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Comparative studies of isozymes in *Oryza sativa*, *O. minuta*, and their interspecific derivatives: evidence for homoeology and recombination

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Abstract. Enzyme electrophoresis was used to compare the isozyme phenotypes of *Oryza sativa*, IR31917 (AA genome), and two *O. minuta* accessions (*Om* 101089 and *Om*101141; BBCC genome) for ten enzyme systems. Between the two species, two systems were monomorphic (isocitrate dehydrogenase and alcohol dehydrogenase) and eight were polymorphic (shikimate dehydrogenase, phosphogluconate dehydrogenase, phosphoglucose isomerase, malate dehydrogenase, glutamate oxaloacetate transaminase, esterase, aminopeptidase, and endopeptidase). Polymorphism between *O. minuta* accessions was detected for shikimate dehydrogenase and glutamate oxaloacetate. As expected, the quaternary structure of the *O. minuta* isozymes was comparable to that of *O. sativa*. Possible allelic relationships with known *O. sativa* alleles and their genomic designation are discussed. Combined with chromosome data, the interspecific variation was exploited to monitor the relative genetic contribution of the two parents in the IR31917/*Om*101141 F_1 hybrids and recurrent (IR31917) backcross progenies. The isozyme content of F_1 hybrid reflected its triploid nature (ABC genome composition), while that of the backcross progenies paralleled the duplication of the A genome and the gradual loss of *O. minuta* chromosomes during the backcrossing process. Evidence is provided for a degree of homoeology between the A, B, and C genomes, and for introgression from *O. minuta* into *O. sativa*.

Key words: *Oryza sativa* – *Oryza minuta* – Interspecific hybrid – Isozyme analysis – Introgression

Introduction

For decades the genetic improvement of cultivated rice (*Oryza sativa* L.; AA genome) has made steady progress by the exploitation of intraspecific genetic variability. The wild *Oryza* species offer an additional reservoir of variability, such as tolerance to biotic and abiotic stresses and novel sources of cytoplasmic male sterility (Sitch 1990). Introgression of alien germplasm has been achieved in rice. A gene for grassy stunt virus resistance was transferred from *O. nivara* into cultivated rice (Khush 1977). Jena and Khush (1989) have developed addition lines of *O. sativa* with chromosomes from *O. officinalis*, leading to the transfer of a gene for brown plant hopper resistance from *O. officinalis*.

Monitoring the introgression of alien germplasm into cultivated varieties can be greatly facilitated by biochemical markers such as isozymes (Tanksley and Rick 1980). When isozyme polymorphism exists between parental species in interspecific hybrids, isozyme analysis can be used both to detect the introgression of genes from the wild germplasm and as a method of applying selection pressure to restore the recurrent parent background (Tanksley and Rick 1980). The usefulness of this technique relies on alien gene expression in interspecific derivatives and the detection of their products in zymograms. In rice, isozyme polymorphism has been used for investigating the evolution and subspecies classification of cultigen (Nakagahra et al. 1975; Glaszmann 1986), as well as phylogeny within the genus *Oryza* (Second 1982; Endo and Morishima 1983).

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O. minuta J. S. Presl ex CB. Presl, an allotetraploid wild species (2n = 48) with a BBCC genome constitution, exhibits resistance to important rice diseases (e.g., bacterial leaf blight and blast) and insect pests (e.g., brown plant hopper and white-backed plant hopper). Amante-Bordeos et al. (1992) described the transfer of bacterial blight and blast resistance from *O. minuta* to cultivated rice. Little work has been done to characterize the isozyme constitution of *O. minuta*. Shahi et al. (1969) detected seven bands each for peroxidase and acid phosphatase. Khush et al. (1991) characterized *O. minuta* (Acc. 101141) at three malate dehydrogenase loci.

In this paper we report: (1) the isozyme profiles of *O. sativa* elite line IR31917 and two *O. minuta* accessions (101081 and 101141) for ten enzyme systems, (2) the quaternary enzyme structure, possible homoeoallelic relationships of the *O. minuta* isozymes with known *O. sativa* alleles, and their genomic designation and, (3) the relative genetic contribution of two parents in the IR31917/*O. minuta* 101141 F_1 hybrids and their backcross derivatives.

