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The intrachromosomal mapping of a glucose phosphate isomerase structural gene, using allelic variation among stocks of Chinese Spring wheat

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SUMMARY

Detectable allelic variation at the *Gpi-1* loci on the short arms of the homoeologous group 1 chromsomes in wheat is not common. However, a variant null allele at the *Gpi-D1* locus is present in some stocks of Chinese Spring. This has allowed the locus to be mapped between the ω-gliadin locus carried distally on the short arm of chromosome 1D, *Gli-D1* (34·5 %) and the high-molecular-weight glutenin subunit locus carried near the centromere on the long arm, *Glu-D1* (36·2 %). The origin of this isoenzyme polymorphism in Chinese Spring stocks is described and its potential significance is discussed in relation to quantitative analysis of aneuploids, alien chromosome addition and substitution lines and intervarietal chromosome substitution lines involving Chinese Spring.

1. INTRODUCTION

The chromosomal locations of structural genes of a large number of enzymes have been identified in wheat (*Triticum aestivum* $2n = 6x = 42$) by analysis of isozyme variation in aneuploids, especially ditelocentric and nullisomic tetrasomic stocks (Hart 1979*a*). However, due to the paucity of evidence for allelic variation at these loci, intrachromosomal mapping has been possible in only a few cases, *Got-Ag 3* (Hart, McMillin & Sears, 1976), α-Amy-B2 (Gale *et al.* 1983), α-Amy-1 (Nishikawa *et al.* 1981) and β-Amy-B2 (Gale, Ainsworth & Baird, 1982).

The location of triplicate structural genes for glucose phosphate isomerase (GPI) on the short arms of the group 1 chromosomes was made by Hart (1979*b*) and improved resolution of the GPI isozymes by isoelectric focussing indicated that the three *Gpi-1* loci each control production of at least two enzyme subunits (Chojecki & Gale, 1982). A variant allele, *Gpi-D1b*, having a null GPI-1D phenotype was found in certain euploid and aneuploid stocks of Chinese Spring (CS), which we have exploited to map the locus against two previously mapped storage protein loci, *Glu-D1* and *Gli-D1* (Payne *et al.* 1982), also located on chromosome 1D.

2. MATERIALS AND METHODS

(i) *Genotypes*

F_2 grains of a cross between the variety Koga II and the Chinese Spring ditelocentric for the short arm of chromosome 1B (CS DT1BS) were investigated. The parents are known to differ at the *Gpi-D1* locus, the *Glu-D1* locus (encoding high-molecular-weight (HMW) subunits of glutenin) and the *Gli-D1* locus (encoding

Table 1. *Parental genotypes*

Phenotype	CSDTI1BS	Koga II
Glucose phosphate isomerase	*Gpi-D1b*	*Gpi-D1a*
HMW subunits of glutenin	*Glu-D1a* (subunits 2 and 12)	*Glu-D1d* (subunits 5 and 10)
ω-Gliadins	*Gli-D1a*	*Gli-D1b*

Note: A complete description of the alleles and phenotypes o the *Glu-1* loci is given in Payne & Lawrence (1983). Complementary catalogues for the *Gli-1* loci are currently being prepared by Payne and co-workers.

ω-gliadins) as summarized in Table 1. All three loci are carried on chromosome 1D. The fact that one parent is ditelocentric for chromosome 1B is incidental to this study.

