Theor Appl Genet (1987) 73:433–439

Chromosomal location of structural genes controlling isozymes in *Hordeum chilense*

1. 6-Phosphogluconate dehydrogenase and malate dehydrogenase

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Received March 5, 1986; Accepted September 10, 1986
Communicated by G. S. Khush

Summary. A study of 6-phosphogluconate dehydrogenase and malate dehydrogenase isozyme expression in *Triticum turgidum* conv. *durum*/*Hordeum chilense* monosomic addition lines has revealed the location of two structural genes, *6-pgd-H^{ch}2* and *Mdh-H^{ch}1*, on chromosome 1H^{ch} of *H. chilense*. The homoeology between 1H^{ch} and other chromosome of Triticeae related species is discussed on the basis of isozyme gene analysis.

Key words: *Hordeum chilense* – Tritordeum – C-banding – 6-phosphogluconate dehydrogenase – Malate dehydrogenase – Isozymes – Isozyme markers

Introduction

Miller et al. (1981) have obtained disomic and ditelosomic addition lines of *Hordeum chilense* chromosomes in common wheat, *Triticum aestivum* cv. 'Chinese Spring' (CS), and the three group 7CS/H^{ch}7 substitutions. However, these authors have not characterized cytologically the chromosomes of *H. chilense*. Such an identification is suitable for gene location studies and has been carried out in the addition series of other species, as, for example, *H. vulgare* (Islam 1980). The C-banded karyotype of *H. chilense* and its amphiploid × *T. turgidum* conv. *durum* was reported by Fernández and Jouve (1984), who also were able to identify the *H. chilense* chromosomes in tetraploid wheat/*H. chilense* monosomic addition forms (Fernández and Jouve 1986).

The easiest and most effective method for the identification of structural genes and their chromosomal location in Triticeae species is the study of isozyme expression in wheat lines which include alien genetic material (Hart 1979). Chromosomal location and genetic control of isozyme genes in common wheat have been the objective of many studies (Hart 1984). Likewise, some structural loci have been identified in *H. chilense* using common wheat addition and substitution lines (Chojecki and Gale 1982; Ainsworth et al. 1984, 1986; Miller et al. 1985).

Chromosomal location studies of isozyme genes using tetraploid wheat addition lines have never been carried out until now. The absence of the D genome of *T. aestivum* could promote the expression of more simple zymograms and would bring about some advantages. In accordance with this, the objectives of our study have been the following: 1) the assignment of biochemical markers to *H. chilense* chromosomes by means of isozyme analysis in tetraploid wheat monosomic addition lines obtained by Fernández and Jouve (1986); 2) the study of the structure and genetic control of the enzymatic systems analyzed and the chromosomal location of their structural genes; and 3) the establishment of linkage groups and, accordingly, the understanding of homoeology relationships between *H. chilense* chromosomes and those of related species.

The first paper deals with the analysis of the location of genes coding for two enzymatic systems, 6-phosphogluconate dehydrogenase (6-PGD, E.C. 1.1.1.44) and malate dehydrogenase (MDH, E.C. 1.1.1.37), on the *H. chilense* chromosomes.

Materials and methods

We have used the following plant material:

Triticum turgidum conv. *durum* (AA BB, 2n = 28), cv. 'Mexican 248 × Andalucía 344' (MA).

Hordeum chilense Brongn var. 'muticum' (Presl) Hauman (H^{ch}H^{ch}, 2n = 14).

The amphiploid *H. chilense* × *T. turgidum* conv. *durum* (AA BB H^{ch}H^{ch}, 2n = 42), line CHMA. This amphidiploid, called Tritordeum, was obtained by chromosome doubling of the hybrid between *durum* wheat cv. MA and the *H. chilense* variety indicated above (Martín and Sánchez-Monge Laguna 1982).

The seven monosomic addition lines of *H. chilense* chromosomes on tetraploid wheat. These plants were obtained by

selfcrossing of the hybrid (AA BB H^{ch}, 2n = 35) between *durum* wheat and Tritordeum, as well as by backcrossing of this hybrid with *durum* wheat (Fernández and Jouve 1986).