Material and methods

Plant material

The following genotypes were used: *O. sativa* IR31917-45-3-2 (hereafter IR31917; AA genome), *O. minuta* (BBCC genome) accessions 101089, 101141 (hereafter *Om*101089 and *Om*101141, respectively), *O. punctata* (BB genome) accessions 103888, 103906, *O. punctata* (BBCC genome) accession 101409, and *O. officinalis* (CC genome) accessions 100896, 101150. Seeds of the wild species were obtained from the International Rice Germplasm Center (IRGC) at the International Rice Research Institute (IRRI), Philippines. The triploid F_1 hybrids, IR31917/*Om* 101089 and IR31917/*Om*101141, and the IR31917/*Om*101141 recurrent (IR31917) backcross derivatives were produced by the IRRI's Wide Hybridization program (Stich et al. 1989). Six BC_1, 21 BC_2, and 22 BC_3 plants were analyzed. The chromosome number of these plants ranged from 46 to 48 in the BC_1, 24 to 37 in the BC_2, and 24 to 29 in the BC_3.

Electrophoresis and enzyme detection

Samples were prepared as described by Glaszmann et al. (1988). Extracts from green leaf tissues were assayed for shikimate dehydrogenase (SDH; EC 1.1.1.25), malate dehydrogenase (MDH, EC 1.1.1.37), esterase (EST, EC 3.1.1.-), aminopeptidase (AMP, EC 3.4.11.1), endopeptidase (ENP, EC 3.4.21-24.-), alcohol dehydrogenase (ADH, EC 1.1.1.1), and isocitrate dehydrogenase (ICD, EC 1.1.1.42), and those from white leaf tissues for phosphogluconate dehydrogenase (PGD, EC 1.1.1.43), phosphoglucose isomerase (PGI, EC 5.3.1.9), glutamate oxaloacetate dehydrogenase (GOT, EC 2.6.1.1) and esterase (EST, EC 3.1.1.-). Electrophoresis was performed in 14% starch gels using the buffer systems described by Glaszmann et al. (1988) and the staining procedures of Shaw and Prasad (1970) and Shields et al. (1983). Electrophoresis of MDH was performed using a 0.009 M Trizma base (Tris) – 0.005 M histidine mono HCl gel buffer (pH 6.0) and a 0.4 M Trizma base (Tris) – 0.105 M Citric Acid, $1H_2O$ electrode buffer (pH 6.0). MDH was visualized by incubating the gel at 30 °C for at least 10 min in a mixture of 5 ml 1 M sodium malate buffer (pH 6.0), 10 ml 0.5 M Tris-HCl (pH 8.5), 2 ml NAD (10 mg/ml), and 33 ml water, freshly added with 1 ml Nitroblue tetrazolium salt (10 mg/ml) and 1 ml phenazine methosulfate (1 mg/ml).

Isozyme and allozyme nomenclature

Isozymes controlled by different loci in *O. sativa* normally show at different band zones in the zymogram. The nomenclature used for the known loci and allozymes of IR31917 and co-focusing isozymes in *Om*101089 and *Om*101141 followed the methods of Glaszmann et al. (1988) and Khush et al. (1991). *O. minuta*-specific isozyme bands were designated M bands followed by an arabic number. For each enzyme system, the fastest migrating band is designated M1 and subsequent slower bands designated successive numbers.

Various forms of the aminopeptidases were assayed using three substrates, namely: DL-alanyl-β-naphthylamide (Ala-N Am), L-arginyl-β-naphthylamide (Ara-N Am), and L-leucyl-β-naphthylamide (Leu-N Am). A single numbering system was adopted for all *O. minuta* aminopeptidase bands detected in the three substrates.

