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The Chromosomal Location of Malate Dehydrogenase Isozymes in Hexaploid Wheat (*Triticum aestivum* L.)

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Summary. The malate dehydrogenase (E.C. no 1.1.1. 37) of *Triticum aestivum* L. cv. Chinese Spring, shows two activity zones. The results obtained support the hypothesis that the malate dehydrogenase isozymes of zone II are dimers composed of the six possible combinations of subunits coded by triplicate genes located in the long arms of chromosomes of the homoeologous group 1.

Key words: *Triticum aestivum* – Wheat – Malate dehydrogenase – Chromosomal location

Introduction

Structural genes for a number of isozymes have been located in specific chromosomes or chromosome arms of hexaploid wheat (*Triticum aestivum* L.) by electrophoretic analyses of appropriate aneuploid strains. The use of nulli-tetrasomic and ditelosomic strains of cultivar Chinese Spring allow study of the effects on isozyme phenotypes of zero from four doses of each chromosome, and of most chromosome arms (Sears 1954; Hart 1970). Several isozymic systems have been located in hexaploid wheat, including alcohol dehydrogenase (Hart 1970; Hart 1973), acid and alkaline phosphatases (Brewer et al. 1969; Hart and Langston 1977), peroxidases (Kobrehel and Feillet 1975; Kobrehel 1978; Benito and Pérez de la Vega 1971), lipoxigenase, endopeptidase, aminopeptidase (Hart and Langston 1977), glutamate oxaloacetate transaminase (Hart 1975), glucosephosphate isomerase (Hart 1979) and alpha-amylases (Nisikawa and Nobuhara 1971). Evidence has also been obtained for triplicate genes for alcohol dehydrogenase (Hart 1970), glutamate oxaloacetate transaminase (Hart 1975), endopeptidase, lipoxigenase (Hart and Langston 1977) and glucosephosphate isomerase (Hart 1979).

Malate dehydrogenase has been studied in hexaploid wheat by Brewer et al. (1969) but no variation was found among the nulli-tetrasomic and ditelosomic strains of cultivar Chinese Spring. Further work by Bergman and Williams (1972) located the malate dehydrogenase band mdh-1 on the long arm of chromosome 1B.

This paper reports the chromosomal location of genes governing malate dehydrogenase isozymes in the embryo plus scutellum and the endosperm of the dry kernel and also in adult plant leaf tissue of *Triticum aestivum* L. cv. Chinese Spring.

Materials and Methods

The materials of this study were the nulli-tetrasomic and ditelosomic series of *Triticum aestivum* L. cv. Chinese Spring, supplied by Professor E. R. Sears. The analyses were carried out with two parts of mature grains, the embryo plus scutellum (E+S), and the endosperm (Ed), and also with adult plant leaf tissue (L). The malate dehydrogenase isozymes were separated using horizontal 12% starch gel slab electrophoresis and also with horizontal 10% polyacrylamide gel slab electrophoresis; the second method yielded better results. Individual samples were crused and immersed in 0.1 M sodium acetate pH 7.2, for 1 h 30 min at 3 °C. Small pieces of filter paper were soaked in the liquid and then inserted into 140×190×10 mm (starch) and 180×280×2 mm (polyacrylamide) gels. The gel buffer was 0.006 M histidine pH 7.0 and the electrode buffer was 0.043 M Tris-citric acid pH 7.0. The samples were electrophoresed at 4 °C for 5 h 30 min. Isozyme migration was from the cathodic to the anodic side. The starch gels were sliced into 2 mm slabs and then stained following methods described by Brewer and Sing (1970).