T. aestivum (AA BB DD, 2n = 42), cv. 'Chinese Spring' (CS).

H. vulgare (NN, 2n = 14), cv. 'Hassan'. Both common wheat and cultivated barley have been used as control patterns in this study.

Chromosomal control of plants was carried out using somatic cells of root tips by the Feulgen method. Identification of the *H. chilense* chromosomes in the corresponding seven monosomic addition lines was made according to C-banded chromosomes described by Fernández and Jouve (1984).

Electrophoretic analysis was carried out on extracts from young leaves 50 mg in weight. Small pieces of paper wicks (Whatman 3MM) were soaked in crude extract and inserted into horizontal starch gels 12% (for 6-PGD and MDH analysis), or polyacrylamide gels 8% (for MDH analysis). Electrophoresis conditions and enzyme staining methods were carried out following techniques published by Salinas and Benito (1983) and Benito and Salinas (1983) for 6-PGD and MDH, respectively. A minimum of ten electrophoretic assays were made for each sample. Photographs of isozyme phenotypes were scanned (=620 nm) using a Joyce and Loebl Chromoscan densitometer.

Results

Chromosome identification

C-banding of monosomic addition lines permits an easy recognition of the alien H^{ch} chromosome, in accordance with the classification of Fernández and Jouve (1984) (Fig. 1).

6-Phosphogluconate dehydrogenase

Although a considerable number of gels were analyzed no intraspecific variation was observed in isozyme phenotypes in any material. 6-PGD zymograms showed two distinct zones on starch gels (Figs. 2 and 4). Zone 1 (most anodic) revealed a single isozyme band, 6-PGD-1, in all materials. Isozymes were present at four migration positions in zone 2 (slow migrating). *T. aestivum* and *T. turgidum* phenotypes exhibited a single isozyme band, 6-PGD-2-w1, and *H. chilense* expressed a 6-PGD-2-ch1 isozyme band. The 6-PGD-2 zymograms of Tritordeum and the addition chromosome V presented three isozymes: *band-1* and *band-3* coincident with 6-PGD-2-w1 and 6-PGD-2-ch1, respectively, and *band-2* with an intermediate migration rate. From them, *band-1* and *band-2* exhibited higher relative staining intensities than *band-3* (see Fig. 5). Finally, the *H. vulgare* zymogram showed the slowest migrating isozyme band.

Malate dehydrogenase

In the polyacrylamide or starch gels, MDH zymograms were invariable in all material and revealed two main electrophoretically active zones, MDH-1 and MDH-2. An additional cathodically migrating zone, faintly stained and unreliable, was excluded from this analysis (Figs. 3 and 4).

Zone 1 (fast migrating) showed three isozyme bands, MDH-1-w1, MDH-1-w2 and MDH-1-w3, in the zymograms of *T. aestivum* and *T. turgidum*. The MDH-1-w3 isozyme band exhibited the highest staining intensity. *H. chilense* presented one slight and faster migrating band, MDH-1-ch1, and another sharp isozyme, MDH-1-ch2, which showed similar migration rate and comparatively higher activity than MDH-1-w1. A third less intensely stained isozyme band, MDH-1-ch3, was also observed in *H. chilense* zymograms. Finally, Tritordeum and the addition chromosome V showed the MDH-1-ch2 band of *H. chilense* (*band-1*), Mdh-1-w3 of *durum* wheat (*band-3*) and a third patent isozyme (*band-2*) with an intermediate migration rate. *Band-2* and *band-3* exhibited higher enzymatic activity than *band-1* (Fig. 6).

Zone 2 (slow migrating) presented four isozymes in *T. aestivum* and *T. turdigum* zymograms. *H. chilense* showed at least two distinct isozyme bands, MDH-2-ch1 and MDH-2-ch2, which exhibited large enzymatic activity. Some of this isozymes also appeared in the MDH-2 zymogram of Tritordeum. No clear differences between MDH-2 isozyme phenotypes of addition lines were found.

