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Chromosome Location of Two Isozyme Loci in *Lolium perenne* Using Primary Trisomics

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Summary. Primary trisomics were used to locate the structural loci coding for particular forms of the dimeric enzymes phosphoglucoisomerase and glutamate oxaloacetate transaminase in *Lolium perenne*. The polymorphy of these loci enabled triallelic trisomics to be produced. Each locus could thus be directly assigned to a particular chromosome without the need to examine segregant progeny. The loci for *GOT/3* and *PGI/2* were found to be located on chromosomes 2 and 6 respectively.

Key words: *Lolium perenne* – Primary trisomics – Isozymes – Glutamate oxaloacetate transaminase – Phosphoglucoisomerase

Introduction

Primary trisomics have been extensively used, in many species, to assign individual gene loci and established linkage groups to particular chromosomes, utilizing the fact that genetic ratios for genes located on the extra chromosome are modified in segregating progenies (Kush 1973). In recent years isozymes have increasingly featured in the field of chromosome mapping and they have several advantages over more conventional morphological characters as genetic markers (Nielsen and Scandalios 1974). The most important of these advantages are that (i) isozyme variants seldom show obvious deleterious effects, (ii) variant alleles are generally codominant making it possible to easily and positively identify heterozygotes as well as homozygotes, (iii) since isozymes generally represent specific gene products, variants are more likely to be single gene mutants than are complex morphological characters.

The location of a specific isozyme locus is particularly facilitated if three or more allelic variants are available. By making suitable crosses, trisomic plants which are also triallelic (identified by their electrophoretic banding pattern) can be obtained, clearly demonstrating that the relevant gene is situated on the chromosome for which the plant is trisomic. The necessity for examining segregant progeny is thereby obviated.

Two loci, each with four alleles, coding for particular forms of the dimeric enzymes phosphoglucoisomerase and glutamate oxaloacetate transaminase respectively, have been described in detail in *Lolium perenne* (Hayward and McAdam 1977; Hayward and Balls 1978) and in *L. perenne* × *L. multiflorum* hybrids (Breese and Thomas 1977). These were designated *PGI/2* and *GOT/3* and the alleles at each labelled a to d according to their electrophoretic mobility; a being the 'fastest' allele.

The present study was undertaken to ascribe *PGI/2* and *GOT/3* to their respective chromosomes using their polymorphy in the way already outlined.

Materials and Methods

Triploid plants (2n = 3x = 21), comprising two genomes of *L. perenne* and one of *L. multiflorum*, were already available at the Welsh Plant Breeding Station. The use of nominally interspecific material was convenient in that the frequency of some of the rarer alleles in *L. perenne* was thereby increased. This is acceptable in the case of these very closely related species since their chromosome sets are karyotypically indistinguishable from one another (Fig. 1).

These triploids were pollinated by diploid *L. perenne* in order to produce trisomic (2n = 15) plants which would be expected to form part of the progeny (Fig. 2). Aneuploids with more than 15 chromosomes (trisomic for two or more chromosomes) were again crossed with diploids to produce further 2n = 15 plants.

During the course of the work, triploids and diploids of suitable *PGI/2* banding patterns became available in sufficient numbers to design a mating programme capable of producing all the possible triallelic progeny from the four alleles. For example, a triploid of phenotype ad, and therefore carrying either a or d in duplicate, could be pollinated with a diploid homozygous for b or c or heterozygous for the two alleles. A more restricted pro-

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Fig. 1. Karyotype of a *L. perenne* × *L. multiflorum* hybrid. The two parental sets of chromosomes are indistinguishable

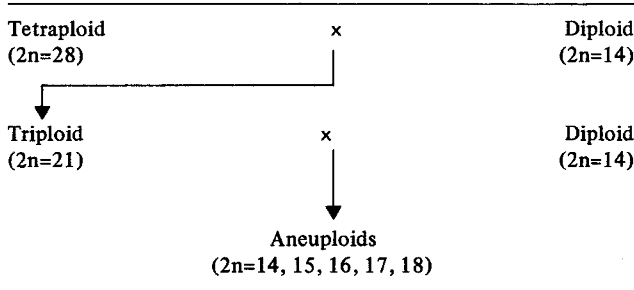

Fig. 2. Crossing programme used to produce trisomics

Fig. 3a and b. a The four triallelic phenotypes for *PGI/2* (left to right: abc, abd, acd and bcd); b the triallelic phenotype obtained for *GOT/3* abc

gramme was carried out for *GOT/3* because of the limited range of suitable parents available.

Root tip chromosome counts were made using the Feulgen staining technique, and the electrophoretic assays for *PGI/2* and *GOT/3* carried out using the technique described by Hayward and McAdam (1977) but with some modifications. These were as follows:

i) only 2 cm of actively growing leaf was used;

ii) the crushing buffer was tris HC1 (pH 7.2, 0.05 M) containing 0.05 ml of mercapto-ethanol per 50 ml;

iii) the gels were run at 35 mA (and 200-220 volts) before removal of the wicks and 50 mA (and approx. 300 volts) afterwards;

iv) in staining for PGI/2, 1.0 ml of $MgC1_2$ replaced the $MgS0_4$ and the reaction was carried out in 0.1 M tris HC1 (pH 7.5) buffer. Trisomics with three variant alleles for either locus were karyotypically analysed, using the Feulgen staining technique after pretreatment for $1\frac{1}{2}$ h in mono bromo-naphthalene, and the respective trisome identified.