Results

Endosperm (Ed) and adult plant leaf tissue (L) both gave the same isozymic pattern for malate dehydrogenase. There were two zones of activity (Fig. 1): zone I,

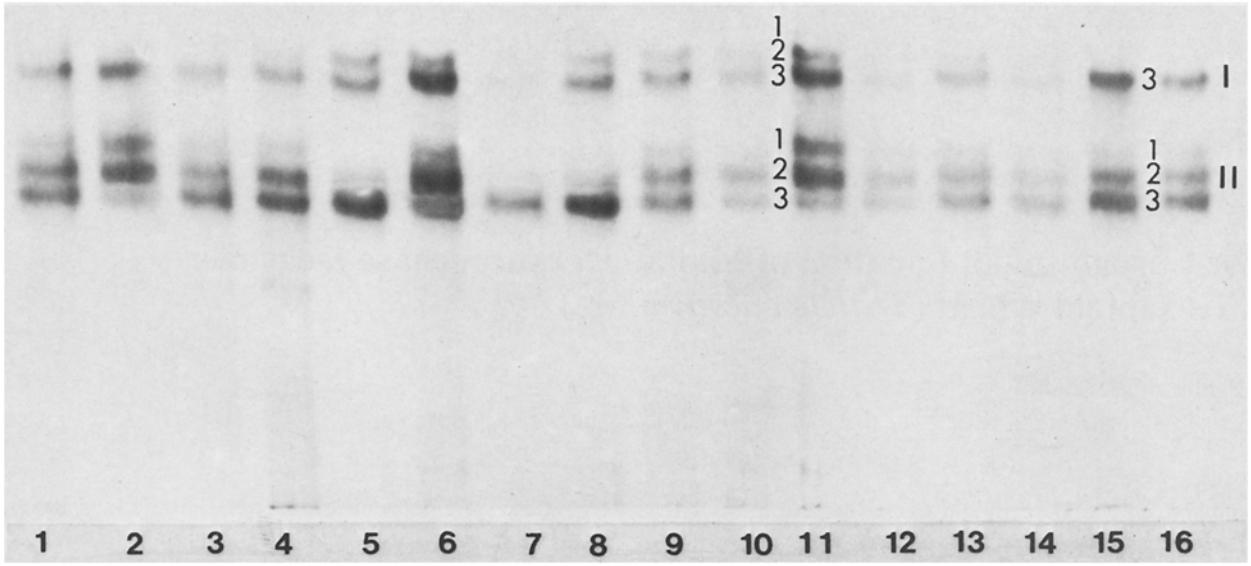

Fig. 1. Different isozymic patterns of MDH in hexaploid wheat. *1.* Euploid "Chinese Spring" Ed, *2.* Nulli-1A-tetra-1B Ed, *3.* Nulli-1A-tetra-1D Ed, *4.* Ditelo-1AL Ed, *5.* Nulli-1B-tetra-1A Ed, *6.* Ditelo-1AS Ed, *7.* Nulli-1B-tetra-1D Ed, *8.* Ditelo-1BS Ed, *9.* Nulli-1BL Ed, *10.* Nulli-1D-tetra-1A Ed, *11.* Nulli-1D-tetra-1B Ed, *12.* Ditelo-1DS Ed, *13.* Ditelo-1DL Ed, *14.* Euploid "Chinese Spring" adult plant leaf tissue, *15* and *16.* Euploid "Chinese Spring" E+S

the faster migrating zone, contained three bands designated MDH1–1, MDH1–2 and MDH1–3 (from faster to slower anodic migration); zone II also contained three bands designated MDH2–1, MDH2–2 and MDH2–3 (from faster to slower anodic migration). The embryo plus scutellum (E+S) also showed two zones of activity: in zone I there was only a single band which had the same migration as MDH1–3; zone II included three bands: MDH2–1, MDH2–2 and MDH2–3 with the same migration as Ed and L (Fig. 1).