Discussion

6-Phosphogluconate dehydrogenase gene location

The 6-PGD enzymatic system has been described as being controlled by two loci in different Triticeae species. Thus,

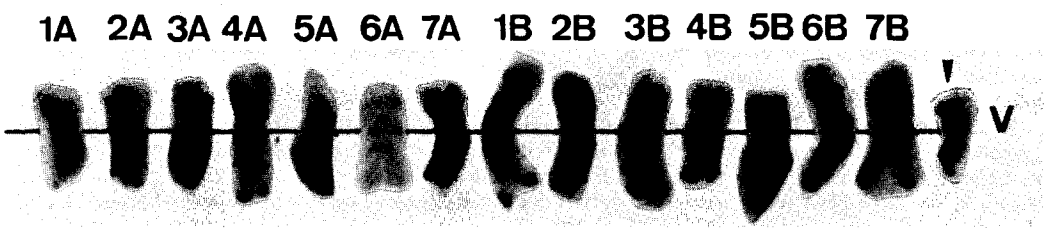

Fig. 1. Giemsa C-banded karyotype of the *durum wheat/H. chilense* addition chromosome V (*arrow* indicates the *H. chilense* chromosome)

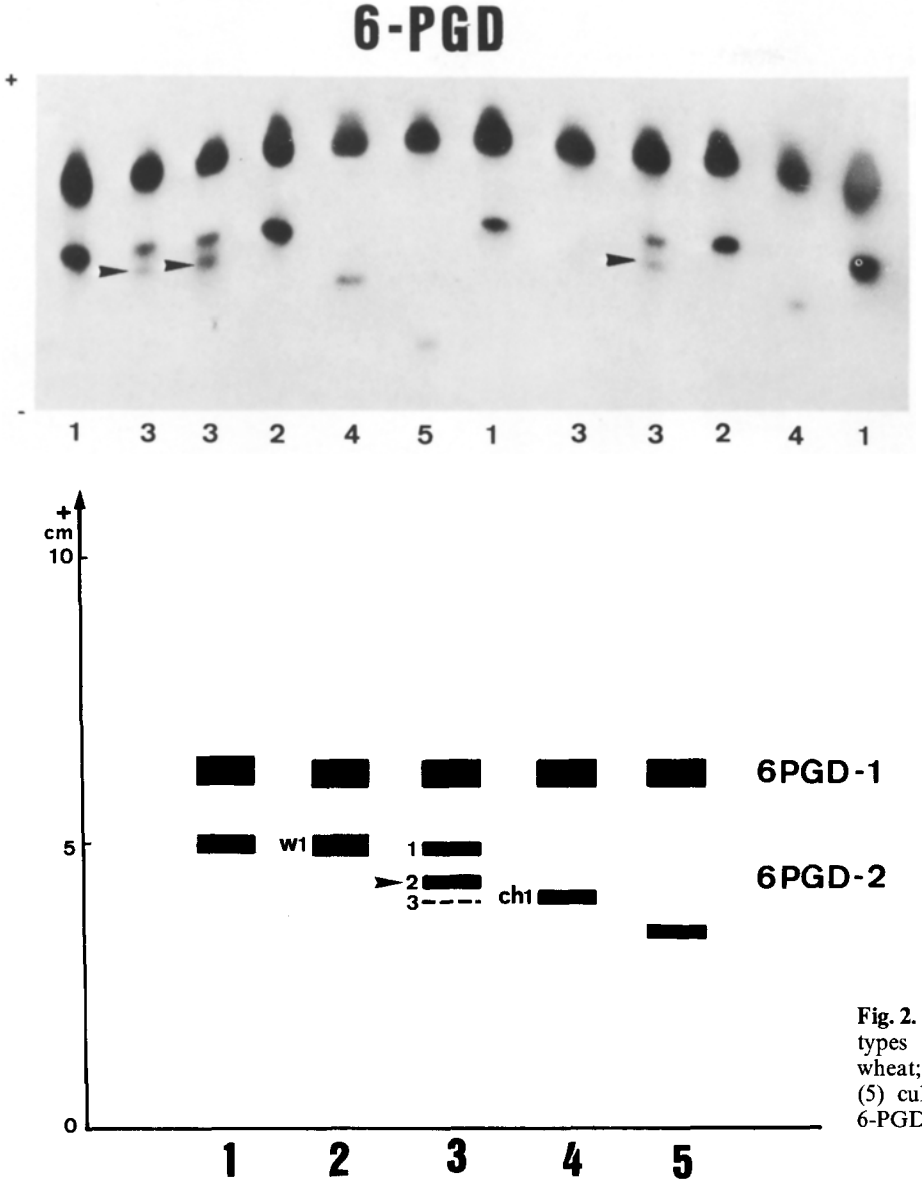

Fig. 2. Starch gel 6-PGD isozyme phenotypes of (1) common wheat; (2) *durum* wheat; (3) Tritordeum; (4) *H. chilense*, and (5) cultivated barley (*arrow* indicates the 6-PGD-2 isozyme marker)

Table 1. Chromosome arms of wheat, rye and barley related to the control of 6-PGD and MDH isozymes

Isozymatic system	Chromosomal location		
	T. aestivum	*S. cereale*	*H. vulgare*
6-PGD-1	7BL, 6A, 6BL (C6)	4RL, 6RL (C8, 6, 9)	–
6-PGD-2	–	2RL (C9)	5H (C4, 2)
MDH-1	1BL (C3)	–	5H (C7)
MDH-2	1AL, 1BL, 1DL (C1, 5)	1RL, 3R (C10)	5H, 3H (C4, 2)

C1 = Benito and Salinas (1983); C2 = Benito et al. (1985); C3 = Bergman and Williams (1972); C4 = Brown and Munday (1982); C5 = Díaz et al. (1986); C6 = Hsam et al. (1982); C7 = Powling et at. (1981); C8 = Rao and Rao (1980); C9 = Salinas and Benito (1983); C10 = Salinas and Benito (1985)

Brody and Mendlinger (1980), in young leaf tissue of *T. monococcum, Aegilops speltoides, Ae. longissima, Ae. bicornis* and *Ae. squarrosa,* demonstrated the expression of two discernable 6-PGD loci. Likewise, Stuber and Goodman (1980) pointed out that 6-PGD isozymes of *Zea mays* were coded for by two unlinked loci. 6-PGD structural genes have been located on different chromosomes of Triticeae species (Table 1).

The 6-PGD-2 isozyme phenotypes showed by Tritordeum and the *durum* wheat/*H. chilense* addition chromosome V reveals the presence of at least one structural gene that we call *6-pgd-H^{ch}2* in accordance with the rules for wheat gene symbolization (McIntosh 1983). This implies the existence of homoeologous genes on barley chromosome 5H and the mentioned chromosome V of *H. chilense.*

Our results on the intensity and distribution of the 6-PGD isozyme bands are in accordance with the assumption of a dimeric structure for the active enzymes, previously proposed by Rao and Rao (1980), Hsam et al. (1982) and Salinas and Benito (1983) for 6-PGD-1, and by Kahler et al. (1981) and Figueiras et al. (1985) for 6-PGD-2, in different Triticeae species.