Results

Plants ranging in chromosome number from 14-18, with 2n = 14, 15 and 16 the most common, were found in the progeny of the triploid × diploid crosses. Nine trisomics which were triallelic at the PGI/2 locus were recovered from a total of 738 plants (derived from tripoid × diploid and aneuploid × diploid). These consisted of abc, abd, acd and bcd; the four possible patterns for these four alleles (Fig. 3a). One *GOT/3* triallelic trisomic was recovered from 79 plants. This had the banding pattern abc (Fig. 3b). These banding patterns confirm the dimeric nature of the two enzymes, in that hybrid bands (heterodimers) for all the alleles present are formed in each case. They also show that the alleles at each locus are codominant in their expression since homodimeric bands are produced by each allele present. The patterns further show that the genes controlling the two enzymes are structural, rather than regulatory or modifying, according to the criteria proposed by Courtwright (1976). Three of the five listed by him: (i) the alteration of electrophoretic mobility in mutants, (ii) codominant expression of alleles in heterozygotes, (iii) hybrid enzyme formation in heterozygotes, are met in the case of both loci.

Karyotype Determinations

Karyotype analysis of a number of the *PGI/2* trisomics clearly showed that the chromosome concerned is the second shortest in the complement. This is a sub-metacentric and was designated chromosome 6 by Malik and Thomas (1966). Figure 4a shows the karyotype of this trisomic.

Analysis of the trisomic plant with three variant alleles at the *GOT/3* locus showed that the extra chromosome was number 2 in the classification of Malik and Thomas (1966). This is the second longest of the three chromosomes which have secondary constrictions (Fig. 4b). Figures 4a and b are from photographs of single cells.

Discussion

The development of techniques enabling the use of isozymes as genetic markers has proved particularly useful in the cytogenetically less well investigated species, such as the material used here, when there is a dearth of other forms of utilizable markers. The many advantages of the

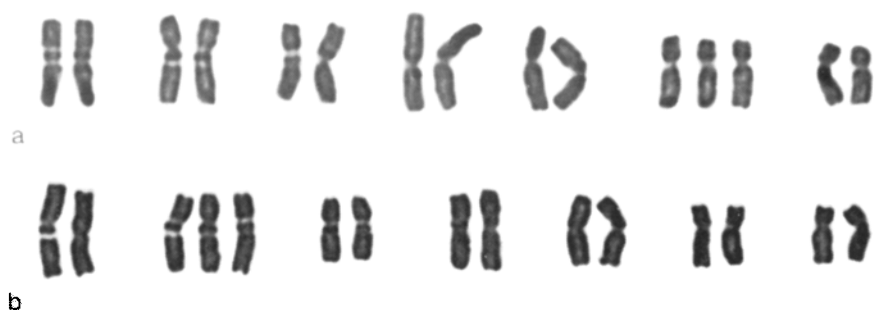

Fig. 4a and b. Karyotype of a single cell, a trisomic for chromosome 6 from a plant triallelic for *PGI/2*; b trisomic for chromosome 2 from the plant triallelic for *GOT/3*

former have been pointed out by Nielsen and Scandalios (1974) and others, and the present study serves to underline the particular value of isozyme loci showing polymorphy and codominance for chromosome labelling.

Courtwright (1976) stressed the importance of distinguishing between structural and regulatory isozyme loci but pointed out the difficulty of doing so directly. He however gave a number of indirect criteria for the identification of the structural gene for a specific enzyme, which depend on altered kinetic and/or physicochemical properties of allozymes. The application of these confirm that the loci studied here are structural.

Since the triploids used in this study were nominally 'interspecific', the consequences of possible 'interspecific' chromosome differentiation, both morphologically and genetically, albeit extremely unlikely, have to be borne in mind in the interpretation of the results.

The results of Malik and Thomas (1966) suggest some difference in the total length of the chromosome complements of these species but the authors expressed considerable reservation on the significance of this difference. A detailed karyotypic analysis of *L. perenne* × *L. multiflorum* hybrids during the course of the present study showed no consistent morphological differences between corresponding chromosomes of the parental genomes.

The possibility that a particular locus may not be located on the 'same' (homoeologous) chromosome in the two species also has to be considered. The composition of the range of banding patterns obtained in the progenies from the triploid × diploid crosses, and more recently from trisomic × diploid combinations, strongly suggests that this is not so. If it had been the case, anomalous banding patterns such as triallelic diploids and possibly even plants carrying no alleles for that locus would have been expected amongst the large numbers of plants assayed. None were found and it is therefore reasonable to rule out this remote possibility.

It is concluded that in *L. perenne* (and *L. multiflorum*) the structural loci for *GOT/3* and *PGI/2* are located on chromosomes 2 and 6 respectively. Furthermore, since it has been shown that *PGI/2* is linked to the S self incompatibility locus in *L. perenne* (Cornish et al., in press), it follows that the latter must also be carried on chromosome 6.

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