Results and discussion

Enzyme polymorphism in the parents

Polymorphism between IR31917 and the two *O. minuta* accessions, *Om*101089 and *Om*101141, was detected in eight out of ten enzyme systems, namely: SDH, PGD, PGI, MDH, GOT, EST, AMP (Ara-N Am and Leu-N Am substrates), and ENP. Polymorphism between *O. minuta* accessions was shown for SDH and GOT. Monomorphic zymogram phenotypes were obtained for ADH, ICD, and AMP (Ala-N Am substrate). IR31917 had 19 detectable isozymes, while *Om*101089 and *Om*101141 had 25 and 26, respectively. The higher number of isozyme bands noted for the *O. minuta* accessions is consistent with their allotetraploid nature.

Zymogram phenotype, enzymatic structure, and genome designation

Shikimate dehydrogenase (SDH). The three parents expressed SDH in the same zymogram zone that corresponds with the *Sdh-1* locus in *O. sativa*. IR31917 showed SDH-1^2, *Om*101089 exhibited SDH-M1, and *Om*101141 had SDH-M1 and SDH-M2 (Fig. 1a). *Sdh-M1* and *Sdh-M2* may be homoeoallelic variants at the *Sdh-1* locus.

IR31917/*Om*101089 F_1 hybrids produced the two parental bands (Fig. 1a). An isozyme band which co-focussed with SDH-M1 was detected in *O. punctata* (BB) 103888, 103906 and *O. officinalis* (CC) 100896, 101150 (data not shown). The absence of intermediate bands in the F_1 hybrids indicates a monomeric structure.