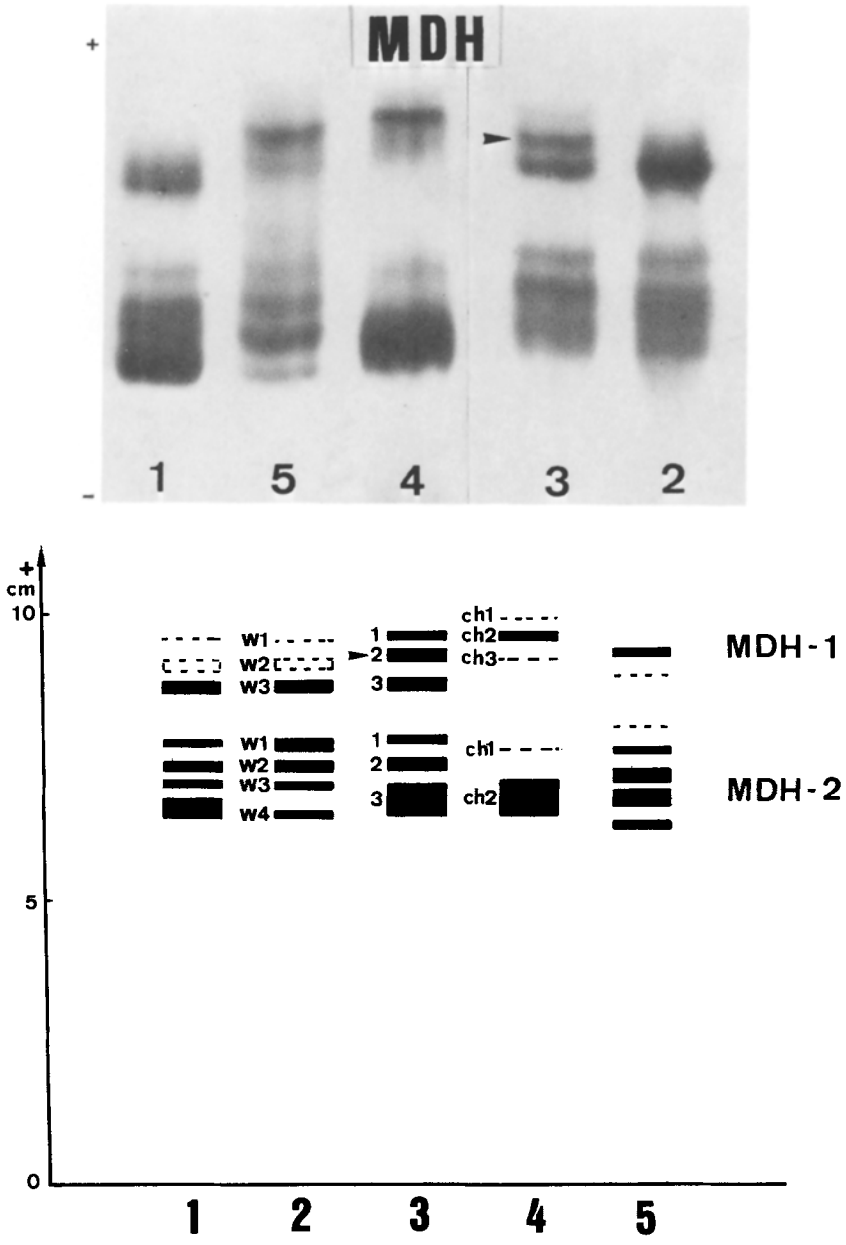

Fig. 3. Polyacrylamide gel and schematic representation showing MDH isozyme phenotypes of (1) *T. aestivum;* (2) *T. turgidum;* (3) Tritordeum; (4) *H. chilense,* and (5) *H. vulgare.* The *arrow* indicates the MDH-1 isozyme marker

The 6-PGD-2 zymograms showed by the amphiploid and the addition chromosome V could be the expression of all possible combinations of protomers α^2 and η^2. The α^2 molecules would be coded for by the same allele of two loci on chromosomes of both A and B genomes. The η^2 protomer would be expressed by the orthologous gene *6-pgd-H^{ch}2.* Thus, the 6-PGD-2-w1 isozyme of tetraploid wheat zymogram (*band-1* in the amphiploid) would be formed by $\alpha^2\alpha^2$ homodimers, and the 6-PGD-2-ch1 isozyme (*band-3* in the amphiploid) would correspond to $\eta^2\eta^2$ homodimers. The *band-2* of the Tritordeum zymogram, with an intermediate migration rate, would be composed of by $\alpha^2\eta^2$ heterodimers. Assuming the same gene dosage effect and a random association of protomers α^2 and η^2, the expected intensity ratios among these three isozymes (1, 2 and 3) should be 4 : 4 : 1, which agrees with the observed values (Fig. 5).

