controlled to avoid misleading variations in the banding patterns. Growth environment of plants can induce changes of zymograms. An example is the action of submersion of the plants on the ADH zymograms in rice. When germinated on water-saturated filter paper, the homozygous seedlings yield single banded zymograms. When they are submerged, even partly, the same seedlings are characterized by multiple ADH bands. Particularly, submerged seedlings homozygous for the allele present in the trisomics could easily be mistaken as heterozygous seedlings carrying two doses of that allele and one dose of the allele which codes for the faster band. Variation of organ age or development stage can also induce inconsistent appearance of secondary bands. Several examples have been described in rice (Second and Trouslot 1980). Organ age is also known to induce variation of the zymograms when there is asynchronous expression of alleles. This has been well documented in rice for peroxidases and acid phosphatases (Endo 1971, 1981). Another source of variation can be the differential sensitivity of enzymes to the external conditions after the sampling of the plant organ, as exemplified by the different thermosensitivity of allelic PGI's of rice after their extraction (Second and Trouslot 1980). Thus perfect control of the growth environment, the stage of the plant analyzed and the technical conditions of protein extraction, electrophoresis and staining is required for dosage effect analysis.

The second source of information in trisomic analysis is the typically distorted segregation. It is the criterion used for localization of most genes whose expression cannot be evaluated at the molecular level. This method is more time-consuming and requires analysis of second generation materials. Moreover, phenomena of distorted segregation independent from trisomy are well known in rice hybrids. They are usually observed in intersubspecific hybrids and are related to gametic selection due to inviability of some haploid genotypes. They can, however, be also observed in intrasubspecific hybrids. Thus the use of trisomics and diploid marker lines of the same varietal group is not sufficient to guarantee that trisomy will be the only cause of segregation distortion. Some of the models explaining selective gametic abortion and the subsequent distortion involve complementary action between independent duplicate genes (Oka 1974). Should one of these genes be carried by the chromosome in triplicate in a given trisomic, there might be distorted segregation of markers linked with the other gene. The variant in excess would be the one coming from the trisomic and that could be taken as a trisomic segregation. Thus, when the only source of information is segregation, it is essential to study progenies of all trisomics to make sure there is only one case of distortion attributable to trisomy. In the present study, we found an example of distortion which cannot be accounted for by trisomy (*Est-8* in progeny of trisomic 9×Chau). We analyzed a large number of F2 plants to make sure that the distortion was not due to insufficient sampling. Whether this distortion will still appear in other F2 populations of the same cross is under investigation. Its appearance only in the combination involving trisomic 9 would demonstrate interchromosomal effects other than those invoked to explain F1 sterility and gametic selection.

Despite this unexplained distortion, there is sufficient evidence to determine the chromosomal location of the four loci investigated. Gene dosage effects and segregation distortions concur to indicate that *Sdh-1* is located on chromosome 6, *Adh-1* on chromosome 11, *Est-8* on chromosome 7, and *Pgi-1* on chromosome 4. Survey of 1688 varieties of *O. sativa* has revealed four alleles at *Sdh-1* locus with two being more preponderant, three alleles for *Adh-1*, one being more common, and two frequent alleles for *Est-8* and for *Pgi-1* (Glaszmann 1987). The large natural polymorphism of these genes as well as their location on chromosomes which are moderately (4 and 11) or very sparsely (6 and 7) populated with mapped genes will probably make them useful marker. In general, it is anticipated that trisomics will hasten localization of isozyme genes and that these will be very helpful to develop the linkage maps of rice further.

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