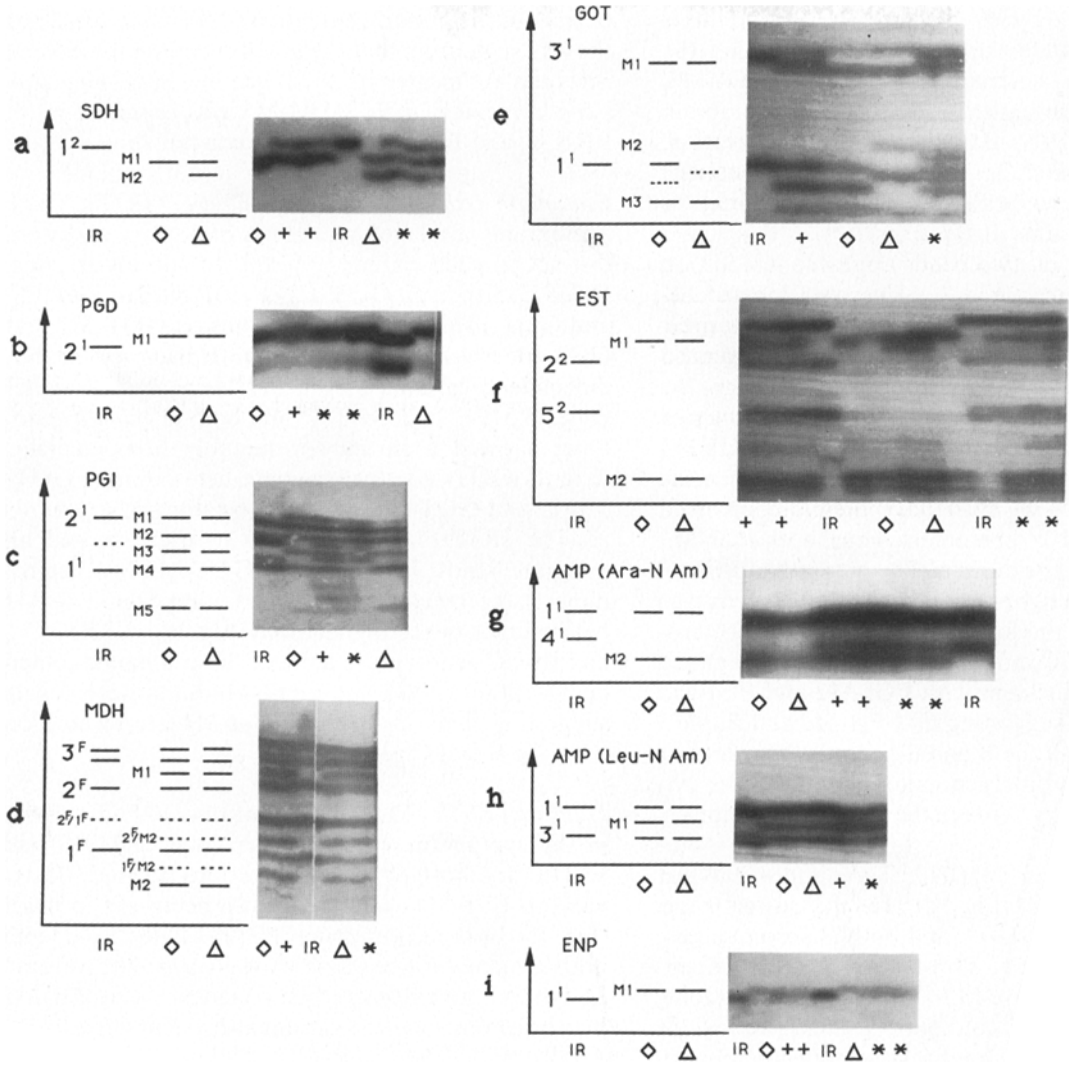

Fig. 1. Zymograms and diagrammatic interpretation of enzyme systems that were polymorphic between IR31917 and *O. minuta* accessions. *IR*, IR31917; ◇, *Om*101089; △, *Om*101141; +, IR31917/*Om*101089 F_1; *, IR31917/*Om*101141 F_1; ----, heterodimeric band

Phosphogluconate dehydrogenase (PGD). PGD zymograms comprised two zones of activity (Fig. 1b). In the higher zone, the *O. minuta* accessions had PGD-M1 while IR31917 showed PGD-2^1. *Pgd-M1* could be a homoeoallelic variant at *Pgd-2*. Although variation was apparent in the lower zone corresponding to PGD-1 in IR31917, resolution was not reproducible.

The F_1 hybrids displayed a wide band in the higher zone, encompassing both parental types (Fig. 1b), and suggesting the presence of an intermediate band (PGD-2^1/M1) and a dimeric structure, as for *O. sativa* PGD isozymes (Glaszmann et al. 1988). All BB, CC, and BBCC genotypes examined showed a band coincident with PGD-M1 (data not shown), indicating that *Pgd-M1* is located on both B and C genomes. The formation of an interspecific heterodimer is evidence of homoeology between genomes.

Phosphoglucose isomerase (PGI). The zymograms of the two species differed considerably (Fig. 1c). IR31917 showed two isozymes, PGI-1^1 and PGI-2^1, and an intermediate intergenic heterodimer (PGI-$1^1/2^1$). The two *O. minuta* accessions had five isozyme bands. Two of these, PGI-M1 and PGI-M4, were coincident with PGI-2^1 and PGI-1^1, respectively, although they did not form an intermediate heterodimer (hence their distinct designations). This is not, however, evidence against their dimeric nature. Markert and Whitt (1968) hypothesized that dimeric enzymes may form heterodimers that can be rejected during the course of evolution. It is also possible that PGI-M4 is itself a

heterodimer formed by PGI-M1 and PGI-M5. If this is so, it would preclude the possibility of forming the PGI-M4/PGI-M1 heterodimer. PGI-M2, PGI-M3, and PGI-M5 distinguished the *O. minuta* zymogram from that of IR31917. Based on isozyme migration, *Pgi-M3*, *Pgi-M4* and *Pgi-M5* may be homoeoallelic variants at *Pgi-1*, while *Pgi-M1* and *Pgi-M2* may be homoeoallelic variants at *Pgi-2*.