Malate dehydrogenase gene location

MDH isozymes have been associated with different cellular structures such as mitochondria or microbodies (Yamazaki and Tolbert 1969; Rocha and Ting 1970; Ting et al. 1975) or they have been recognised as soluble cytoplasmic molecules. McDaniel (1969) pointed out that most anodic MDH isozymes of wheat and barley zymograms correspond to cytoplasmic forms. Yang and Scandalios (1975) confirmed the soluble

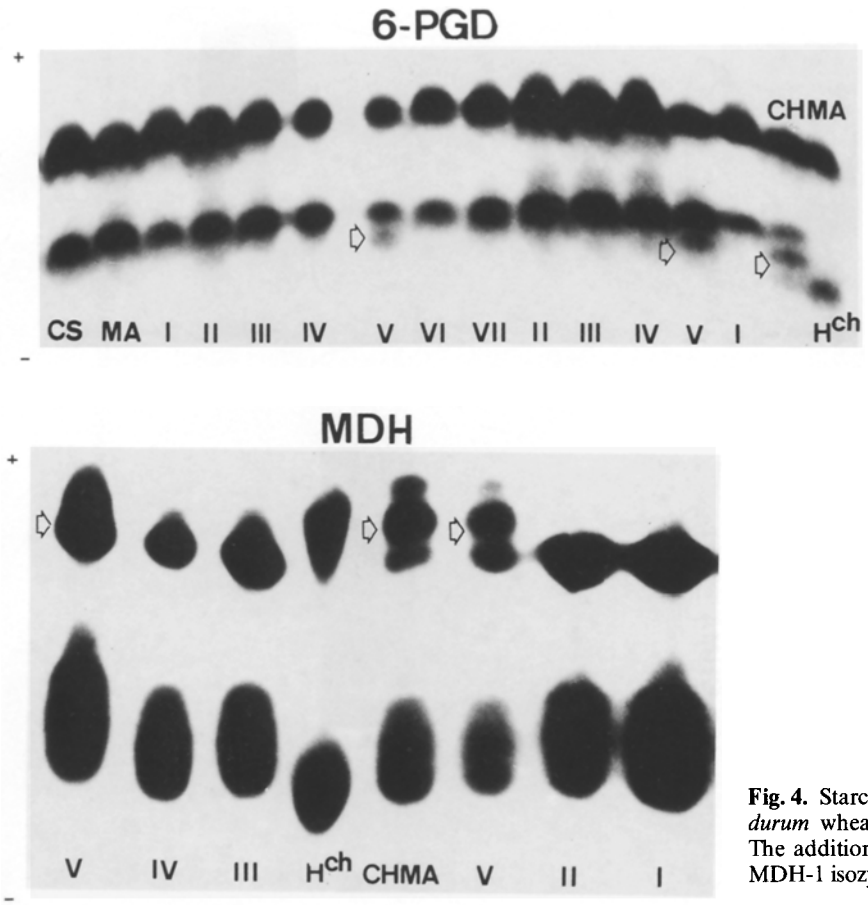

Fig. 4. Starch gel zymograms of 6-PGD and MDH in *durum* wheat/*H. chilense* monosomic addition lines. The addition chromosome V shows the 6-PGD-2 and MDH-1 isozyme markers, as is indicated by *arrows*

nature of these isozymes and demonstrated their nuclear control. MDH gene location analysis has been carried out in some Triticeae species (Table 1).

MDH isozymes have been described as dimers in maize (Yang and Scandalios 1975; Yang et al. 1977). Benito and Salinas (1983) reported dimeric MDH-2 isozymes in wheat, but, conversely, Díaz et al. (1986) confirmed a monomeric structure for these molecules in *durum* and common wheats. The observations of the present report suggest a dimeric behaviour for MDH-1 isozymes of *H. chilense* and Tritordeum, whereas MDH-2 isozymes seem to be monomers. These results are in agreement with previous descriptions of other Triticeae species (Powling et al. 1981; Pérez de la Vega and Allard 1984; Salinas and Benito 1985; Benito et al. 1985).