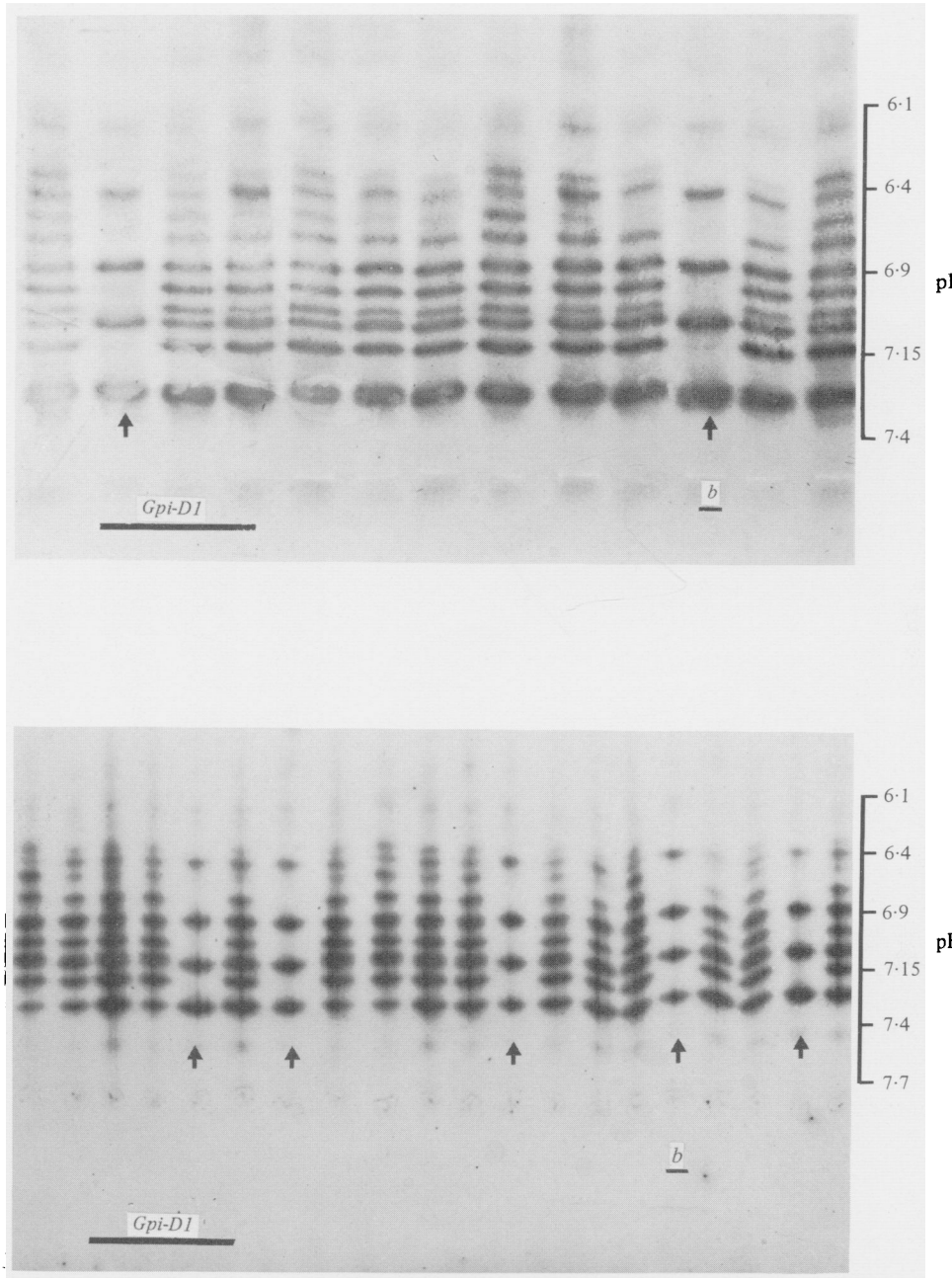
(ii) *Glucose phosphate isomerase*

The GPI phenotypes of the F_2 progeny were ascertained using isoelectric focussing (IEF) and specific enzyme staining as described previously (Chojecki & Gale, 1982), but with a modified preparative procedure. A 2–3 mm portion was removed from the distal end of each grain and placed cut face down on the gel surface, 2 cm in from the cathode. The portions were removed after 1 h of electrofocussing and retained. This method considerably reduces the possibility of artifacts of isozyme pattern caused by procedures of sample preparation or storage (Jones, 1982), as well as saving time. In addition, more genotypes were screened per unit width of gel, since the portions were narrower than the paper wicks used previously. Furthermore, the grain portions can be re-used, for example as in this case, in the preparation of samples for analysis of endosperm storage proteins, and the embryo (plus an adjoining piece of endosperm) can be retained to be grown into plants for progeny testing, if required.

The zymograms produced by this direct method and the more extensive extraction procedure are compared in Plate I.

(iii) *Endosperm storage proteins*

HMW subunits of glutenin were analysed using sodium dodecyl sulphate, polyacrylamide-gel electrophoresis (SDS-PAGE) as described by Payne, Law & Mudd (1980). The ω-gliadins were analysed by ACID PAGE (A-PAGE) using the method of Payne *et al.* (1982).



(*a*) GPI zymograms produced by F_2 progeny segregating at the *Gpi-D1* locus. The arrows indicate the homozygous *Gpi-D1b* genotypes. The heterozygotes and homozygous *Gpi-D1a* genotypes are qualitatively indistinguishable by IEF. The samples were prepared using the full extraction procedure, and applied to a narrow pH-range gel using paper wicks. (*b*) As plate I*a*, except using the direct method of sample application and a wide pH-range gel. The same width of gel is shown. The marks left by the cut grain sections are discernible in the lower half of the plate.