In the F_1 hybrids, two bands appeared in addition to the parental types (Fig. 1c). One was located between PGI-M5 and PGI-1^1 or PGI-M4. This most likely represented a PGI-M5/1^1 heterodimer, and not a PGI-M5/M4 heterodimer since it did not appear in the *O. minuta* parents. The second heterodimer was located midway between the PGI-M3 and PGI-1^1 or PGI-M4 bands. Again, this heterodimer was not present in the *O. minuta* parent and must have therefore involved PGI-M3 and PGI-1^1 protomers. Thus, both PGI-M3 and PGI-M5 must be dimeric. The presence of PGI-1^1 and PGI-2^1 in the hybrids and backcross progeny was detected through the formation of PGI-$1^1/2^1$ heterodimers. All BB, CC, and BBCC genotypes examined showed bands coincident with PGI-M2 and PGI-M3 (data not shown), suggesting that *Pgi-M2* and *Pgi-M3* are located on both the B and C genomes. The formation of apparently intergenomic heterodimers is evidence of homoeology between the A, B, and C genomes.

Malate dehydrogenase (MDH). Three zones of MDH activity were noted (Fig. 1d). In the lower zone, IR31917 showed MDH-1^F, and both *O. minuta* accessions had MDH-1^F and a slower (presumably homoeoallelic) variant, MDH-M2. In the middle zone, IR 31917 had MDH-2^F, and both *O. minuta* accessions also exhibited MDH-2^F and a faster (presumably homoeoallelic) variant, MDH-M1. In the higher zone, IR31917 and both *O. minuta* accessions displayed MDH-3^F. As expected, the allotetraploid *O. minuta* parents produced more isozymes than the diploid IR31917, reflecting the divergence of their constitutent genomes for this enzyme system. The intermediate heterodimers MDH-$2^F/1^F$, MDH-2^F/M2 and MDH-1^F/M2 denote a dimeric structure.

The F_1 hybrids between the two species showed the same MDH zymogram as for the tetraploid *O. minuta* (BBCC) parents despite the incorporation of the A genome from IR31917. This is because all of the MDH bands in IR31917 were already present in the *O. minuta* parents. Indeed, the absence of new heterodimers in the F_1 hybrids shows that the IR31917 bands are identical with the coincident *O. minuta* bands since in the presence of other MDH isozymes the same set of heterodimers was formed in both the triploid F_1 (ABC) and tetraploid parents (BBCC) (Fig. 1d).

Bands coincident with MDH-1^F, MDH-2^F and MDH-M2 were also observed in diploid and tetraploid *O. punctata* accessions, but not in *O. officinalis* (data not shown), suggesting that the genes encoding these three isozymes are located on the B genome in *O. minuta*. A band coincident with MDH-M1 was observed in all BB, CC, and BBCC genotypes (data not shown).

Glutamate oxaloacetate transaminase (GOT). GOT zymograms displayed two zones of activity and were distinct in each parent (Fig. 1e). In the lower zone, IR31917 displayed GOT-1^1, *Om*101089 had GOT-1^1 and GOT-M3, and *Om*101141 showed GOT-M2 and GOT-M3. *Got-M2* and *Got-M3* appear to be homoeoallelic variants of *Got-M1* locus. In the higher zone, IR31917 had GOT-3^1 and both *O. minuta* genotypes showed a slower (presumably homoeoallelic) variant, GOT-M1. Intermediate heterodimers GOT-1^1/M3 and GOT-M2/M3 denote a dimeric structure.

The IR31917/*Om*101089 F_1 hybrids showed all parental bands, including the GOT-M2/M3 heterodimer in the lower zone, and an additional GOT-3^1 M1 heterodimer in the higher zone (Fig. 1e). All BB, CC, and BBCC genotypes examined showed bands coincident with GOT-M3 and GOT-M4 (data not shown), suggesting that *Got-M3* and *Got-M4* are located on both the B and C genomes.

Esterase (EST). There were two zones of EST activity in the zymogram. In the higher zone, IR31917 had EST-2^2 and both *O. minuta* accessions showed a faster variant, EST-M1, both allozymes being red in color (Fig. 1f). In the lower zone, IR31917 had EST-5^2 and both *O. minuta* accessions showed a slower variant, EST-M2, both allozymes being black in color. *Est-M1* may be a homoeoallelic variant at *Est-2*, and *Est-M2* at *Est-5*.