The isozyme phenotypes presented by the amphiploid and the addition chromosome V reveal the existence of at least a locus on this chromosome, that we called *Mdh-H^{ch}1*. It would be responsible for the production of protomers η^1, which would be associated among themselves or with other subunits (α^1), coded by wheat orthologous genes, to constitute active dimeric MDH-1 enzymes. As in the above mentioned model for 6-PGD-2 isozyme expression, a duplicated gene system could be assumed for MDH-1 structural genes in A and B wheat genomes, which coded for α^1 protomers. Thus, the MDH-1-ch2 isozyme (*band-1* in the amphiploid) would be formed by $\eta^1\eta^1$, whereas the MDH-1-w3 isozyme (*band-3*) would correspond to $\alpha^1\alpha^1$ homodimers. Isozyme *band-2,* that appears in the amphiploid and the addition chromosome V zymograms, would be constituted by $\alpha^1\eta^1$ heterodimers formed by random association of α^1 and η^1 subunits. Expected intensity ratios between band (1 : 4 : 4) agree with the observed values if we assume the same enzymatic activity of gene products.

Chromosomal homoeology

The location of *6-pgd-H^{ch}2* and *Mdh-H^{ch}1* structural genes on *H. chilense* chromosome V suggests homoeology between this chromosome and the mentioned group 1 of wheat and barley chromosome 5H. In addition, some morphological traits which have also been associated with *H. chilense* chromosome V are controlled by group 1 chromosomes in wheat (Fernández and Jouve 1986).

Generally chromosomes of Triticeae species have been named in accordance with their homoeology with *T. aestivum* groups. Previous studies carried out in the Plant Breeding Institute (Cambridge, U.K.) have classified two *H. chilense* chromosomes (A and G) on the basis of their capacity to replace their homoeologues of

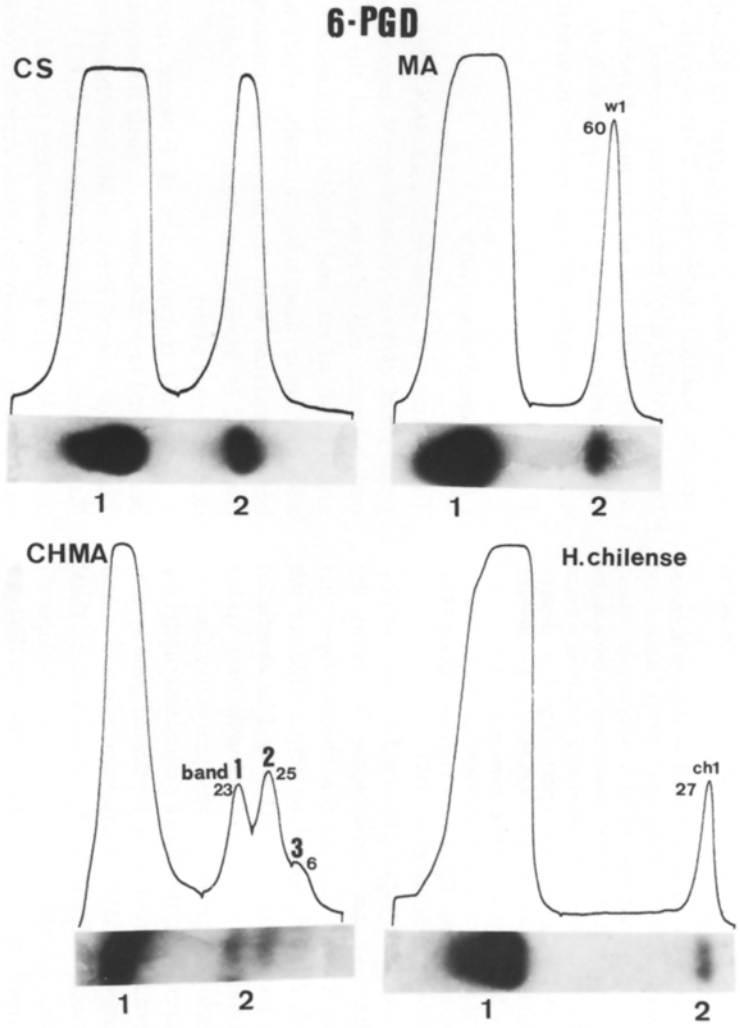

Fig. 5. Densitograms and densitometric values of 6-PGD-2 isozyme patterns of *T. aestivum* cv. CS, *T. turgidum* conv. *durum* cv. MA, Tritordeum line CHMA and *H. chilense*

