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NADH dehydrogenase: a new molecular marker for homoeology group 4 in Triticeae. A map of the 4RS chromosome arm in rye

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Summary. Structural gene loci encoding the monomeric isozymes nicotin adenin dinucleotide dehydrogenase (NADH dehydrogenase or NDH) have been located on the 4AL, 4Bα, and 4DS chromosome arms of *Triticum aestivum* cv "Chinese Spring", on the 4RS chromosome arm of *Secale cereale* cultivars "Imperial", "King II", "Dakold," and "Ailes," on the $4S^1$ S/$7S^1$ chromosome of *Aegilops longissima,* the 4E of *Elytrigia elongata,* and the CSU-A of *Aegilops umbellulata.* All the results support the homoeologous relationships among these chromosomes in the five species studied. In addition, a map of the 4RS chromosome arm in cv "Ailes" has been realized, linking loci *Pgm-1* (located on the 4RS chromosome arm) and *Ndh-1* (17.91 cM), with an estimated distance between both loci and the centromere of 20.00 cM and 32.12 cM, respectively.

Key words: NADH dehydrogenase – Isozyme marker – Chromosomal location – Triticeae – Homoeology group 4

Introduction

In genetic studies, isozyme markers can be used to detect the presence of specific chromosomes or chromosome segments, thus providing information for chromosome homology and homoeology as well as genetic relationships among related species. These markers can also be used to map and identify the chromosomes involved in structural rearrangements such as translocations.

NADH dehydrogenase (EC. 1.6.99.3) isozymes belong to electronic transport chains. Their function is to hold electrons from NADH and subsequently transfer them to the first acceptor: coenzyme Q or ubiquinone.

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This paper reports the results on the chromosomal location of structural genes encoding NDH isozymes of zone I in some Triticeae species, as well as a map of the *4RS* chromosome arm in rye cultivar "Ailés."

Materials and methods

The chromosomal location was carried out with the nulli-tetrasomic and ditelocentric series of *Triticum aestivum* cv "Chinese Spring" (CS) (supplied by Prof. E.R. Sears), except for the nulli 4B stock, and the disomic addition lines between wheat and the five species represented in Table 1. the nomenclature used to designate the wheat genomes is that proposed by Dvořák et al. (1990). Thus, chromosomes 4A and 4B are now called 4B and 4A, respectively.

The mapping of the 4RS chromosome arm of rye "Ailés" was carried out by analyzing the offspring of a testcross between a female plant of cv "Ailés," heterozygous for both a reciprocal translocation for chromosomes 4R and 5R (4RL/5RL), and several isozymatic loci located in six of the seven chromosomes of the rye, haploid complement: *Pgi-1* (1RS), *Mdh-1* (1RL), *Got-3* (3R), *Pgm-1* (4RS), *NdH-1* (4RS, data this paper), *Aco-2* (5RL), *Aco-1* (6RL), and *Acph* (7RL), and a male plant belonging to inbred line "RioDeva," homozygous for both the standard chromosome arrangement and the isozyme loci. For characteristics of cv "Ailés" and loci location data, see Figueiras et al. (1990, 1991).

The procedure for the isozyme analyses was described in Figueiras et al. (1985). The NDH isozymes were electrophoresed in *TRIS*-citric acid (0.043 *M*, pH 7.0) as the electrode buffer, and histidine (0.006 *M*, pH 7.0) as the gel buffer, and stained using the following solution: 10 ml of 1 *M* TRIS-HCl (pH 8.0), 20 mg of NADH, 20 mg of MTT, 5 mg of DCPIP, and 80 ml of distilled water.

Results and discussion

Chromosomal location

The NADH dehydrogenase phenotype of euploid CS consists of two bands with different staining intensities

(1:2). The faster band (band 1) is less intense than the slower (band 2) (Figs. 1A, 2A, and 2J).

All of the nullisomic and ditelocentric series of CS showed the same pattern as the euploid, excluding those that involved group 4 chromosomes. A correlation between absence of chromosome 4A (*N4A-T4B* and *N4A-T4D*) and lack of band 1 was found (Figs. 1B, 2D, and 2E). However, this band was present in ditelocentric strains for the 4AL chromosome arm, indicating that band 1 is encoded by structural genes located on this chromosome arm.

Band 2 does not disappear completely in the absence of group 4 chromosomes, but its relative staining intensity decreases when the number of chromosome arms 4Bα and 4DS is reduced below the four sets present in the euploid. Thus, band 2 presents the same staining intensity as band 1 in 4DL ditelocentric stocks (Figs. 1D and 2F) but not in *N4D-T4B*, ditelo 4DS, or ditelo 4Bα (Figs. 1A, 2B, 2G, and 2I). Therefore, we can conclude that both 4Bα and 4DS chromosome arms carry a gene coding an isozyme with the same mobility as that of band 2.