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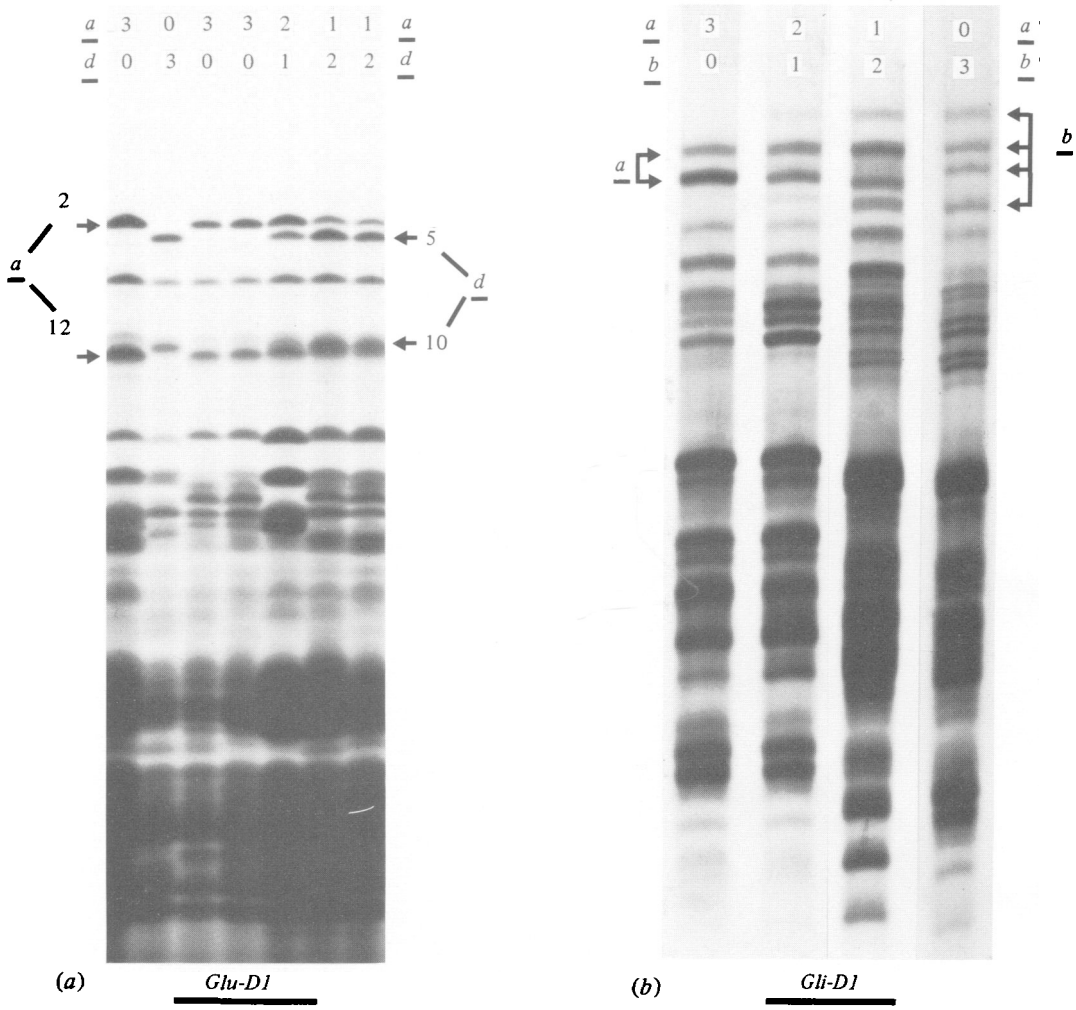

(*a*) SDS-PAGE analysis of F_2 progeny segregating at the *Glu-D1* locus. The locus encodes the HMW small subunits of glutenin (*Glu-D1a* codes for subunits 2 and 12 and *Glu-D1d* codes for subunits 5 and 10, as indicated). Both homozygotes and both heterozygotes (see Results, ii) in the triploid endosperm are distinguishable. The different allelic doses (given at the top of each track) produce bands of different intensities. (*b*) A-PAGE analysis of F_2 progeny segregating at the *Gli-D1* locus which encodes the ω-gliadins. *Gli-D1a* codes for two ω-gliadins and *Gli-D1b* codes for four, as indicated). Triploid endosperm dosages of the two alleles are given at the top of each track.

