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The chromosomal location of a third set of malate dehydrogenase loci, *Mdh-3*, in wheat, barley and related species

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Summary. A third set of malate dehydrogenase loci have been identified and located on the short arms of homoeologous group 5 chromosomes in wheat. Allelic differences have been found at each of the three *Mdh-3* loci. However, *Mdh-D3* appears to be least variable, with a second allele found only in Sears' 'Synthetic' among a survey of 42 varieties. Homoeoloci were identified on chromosome *7 (5H)* of *Hordeum vulgare*, the short arm of *5E* in *Agropyron elongatum* and *5U* in *Aegilops umbellulata*.

Key words: Wheat – Malate dehydrogenase – Isozymes – *Triticeae*

Introduction

The need for marker genes in wheat for improving the precision of genetic analysis, chromosome manipulation and selection (Ainsworth and Gale 1987; Gale and Sharp 1988) has resulted in the identification, over the last 20 years, of some 150 genes encoding enzymes or other proteins. It is probable that the number of markers available will increase rapidly as restriction fragment length polymorphisms (RFLPs) are identified (Gale et al. 1989; Chao et al. 1988). Nevertheless, protein marker loci will continue to be valuable, especially those which display allelic variation between cultivars which can, therefore, be precisely mapped within chromosomes. It is likely that proteins will continue to be, for some time yet, more rapidly and economically assayed in experimental or breeding populations than is possible with RFLP techniques.

Malate dehydrogenase (E.C.1.1.1.37; MDH) was first studied in wheat by Brewer et al. (1969), who observed two zones of activity following electrophoresis on starch gels. One of these, now recognised as *Mdh-1*, has been shown to be controlled by genes on the long arms of the homoeologous group 1 chromosomes. However, aneuploid analysis failed to identify any of the loci controlling isozymes in the second zone (Bergman and Williams 1972; Benito and Salinas 1983).

In related species, MDH loci have been reported on chromosomes *1R* and *3R* of rye, *Secale cereale* (Salinas and Benito 1985), and chromosomes *5 (1H)* (Powling et al. 1981) and *3 (3H)* of barley, *Hordeum vulgare* (Benito et al. 1985). This evidence led to the naming of a second set, *Mdh-2*, on homoeologous group 3 chromosomes. This set has not yet, however, been demonstrated in hexaploid wheat. In this paper, we describe a reexamination of the genetic control of MDH in wheat and related species, using isoelectric focusing (IEF) rather than native electrophoresis.

Materials and methods

Genetic stocks

The genotypes used in this study included: (1) all the available nullisomic-tetrasomic lines (N-T) of 'Chinese Spring' ('CS') and the three group 5 long-arm ditelosomic lines of 'CS' (i.e. CSDT5AL, CSDT5BL and CSDT5DL); (2) 42 hexaploid wheat genotypes, listed in Table 1; (3) two series of homoeologous group 5 intervarietal chromosome substitution lines: 'CS' ('Hope'), developed by E. R. Sears (University of Missouri) and 'CS' ('Synthetic'), where the donor genotype (IPSR 1190903) is the synthetic hexaploid derived from *Triticum dicoccum* × *Aegilops squarrosa* (McFadden and Sears 1946), produced by C. N. Law and A. J. Worland (Institute of Plant Science Research, Cambridge); and (4) alien-wheat single-chromosome addition series: 'CS'/*Hordeum vulgare* cv 'Betzes' (Islam et al. 1975), 'CS'/*Secale cereale* cultivars 'King II' and 'Imperial' (Driscoll and Sears 1971; Miller 1973), 'CS'/*Agropyron elongatum* (Dvorak and Knott 1974) and 'CS'/*Aegilops umbellulata* (Kimber 1967).

Table 1. Allelic variation at MDH-3 loci in hexaploid wheat

Type	Variety	Allele		
		Mdh-A3	*Mdh-B3*	*Mdh-D3*
1 (A)	CS, Starke, Timstein, Timgalen, P168, Highbury, *T. macha* (IPSR 124005)	a	a	a
2 (D)	Ciano67, RL4137, Chris	a	b	a
3 (B)	Berśee, Sava, Champlein, Luna, Poros, Bezostaya 1, Favorits, Atlas 66, Mara, Lutescens 62, Cheyenne, Cappelle-Desprez, Moulin, Purple Pericarp, Carmen, Sicco, April Bearded, Holdfast, Hobbit 'S', Koga II, Perziven, Vilmorin 27, Karcag, Grana, *T. spelta* (IPSR 1220017)	b	a	a
4 (C)	Hope, Maris Huntsman, C591, Rendevous, Thatcher, Spica	b	b	a
5 (E)	Synthetic (IPSR 1190903)	b	b	b

Methods

The embryos of two mature dry grains of each genotype were crushed in a microhammer mill and incubated in 70 µl of 20% sucrose solution containing 0.01 *M* dithiothreitol at room temperature for 1 h. The extract was then centrifuged briefly prior to application to the gel.