Isozyme patterns were consistently the same for zone I for Ed, E+S, and L in all nulli-tetrasomics and ditelosomics; consequently it was not possible to relate these isozymes to a specific chromosome. However, for zone II, four different patterns were observed in nulli-tetrasomic and ditelosomic strains with respect to pres-

PHENOTYPE	I	II	III	IV
BAND				
MDH2-1	1	4		1
MDH2-2	4	4		2
MDH2-3	4	1		1
DOSAGE OF				
1AL	2 0 4	0 2	4 2 2	2 0
1BL	2 2 2	4 4	0 0 0	2 2
1DL	2 4 0	2 0	2 4 2	0 2

Fig. 2. Diagram showing the relationships between dosages of the long arm of chromosomes of homoeologous group 1 and the MDH zone II isozymatic patterns produced

ence or absence, and for the relative staining intensities of MDH bands. The nulli-tetrasomic and ditelosomic of homoeologous groups 2, 3, 4, 5, 6 and 7 have the same pattern as euploid Chinese Spring. This pattern features three bands in zone II, MDH2–1, MDH2–2 and MDH2–3 with a relative staining intensities of 1 : 4 : 4, respectively (Figs. 1 and 2). On electrophoresis of the nulli-tetrasomic and ditelosomic of homoeologous group 1, the nulli-1A-tetra-1D, nulli-1D-tetra-1A, ditelo-1AL, ditelo-1BL and ditelo-1DL showed the same isozymic pattern as Chinese Spring (Figs. 1 and 2). When chromosome 1B -tetra-1A and nulli-1B-tetra-1D) or its long arm (ditelo-1B) were absent, bands MDH2–1 and MDH2–2 did not appear (Figs. 1 and 2). On the other hand, nulli-1A-tetra-1B and nulli-1D-tetra-1B (both tetrasomics for chromosome 1B) had the same three bands MDH2–1, MDH2–2 and MDH2–3 as disomic Chinese Spring, but their relative staining intensities were 4 : 4 : 1 (Figs. 1 and 2). Finally, when the long arm of chromosome 1A or the long arm of chromosome 1D was absent (ditelo-1AS and ditelo-1DS respectively) the relative staining intensities observed for MDH2–1, MDH2–2 and MDH2–3 were 1 : 2 : 1, respectively (Figs. 1 and 2).

Discussion

The results of this study demonstrate that each of the long arms of the chromosomes of homoeologous group 1 carries a gene (or genes) involved in the

Table 1. Hypothesis for the subunits composition of MDH zone II isozymes produced by Chinese Spring and nulli-tetrasomic and ditelosomic strains of homoeologous group 1. The expected quantitative distribution of the isozyme is indicated by the ratios preceding the dimers

	Chinese Spring	Nulli-1A Tetra-1B	Nulli-1A Tetra-1D	Nulli-1B Tetra-1A	Nulli-1B Tetra-1D	Nulli-1D Tetra-1A	Nulli-1D Tetra-1B
Isozymes							
MDH2-1	$1/9\beta\beta$	$4/9\beta\beta$	$1/9\beta\beta$			$1/9\beta\beta$	$4/9\beta\beta$
MDH2-2	$4/9\alpha\beta, \beta\delta$	$4/9\beta\delta$	$4/9\beta\delta$			$4/9\alpha\beta$	$4/9\alpha\beta$
MDH2-3	$4/9\alpha\alpha, \alpha\delta, \delta\delta$	$1/9\delta\delta$	$4/9\delta\delta$	$\alpha\alpha, \alpha\delta, \delta\delta$	$\alpha\alpha, \alpha\delta, \delta\delta$	$4/9\alpha\alpha$	$1/9\alpha\alpha$
Dosage of							
$Mdh2_A$	2	0	0	4	2	4	2
$Mdh2_B$	2	4	2	0	0	2	4
$Mdh2_D$	2	2	4	2	4	0	0
	Chinese Spring	Ditelo 1AL	Ditelo 1AS	Ditelo 1BL	Ditelo 1BS	Ditelo 1DL	Ditelo 1DS
Isozymes							
MDH2-1	$1/9\beta\beta$	$1/9\beta\beta$	$1/4\beta\beta$	$1/9\beta\beta$		$1/9\beta\beta$	$1/4\beta\beta$
MDH2-2	$4/9\alpha\beta, \beta\delta$	$4/9\alpha\beta, \beta\delta$	$2/4\beta\delta$	$4/9\alpha\beta, \beta\delta$		$4/9\alpha\beta, \beta\delta$	$2/4\alpha\beta$
MDH2-3	$4/9\alpha\alpha, \alpha\delta, \delta\delta$	$4/9\alpha\alpha, \alpha\delta, \delta\delta$	$1/4\delta\delta$	$4/9\alpha\alpha, \alpha\delta, \delta\delta$	$\alpha\alpha, \alpha\delta, \delta\delta$	$4/9\alpha\alpha, \alpha\delta, \delta\delta$	$1/4\alpha\alpha$
Dosage of							
$Mdh2_A$	2	2	0	2	2	2	2
$Mdh2_B$	2	2	2	2	0	2	2
$Mdh2_D$	2	2	2	2	2	2	0