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3. RESULTS

(i) *Gpi-D1*

The *Gpi-D1a* allele (Koga II) is dominant to the *Gpi-D1b* allele (CS DT1BS) and so the GPI phenotypes of the heterozygote and dominant homozygote are indistinguishable by IEF, both having the full GPI phenotype. Of 231 F_2 grains examined, 58 were identified as recessive homozygotes (*Gpi-D1b*) having the

Table 2*a*. *Segregation at Glu-D1 and Gli-D1 loci among 58 Gpi-D1b homozygotes identified among F_2 progency of Koga II* (*Gpi-D1a*, *Glu-D1d*, *Gli-D1b*) × *CS DT1BS* (*Gpi-D1b*, *Glu-D1a*, *Gli-D1a*)

Glu-D1	ddd	dda	daa	aaa	Total
Gli-D1					
bbb	1	2	1	4	8
bba	1	3	3	4	11
baa	0	6	4	5	15
aaa	3	4	7	10	24
Total	5	15	15	23	58

Table 2*b*. *Frequencies of recombination between Gpi-D1 and storage protein loci among F_1 gametes*

Glu-D1	dd	da	aa	Total
Parental	0	30	46	76
Recombinant	10	30	0	40
Gli-D1	bb	ba	aa	Total
Parental	0	26	48	74
Recominant	16	26	0	42

null-GPI-D1 phenotype (plate 1*a*). The ratio of heterozygotes and dominant homozygotes to recessive homozygotes is 173 : 58 which is not significantly different from the 3 : 1 Mendelian ratio expected for single gene segregation in an F_2. Because of the incomplete classification of *Gpi-D1*, only the 58 *Gpi-D1b* homozygotes were selected for analysis for the protein gene markers *Glu-D1* and *Gli-D1*, these being the genotypes which provide most information in an assessment of linkage.

(ii) *Glu-D1 and Gli-D1*

Complete classification of F_2 progeny is possible for both these loci, using PAGE. In addition, due to the nature of the triploid inheritance in the endosperm, there are two classes of heterozygotes (one dose from one parent and two from the other, and *vice versa*). These classes can be distinguished from each other by the intensity of bands visualized using PAGE (plate II*a* and *b*). This permits a check to be made for differential transmission of alleles through male or female gametes, which would result in a departure from the expected 1 : 1 ratio of the two heterozygote classes, for either protein locus. The results of PAGE analysis of the 58 *Gpi-D1b* homozygotes are summarized in Table 2*a*. There is no significant departure from

the expected ratios of heterozygote classes for either locus, indicating that there is no differential transmission. Ratios of 1:2:1 for homozygous and heterozygous storage protein genotypes would be expected if the *Glu-D1* and *Gli-D1* loci were independent of *Gpi-D1*. However these ratios are plainly disturbed in favour of the CS DT1BS genotype indicating that both genes are linked with the enzyme locus.

(iii) *Estimation of linkage*

Since all possible gametes can be identified, the frequencies of recombination between *Gpi-D1* and each of the two protein loci can be ascertained directly (Table 2*b*). The values obtained are $36{\cdot}2 \pm 4{\cdot}5\,\%$ recombination between *Gpi-D1* and *Glu-D1* and $34{\cdot}5 \pm 4{\cdot}4\,\%$ between *Gpi-D1* and *Gli-D1*.

To obtain a direct estimate of recombination between *Glu-D1* and *Gli-D1*, a random sample of 209 of the 231 progeny used in the GPI investigation were also analysed by electrophoresis. The value obtained of $48{\cdot}3 \pm 2{\cdot}4$ is similar to the $43{\cdot}0 \pm 1{\cdot}4\,\%$ recombination between the homoeologous storage protein genes in chromosomes 1A and 1B (Payne *et al.* 1982). Thus, considering the present data, *Gpi-D1* would appear to lie between *Glu-D1* and *Gli-D1*. An indirect estimate of the recombination frequency between these loci can be obtained using the formula of Trow (1913), $y_{1+2} = y_1 + y_2 - 2y_1 y_2$, where y_1 and y_2 are recombination frequencies in consecutive segments and y_{1+2} is that over them both. This gives a value for recombination between *Glu-D1* and *Gli-D1* of $45{\cdot}8\,\%$, which is close to the direct estimate of $48{\cdot}3\,\%$.

This calculation assumes no interference between the two chromosome segments (*Gpi-D1* to *Glu-D1* and *Gpi-D1* to *Gli-D1*). The absence of interference is directly confirmed in these data, by the insignificant value of χ^2 ($P > 0{\cdot}3$) in a simple test for independence of cross-over frequencies in the two segments.