IEF was carried out on polyacrylamide gels, 0.25 mm thick with 16 cm between electrodes, containing 3% w/v ampholyte (Ampholine 5–7, Isolyte 6–8 and Isolyte 6–10 in the ratio 1.5:1.5:1). NaOH (1 *M*) and citric acid (0.33 *M*) were used as catholyte and anolyte, respectively. A constant power of 1 W/cm gel length was applied with a maximum voltage of 3,000 V. The gels were prefocused for 800 V/h and 35 µl of each sample was loaded 1.5 cm from the anode, using a silicone rubber tape with 7 mm wide wells. IEF was terminated at 11,000 V/h.

MDH activity was visualised with a mixture of 220 mg L-malic acid, 20 mg β-nicotinamide adenine dinucleotide (NAD), 20 mg thiazolyl blue (MTT) and 10 mg phenazine methosulphate (PMS), made up to 100 ml with 0.1 *M* TRIS-HCl (pH 8.5). Gels were incubated in this solution in the dark at 37 °C for 30 min.

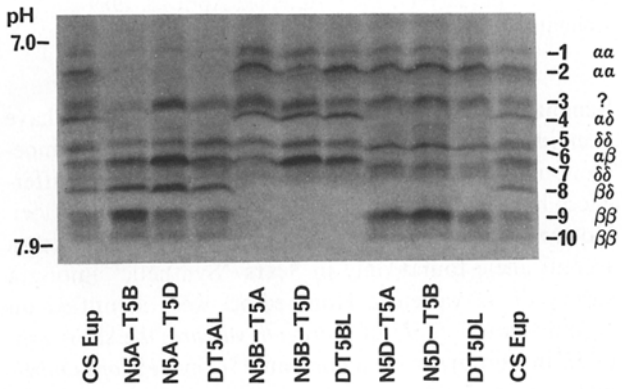

Fig. 1. MDH-3 IEF phenotypes of homoeologous group 5 aneuploid lines of 'Chinese Spring'. Isozyme numbers are indicated on the right. αα, ββ and δδ identify homodimers encoded by chromosomes *5A*, *5B* and *5D*, respectively; αβ, αδ and βδ identify heterodimers

Results

On wide pH range gels (pH 3–8), a complicated MDH zymogram with more than 25 isozymes is observed. The analysis described below concentrates on the cathodal group of isozymes focusing in the pH 7–8 region.

Analysis of aneuploid lines of 'Chinese Spring'

Analysis of nullisomic-tetrasomic and ditelosomic stocks of 'CS' shows clearly that at least nine of the ten cathodal isozymes are controlled by genes on the short arms of the group 5 chromosomes (Fig. 1). These loci are described here as the *Mdh-3* set. The MDH-3 zymograms show that the enzyme is dimeric with at least two protomers produced at each of the three loci. Although all 21 possible dimers from such a protein system cannot be discerned, it is clear that the αα homodimers focus at the anodal end of the group, the δδ dimers in the middle and the ββ dimers at the cathodal end. The resolvable heterodimers focus, as expected, at intermediate isoelectric points. The band labelled 3, which is not entirely removed with the absence of any single chromosome, probably represents more than one isozyme. One of these is likely to be an αδ heterodimer, since the intensity of band 3 varies with the dosage of both chromosomes *5A* and *5D*. Other weaker isozymes, such as those between isozymes 7 and 8, one of which is clearly a further αβ heterodimer, have been ignored in this analysis, because they were obscured in some genotypes by other major isozymes.