The F_1 hybrids showed only the parental bands (Fig. 1f), indicating a typical monomeric structure. A band which co-focussed with EST-M1 was observed in *O. punctata* (BB) 103888, 103906 and *O. officinalis* (CC) 100896, 101150 (data not shown). Similarly a band of comparable mobility to EST-M2 was observed in *O. punctata* (BB) 103888 and in *O. officinalis* (CC) 100896 (data not shown). It is likely therefore that copies of both *Est-M1* and *Est-M2* are found within both the B and C genomes.

Aminopeptidase (AMP). Two-zone AMP (Ala-N Am) zymograms were monomorphic for the three parents. IR31917 and both *O. minuta* accessions showed AMP-1^1 and AMP-2^1. AMP (Ara-N Am) zymograms displayed two zones of activity in both species (Fig. 1g). The higher zone represented AMP-1^1 in IR31917 and *O. minuta*. The lower zone showed AMP-4^1 for IR31917, and a lower (presumably homoeoallelic) variant band AMP-M2 for both *O. minuta* accessions. With Leu-N Am substrate, IR31917 and both *O. minuta*

accessions showed AMP-1^1 and AMP-3^1 (Fig. 1h). The *O. minuta* genotypes also displayed a variant isozyme band, AMP-M1, between AMP-1^1 and AMP-3^1.

Only parental bands appeared in the F_1 hybrids (Fig. 1g, h), signifying a monomeric isozyme structure, as shown in *O. sativa* (Glaszmann et al. 1988). A band which co-focussed with AMP-M1 was observed in *O. officinalis* (CC) 100896, 101150, and in tetraploid *O. punctata* (BBCC) 101409. The diploid *O. punctata* (BB) accessions showed variant bands in this zone (data not shown). These observations suggest that *Amp-M1* is located on the C genome.

Endopeptidase (ENP). A single band characterized the ENP zymograms for each species (Fig. 1i). IR31917 had ENP-1^1 and both *O. minuta* accessions exhibited a slightly faster band ENP-M1 in the same zone. *Enp-M1* may be a homoeoallelic variant at *Enp-1*.

The F_1 hybrids showed only the parental types (Fig. 1i), denoting a monomeric structure. A band coincident with ENP-M1 was observed in *O. officinalis* CC genotypes and also in the *O. punctata* BBCC genotype, but not in the *O. punctata* BB accession (data not shown), suggesting that *Enp-M1* is located on the C genome.

Evidence for homoeology

For all enzyme systems studied, *O. minuta*-specific bands were fully expressed in the cultivar background, This has been observed in many species such as the *Triticeae* (Tang and Hart 1975; Hart and Tuleen 1983a), *Brassica* (Chen et al. 1989), *Lycopersicon* (Rick 1969), and *Gossypium* (Stephens 1949). Failure of alien isozyme gene expression in interspecific derivatives may be associated with cytoplasmic control of that expression (Bergman and Maan 1973).

Despite the different genomic constitutions of the two parents, coincidence was observed with eight known *O. sativa* genes: *Adh-1*, *Icd-1*, *Amp-1*, *Amp-2*, *Amp-3*, *Got-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Pgi-1*, and *Pgi-2*. Possible *O. minuta*-specific homoeoallelic variants were detected for *Amp-4*, *Enp-1*, *Est-2*, *Est-5*, *Got-1*, *Mdh-1*, *Mdh-2*, *Pgd-2*, *Pgi-1*, *Pgi-2*, and *Sdh-1*. This indicates a degree of homoeology between the A, B, and C genomes. This is further supported by the fact that interspecific heterodimers formed for two enzymes: phosphoglucose isomerase (PGI-1^1/M5 and PGI-1^1/ M3) and phosphogluconate dehydrogenase (PGD-2^1/M1).