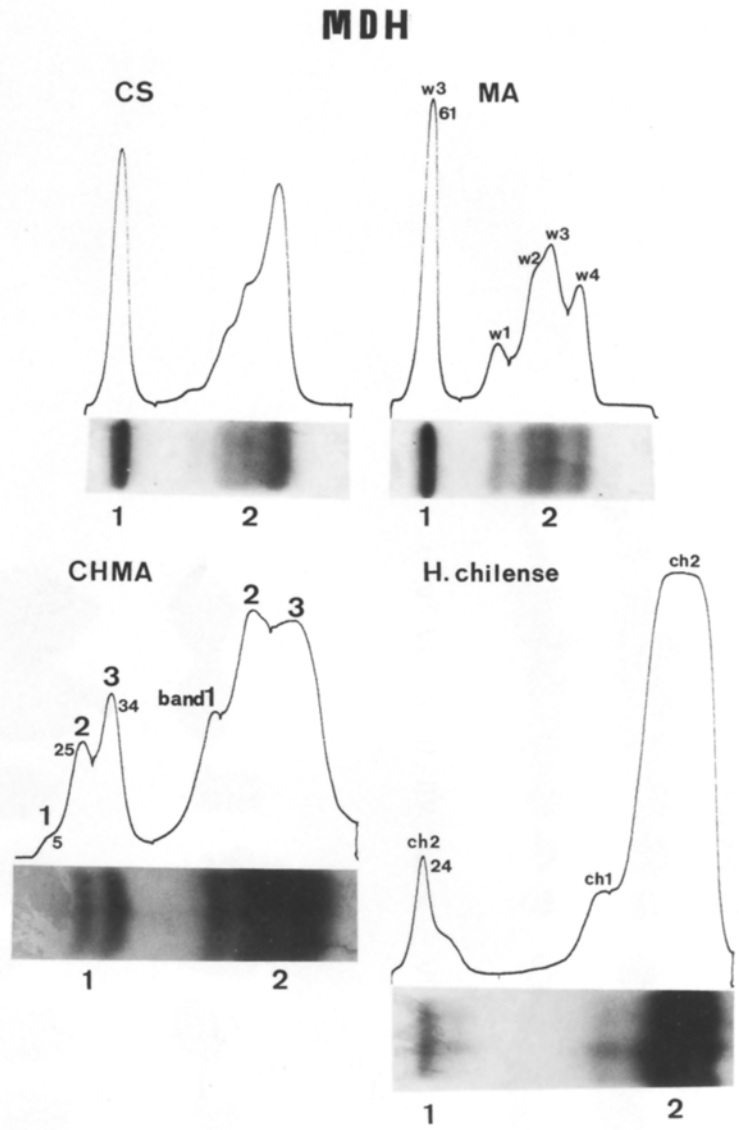

Fig. 6. Densitograms and densitometric values of MDH-1 isozyme patterns in the materials analyzed

wheat, being named $7H^{ch}$ and $1H^{ch}$, following the general criterion of Driscoll (1983). The presence of a glucose phosphate isomerase locus *Gpi-H^{ch}1*, a peroxidase locus *Per-H^{ch}1*, and a gene of high molecular weight glutenins on chromosome $1H^{ch}$, and its homoeology with group 1 chromosomes of wheat and 1R of rye (Miller et al. 1981; Chojecki and Gale 1982; Ainsworth et al. 1984) has been demonstrated. All these data permit us to establish clear homoeology relationships between the $1H^{ch}$ (chromosome V) of *H. chilense* and group 1 of wheat, rye and 5H of barley. It agrees with the little divergence in the location of isozyme structural genes and the maintenance of linkage groups throughout the evolution of Triticeae mentioned by Hart (1979).

Acknowledgements. We are deeply indebted to Dr. A. Martín for his supply of plant material and to Dr. C. Benito for his technical assistance. English correction of manuscript was made by Miss Ruth Johnson. This study has been financed by a grant of the "Plan de Formación del Personal Investigador (B.O.E. 1-8-82), M.E.C., Spain".

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