4. DISCUSSION

The *Gpi-D1b* allele is present in several Chinese Spring aneuploid stocks but probably first occurred in the plant from which Dr E. R. Sears extracted the first monosomic, mono 1B (formerly identified as mono 1). It is not present in any subsequently discovered monosomic lines. However *Gpi-D1b* has been found in three other stocks, all traceable to monosomic 1B, namely CS ditelosomic 1BS, CS ditelosomic 5BS and the CS euploid stock maintained at the Plant Breeding Institute (PBI). CSDT1BS was probably derived directly from mono 1B by Dr Muramatsu, the PBI stock of CS euploid was derived from two disomic plants extracted from mono 1B in 1961 by Mr V. Chapman, and CSDT5BS was identified by Mr T. E. Miller in the PBI CS euploid background. Our survey of many of the Chinese Spring aneuploids has not been exhaustive and it is probable that *Gpi-D1b* will be found in other stocks, especially since euploid grain from the PBI has been distributed worldwide.

As described by Chojecki & Gale (1982), the *Gpi-1* loci are probably compound and each produce at least two enzyme protomer subunits. The *Gpi-D1b* allele produces a null phenotype and therefore could represent an interstitial deletion

on chromosome 1D. However, the agreement between the combined estimate of recombination between the *Glu-D1* and *Gli-D1* obtained here and the values previously obtained for the homoeologous loci on chromosomes 1A and 1B indicate that, if a deletion is involved, it is not large. The genetic map of chromosome 1D is summarized in figure 1.

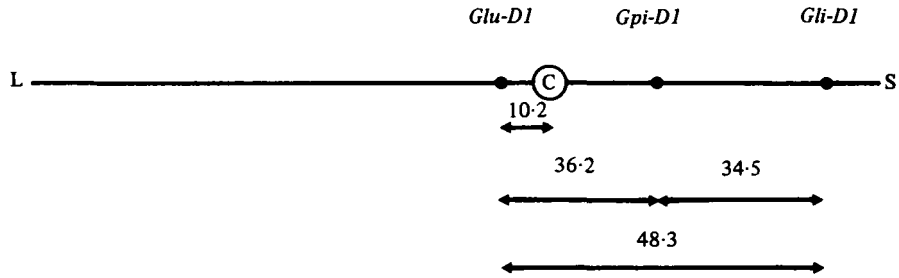

Fig. 1. Genetical map of chromosome 1D, with recombination frequencies (%). L, C and S indicate the long arm, centromere and short arm respectively.

The polymorphism among Chinese Spring stocks means that many derivatives, such as alien chromosome addition lines and substitution lines and intervarietal chromosome substitution lines, may have different *Gpi-D1* background genotypes. In fact the *Gpi-D1b* background has proved to be an advantage in recognizing, for example, the presence of *Gpi-R1* and *Gpi-H^{ch}1* on the group 1 homoeologues of rye and *Hordeum chilense* (Chojecki & Gale, 1982), because the alien homodimers and heterodimers cofocus with dimers involving products of *Gpi-D1a*. The background of CS based alien-wheat chromosome addition series depends upon the laboratory from which they originated. Thus, among the wheat-rye series, Chinese Spring/*Secale cereale* cv King II and Chinese Spring/*S. montanum* carry *Gpi-D1b* while Chinese Spring/*S. cereale* cv Imperial carries *Gpi-D1a*. Similarly the Chinese Spring/*Hordeum chilense* addition series developed at the PBI carries *Gpi-D1b* while the Chinese Spring/*Agropyron elongatum* series developed elsewhere by Dvorak & Knott (1974) carries *Gpi-D1a*, although two new *Agropyron* additions, 3E and 5E, developed by Hart & Tuleen (1983) have been produced in a *Gpi-D1b* background. The one anomaly so far encountered is the Chinese Spring/*Aegilops umbellulata* addition series developed at the PBI by Kimber (1967) which carry *Gpi-D1a*. This situation could arise where pollen from a CS monosomic was used in the backcrossing rather than from the euploid stock.

The significance of this single allelic difference among Chinese Spring stocks is not clear. It may be an isolated effect and certainly does not involve the deletion of a major part of the short arm of chromosome 1D. However it is possible that there may be associated pleiotropic effects on other important characters, particularly in view of the involvement of GPI in starch synthesis, thus it may be prudent to employ the correct Chinese Spring stock in future comparative analyses. The correct stock for use with intervarietal chromosome substitution lines in a Chinese Spring background will usually be the *Gpi-D1a* line, since these genotypes are developed by backcrossing onto monosomic stocks rather than euploids.

Pure lines of both Chinese Spring genotypes will shortly be available for distribution from this laboratory.

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