This phenomenon of intergenomic homoeology has been extensively observed in the *Triticeae*. Recently, Gale and Miller (1987) summarized evidence for orthology of 40 protein structural genes in *Triticum aestivum* (genomes A, B, and D), *Secale cereale* (genome R), *Hordeum vulgare* (genome H), and five wild species, *Agropyron elongatum* (*Elytrigia elongata*; genome U), *Ae. longissima* and *Ae. sharonensis* (genome S^1), and *Ae. bicornis* (genome Sb). The strongest evidence for orthology has been for the multimeric enzymes *Adh-1*, *Adh-3*, *Est-1*, *Gpi-1*, *Got-2*, *Got-3*, and *Tpi-1*, where active enzymes consisting of wheat and alien protomers were formed (Hart and Tuleen 1983a). Unfortunately, the genomes of *O. sativa* and those of the related species have not been so extensively characterized. Although a restriction fragment length polymorphism (RFLP) map of the *O. sativa* genome is available (McCouch et al. 1988), molecular relationships between the AA genome of *O. sativa* and related genomes largely remain unexplored. Homoeology between genomes is implicated by morphological similarities between *O. officinalis* addition lines into *O. sativa* and the corresponding primary trisomics of *O. sativa* (Jena and Khush 1989).

Changes in the O. minuta genetic contribution between generations

The number and frequency of *Om*101141-specific bands decreased from 60% in the F_1, to 58% in the BC_1, to 43% in the BC_2, and finally 9% in the BC_3; all IR31917 isozyme bands remained throughout the various generations (Table 1). The proportions of *O. sativa* and *O. minuta* isozyme bands directly reflect the presence of one copy of the IR31917 A genome (33% of the total genomic composition) and one copy of each of the two *Om*101141 genomes BB and CC (67% of the total genomic composition) in the F_1, the maintenance of the majority of *O. minuta* chromosomes in the BC_1 generation, and the subsequent loss of *O. minuta* chromosome during backcrossing. A high correlation ($r = 0.97$, $n = 57$) between the number of *Om*101141 chromosomes and the number of isozymes present in the progenies was noted (Fig. 2). On average, the BC_3 progeny had recovered 90% of the IR31917 isozyme genotype, reflecting a high return rate to the cultivar genetic background (Table 1).

Table 1. Number and percentage of parent-specific isozymes in IR31917/*Om*101141 F_1 hybrids and backcross progenies

Generation (no. of plants)	IR31917		*Om*101141	
	Number	%	Mean (range)	%
F_1 (12)	11	40	16	60
BC_1 (6)	11	42	15 (12–16)	58
BC_2 (21)	11	57	8 (0–13)	43
BC_3 (22)	11	91	1 (0–8)	9

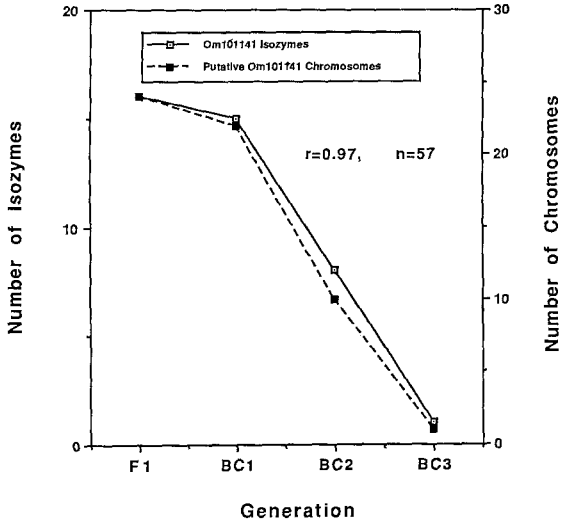

Fig. 2. Correlation between number of *Om*101141-specific isozymes and number of putative 101141 chromosomes in the IR31917/*Om*101141 F_1 and backcross derivatives