Varietal differences in MDH-3 phenotype and variation at Mdh-3 loci

Five phenotypes, A-E (Fig. 2) were observed among 42 varieties (Table 1). Phenotype E was found only in 'Synthetic'. As described below, analysis of intervarietal chromosome substitution lines allowed these differences to be described in terms of alleles at the *Mdh-3* loci. The

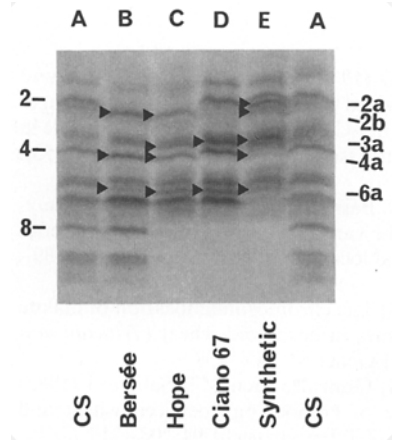

Fig. 2. Five MDH-3 phenotypes found in a sample of 42 varieties. Standard isozyme numbers are indicated on the left. *Arrows* indicate those isozymes not present in 'CS'

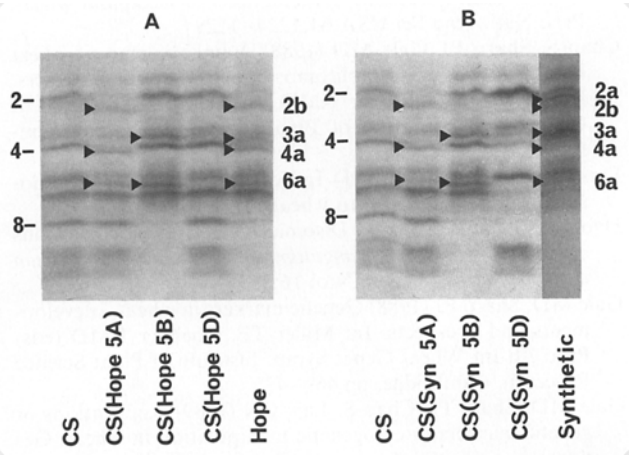

Fig. 3A and B. MDH-3 IEF phenotypes of the homoeologous group 5 intervarietal chromosome substitution lines of **A** 'CS' ('Hope') and **B** 'CS' ('Synthetic'). *Arrows* indicate those isozymes not present in 'CS'. Standard isozyme numbers are marked on the left

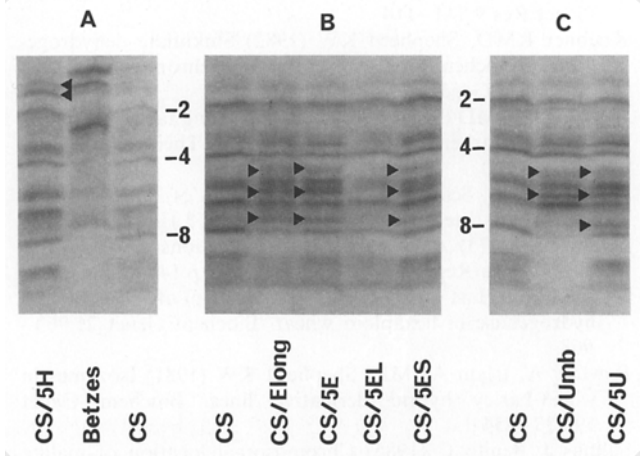

Fig. 4A–C. MDH-3 IEF phenotypes of **A** 'CS'/*H. vulgare* cv 'Betzes', **B** 'CS'/*Ag. elongatum* and **C** 'CS'/*Ae. umbellulata*. Isozymes not present in 'CS' are marked

genotype of 'CS' is, by defintion, *Mdh-A3a, Mdh-B3a* and *Mdh-D3a*.

Mdh-A3: Phenotypes B, C and E all have a strong novel isozyme, 2b. In B and C, this replaces isozyme 2 ($\alpha\alpha$). These three phenotypes also have isozyme 4 ($\alpha\delta$) replaced by a slightly more basic isozyme, 4a, and another isozyme, 6a, which focuses between isozymes 6 and 7. These differences arise from an allelic difference at the locus on chromosome 5A, designated *Mdh-A3b,* as shown by the analysis of both the 'CS' ('Hope') and 'CS' ('Synthetic') group 5 substitution lines (Fig. 3). Isozyme 2a is a $\alpha\alpha$ homodimer, while 4a and 6a are $\alpha\delta$ and $\alpha\beta$ heterodimers involving the allelic α protomer.

The presence of isozyme 2 in the 'Synthetic' parental zymogram, but not in the 'CS' ('Synthetic' 5A) substitution, is perplexing. It can only be concluded that the parent presently available differs from that originally used to construct the substitution lines. Several repeated analyses have ruled out the possibility that the 'parental' genotype is a *Mdh-A3a/b* heterozygote.