production of MDH zone II. The MDH bands of this zone (MDH2–1, MDH2–2 and MDH2–3) appear to be the result of six dimeric combinations of three subunits (α, β and δ) coded by triplicate genes located in the long arm of the chromosomes of homoeologous group 1. This hypothesis is supported by the relationship between chromosomal constitution and the relative staining intensities of MDH bands of zone II for each strain examined. This hypothesis assumes: i) that each long arm of the chromosomes of homoeologous group 1 possesses a gene that codes for an MDH polypeptide chain, or subunit; ii) that the MDH isozymes are dimers produced by the random association of subunits, and iii) that each MDH gene produces approximately equal amounts of its respective MDH subunit. The behavior of MDH zone II of hexaploid wheat is very similar to the behavior of the alcohol dehydrogenase and glucosephosphate isomerase isozymes described by Hart (1970, 1979).

Table 1 presents a schematic model for the subunit composition of MDH isozymes of the zone II in Ed, E+S and L of disomic Chinese Spring, and for nulli-tetrasomic and ditelosomic strains for homoeologous group 1. MDH structural genes located on chromosome arms 1AL, 1BL and 1DL are designated, respectively, $Mdh2_A$, $Mdh2_B$ and $Mdh2_D$ and the polypeptide chains they code are designated α, β and δ, respectively.

The association of $\beta\beta$ dimers with the MDH2–1 band and the $\alpha\beta$ and $\delta\beta$ dimers with the MDH2–2 band are consistent with the finding that the long arm of chromosome 1B is involved in the production of MDH2–1 and MDH2–2 bands. Also when chromosome 1B is present in four doses (tetrasomic for 1B) and chromosome 1A or 1D is absent, the relative staining intensities of MDH2–1, MDH2–2 and MDH2–3 are 4:4:1 respectively. This result is consistent with the appearance of the $\alpha\alpha$, $\alpha\delta$ and $\delta\delta$ dimers in the MDH2–3 band. In the same way, the relative staining intensities of 1:2:1 observed in ditelo-1AS and ditelo-1DS support the deduction that the MDH2–3 band results from overlap of the $\alpha\alpha$, $\alpha\delta$ and $\delta\delta$ dimers.

Our results agree with those of Bergman and Williams (1972) concerning the association of chromosome 1B with band MDH-1 but these authors did not find the relationship between chromosome 1A and 1D and MDH patterns. Brewer et al. (1969) also observed two activity zones of MDH, but found no variation among the nulli-tetrasomic and ditelosomic strains of cultivar Chinese Spring; they used a system of electrophoresis that did not have great resolving power for the MDH isozymes.

The hypothesis we propose in explanation of zone II MDH isozymes is the same as that proposed by Hart (1970, 1979) for alcohol dehydrogenase and

glucosephosphate isomerase. However, our hypothesis assumes that the MDH isozymes are dimers, an assumption which is consistent with the results reported in maize by Yang and Scandalios (1975) and Yang et al. (1977). We have not yet found variability in MDH isozyme patterns among the 40 hexaploid wheat cultivars we have examined; consequently genetic segregation data are not available for analysis of the dimeric nature of wheat malate dehydrogenase isozymes.

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