Identification of addition lines and evidence of introgression

Isozymes have been used as markers for identifying wheat-rye (Tang and Hart 1975), wheat-barley (Hart et al. 1980), wheat-*Elytrigia elongata* (Hart and Tuleen 1983b), and *Allium* (Peffley et al. 1985) alien addition lines. Two *O. minuta*-derived plants, with a chromosome number of 2n = 25, exhibited two (ENP-M1 and PGD-M1) and one (SDH-M1) *Om*101141-specific isozymes, respectively. These plants presumably represent addition lines, each with an extra chromosome coming from *Om*101141. Although these results suggest that ENP-M1 and PGD-M1 are encoded by genes on the same chromosome, these two isozymes showed co-transmission in only 11 of the 18 BC_2 progeny (data not shown). It is therefore more likely that one locus has been introgressed into the *O. sativa* genome by recombination and the other is carried on the additional monosomic *O. minuta* chromosome. Indeed, in *O. sativa*, *Pgd-2* (possibly homoeoallelic to *Pgd-M1*) has been located on chromosome 6 (Khush et al. 1991), and *Enp-1* (possibly homoeoallelic to *Enp-M1*) has been located on chromosome 3 (IRRI 1990). Another possibility is that both *Enp-M1* and *Pgi-M1* were introgressed by recombination, and that the extra chromosome either came from *O. sativa* or was an unmarked *O. minuta* chromosome.

The absence of any alien isozyme in four presumptive alien addition lines (2n = 25) shows the limitation of the electrophoretic analysis used. The 16 *Om*101141-specific isozymes do not mark all 24 *O. minuta* chromosomes. This limitation also applies to *O. sativa*, where isozyme genes have not been identified on chromosomes 9 and 10 (Wu et al. 1988; Ishikawa 1991; Khush et al. 1991).

Two euploid plants (2n = 24) exhibiting *Om*101141-specific isozymes were also identified. One plant ('69-8') had two alien isozymes (SDH-M2 and PGI-M5), and another ('78-1') had seven *O. minuta*-specific isozymes (SDH-M2, EST-M1, EST-M2, PGD-M1, PGI-M2, PGI-M3, PGI-M5). In *O. sativa*, *Pgd-2* (possibly homoeoallelic to *Pgd-M1*) is located on chromosome 6 (Khush et al. 1991), *Sdh-1* (possibly homoeoallelic to *Sdh-M2*) is located on chromosome 6 (Ranjhan et al. 1988), *Pgi-2* (possibly homoeoallelic to *Pgi-M2*) is located on chromosome 3 (Sano and Morishima 1984), *Est-2* (possibly homoeoallelic to *Est-M1*) is located on chromosome 3 (Sano and Morishima 1984), and *Est-5* (possibly homoeoallelic to *Est-M2*) is located on chromosome 1 (Wu et al. 1988). Bearing in mind the chromosome location of the homoeologous *O. sativa* loci, it is clear that a considerable amount of introgression has occurred in a few backcross progeny, presumably involving more than one *O. minuta* chromosome. This introgression presumably resulted from either recombination or centric break-fusion. Studies of chromosome pairing in *O. sativa*/*O. minuta* F_1 hybrids commonly reveal low levels of pairing at metaphase-I (Li et al. 1962). Indeed, Sitch et al. (1989) observed a mean chiasma frequency of 3.93 per cell at pachytene whereas the frequency of pairing was reduced to a mean of 3.49 chiasma per cell at diakinesis and to 2.59 chiasma per cell at metaphase-I. These results suggest that a low frequency of recombination may occur. The translocation, through centric break-fusion, of an *O. minuta* chromosome arm onto a complete or incomplete *O. sativa* chromosome, could have occurred during F_1 meiosis, when chromosomes of both parents remain largely unpaired, or during the BC_1 meiosis, when the majority of *O. minuta* chromosomes and occasional *O. sativa* chromosomes remain unpaired (Sitch, unpublished).

The introgression of a single isozyme locus, such as *Sdh-M1*, could have occurred through recombination, centric break-fusion, or through the substitution of a whole *O. minuta* chromosome. As with centric break-fusion, *Om*101141 chromosome substitution could have occurred during the F_1 or BC_1 meioses.

In conclusion, isozyme analysis has provided definitive evidence for a degree of homoeology between the two species and proved a valuable tool for monitoring the transmission of the *O. minuta* genome during the gene transfer process and for detecting alien introgression. More enzyme systems need to be examined to more fully mark the *O. minuta* genomes. Molecular analysis will provide a more complete marker system, either through the use of species-specific DNA probes for in-situ hybridization analysis or through restriction fragment length polymorphism analysis. Both these

analyses are now underway to determine further the nature and extent of introgression.

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