Mdh-B3: Phenotypes C, D and E all lack isozymes 9 and 10 ($\beta\beta$) and the heterodimers involving β protomers, 8 ($\beta\delta$) and 6 ($\alpha\beta$). They each have isozyme 6a. The 'Hope' and 'Synthetic' substitutions indicate that this phenotype results from an allele, *Mdh-B3b,* in which the *a* allele $\beta\beta$ homodimers are replaced by a novel homodimer focusing at the same position as the 6a isozyme, which comprises part of the *Mdh-A3b* product. The novel isozyme 3a is presumably a new $\alpha\beta$ heterodimer.

Mdh-D3: The E phenotype differed from all other genotypes examined in that isozymes 7 ($\delta\delta$) and 8 ($\beta\delta$) were absent. This partial null phenotype, identified as due to *Mdh-D3b,* is demonstrated clearly in the 'CS' ('Synthetic' 5D) substitution line (Fig. 3B).

Homoeologous Mdh-3 loci in related species

Of the five series of alien-wheat addition lines analysed, three – 'CS'/*Hordeum vulgare* cv 'Betzes', 'CS'/*Ag. elongatum* and 'CS'/*Ae. umbellulata* – expressed *Mdh-3* isozymes not present in 'CS'. The genes controlling production of these novel isozymes, designated *Mdh-H3, Mdh-E3* and *Mdh-U3,* were all located on the group 5 chromosomes, as in wheat (Fig. 4). *Mdh-E3* has been further located on the short arm of chromosome *5E,* where the telocentric addition *5EL* exhibited the 'CS' pattern, while addition *5ES* showed an MDH-3 zymogram identical to that of the amphiploid and the whole chromosome *5E* addition (Fig. 4B).

'CS'/'Betzes' addition *5H* (barley chromosome 7) expressed two novel isozymes. The one with lower pI was also expressed in 'Betzes' itself, and must therefore be a homodimer controlled by *Mdh-H3*. The other one with a higher pI was only expressed in the addition *5H* and must be a heterodimer between barley and wheat protomers.

The group 5 additions of 'CS'/*Ag. elongatum* and 'CS'/*Ae. umbellulata* showed similar MDH-3 patterns (Fig. 4B and D). Because their alien parents were not available in this study, it is not clear which isozymes are homodimers and which are heterodimers. Interestingly, however, the 'CS'/*Ae. umbellulata* amphiploid lacked the isozymes controlled by *Mdh-B3*. The most likely explanation for this is that the amphiploid has suffered a deletion of part of the short arm of 5B, subsequent to its use in the production of the addition series. If this is the case, the additional isozyme with the highest pI is a *βu* heterodimer (Fig. 4C).

Discussion

The *Mdh-3* loci provide useful markers on a region of the wheat genome as yet relatively devoid of known biochemical loci. Only shikimate dehydrogenase, *Skdh-1*, has been located on the short arms of the group 5 chromosomes (Koebner and Shepherd 1982; Neuman and Hart 1983) and, as yet, no intervarietal variation has been demonstrated for this system. Experiments are now underway to map *Mdh-3* within the group 5 chromosomes. Other loci, including iodine binding factor, *Ibf-1* (Liu and Gale 1989), *β-Amy-A1* (Ainsworth et al. 1983) and a number of RFLPs, are already located to the same chromosome and are, therefore, available for mapping.

The genetic control of the remaining MDH isozymes resolved with IEF in the pI range 3–8 has been studied in the 'CS' nullisomic-tetrasomic lines (C. J. Liu and M. D. Gale, unpublished results). Although some isozymes with low pIs, indicated by strong activity around the anodal end of the gel, were not included in the analysis, only a single isozyme out of about 20 could be unequivocally assigned chromosomal control. This isozyme, focusing in the pH 5–6 region, was controlled by chromosome *1B* and is, thus, probably a product of *Mdh-B1*, as found by Bergman and Williams (1972). No evidence was observed for any *Mdh-2* activity in wheat, even though novel isozymes in the 'CS'/*S. cereale* cv 'Imperial' *3R* and 'CS'/*H. vulgare* cv 'Betzes' *3H* additions were observed, as reported by Benito and Salinas (1983). These isozymes had pIs in the more basic region of the total MDH zymogram and any corresponding wheat *Mdh-2* isozymes, had they been present in that region, should have been observed.

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