The wheat location data indicate that NDH isozymes are encoded by three gene loci (*Ndh-A1*, *Ndh-B1*, and *Ndh-D1*) situated on the 4AL, 4Bα, and 4DS chromosome arms, respectively. This confirms the homoeologous relationships among these chromosome arms already described by Hart (1979) and Benito et al. (1984).

In rye, the chromosomal location of NDH isozymes was carried out with the disomic addition lines listed in Table 1. The cv "Imperial" showed three distinct phenotype patterns: strains with only one band of the same (band 2, Figs. 1G and 3C) or shorter (band 3, Figs. 1E and 3A) migration than band 2 of CS, and strains that simultaneously presented both bands (Figs. 1F and 3K). All disomic addition lines showed the CS phenotype, excluding line 4R, where a third band was present exhibiting the same mobility as band 3 of cv "Imperial" (Figs. 1H and 3J).

The "Kharkov" and "Holdfast" wheat cultivars had two bands, designated 1 and 2, with a 2:1 relative staining intensity (Fig. 1I). The "King II" and "Dakold" rye cultivars showed only one band, with the same migration as band 2 of the "Holdfast" and "Kharkov" wheats (Fig. 1G). All of the disomic addition lines had the wheat pattern, except line *4R* in which the relative staining intensity of bands 1 and 2 was 1:1 (Fig. 1J).

The results obtained in the three cultivars of rye indicate that NDH isozymes are encoded by one locus (*Ndh-R1*) located on chromosome 4R. Taking into account the homoeologous relationships between homoeology group 4 chromosome arms of hexaploid wheat and the short arm of rye chromosome 4R (Salinas and Benito 1985), the *Ndh-R1* locus could be tentatively located on 4RS chromosome arm.

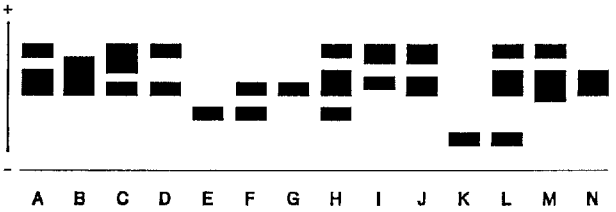

Fig. 1. Diagrams of the NDH zymogram phenotypes. *A*: *T. aestivum* cv "Chinese Spring" (CS), ditelo 4Bα, ditelo 4AL, ditelo 4DS, *N4D-T4B*, *CS-I*, CS-*Ae. longissima*, CS-*E. elongata*, and CS-*Ae. umbellulata* disomic addition lines, with the exception of 4R, $4S^1$ $S/7S^1$ L, 4E, and *CSU-A*; *B*: *N4A-T4B*, *N4A-T4D*; *C*: *N4D-T4A*; *D*: ditelo 4DL; *E* and *F*: *S. cereale* cv "Imperial" (I); *G*: *S. cereale* cultivars "Imperial" (I), "King II" (KII), and "Dakold" (D), *Elytrigia elongata*, *Aegilops umbellulata*; *H*: 4R CS-I disomic addition line; *I*: *T. aestivum* cultivars "Holdfast" (H) and "Kharkov" (K), H-KII, and K-D disomic addition lines, excluding 4R; *J*: 4R H-KII and K-D disomic addition lines; *K*: *Aegilops longissima*; *L*: $4S^1$ $S/7S^1$ L CS-*Ae. longissima* disomic addition line; *M*: 4E and *CSU-A* disomic addition lines of CS-*E. elongata* and CS-*Ae. umbellulata*; *N*: *T. aestivum* cv "Vilmorin" (V), *Agropyron intermedium*, and V-*A. intermedium* disomic addition lines

Table 1. Hexaploid wheat recipient variety, donor species, and original source of addition lines studied

Recipient variety	Donor species	Original source
"Chinese Spring"	*S. cereale* cv "Imperial"	E.R. Sears
"Holdfast"	*S. cereale* cv "King II"	J.P. Gustafson
"Kharkov"	*S. cereale* cv "Dakold"	J.P. Gustafson
"Chinese Spring"	*S. Aegilops longissima*	M. Feldman
"Chinese Spring"	*Elytrigia elongata*	J. Dvorak
"Chinese Spring"	*Aegilops umbellulata*	G. Kimber
"Vilmorin 27"	*Agropyron intermedium*	Y. Cauderon

The NDH phenotype of *Aegilops longissima* showed a single band of shorter migration than band 2 of euploid CS wheat (Figs. 1K and 3M). All of the disomic addition lines exhibited the same pattern as CS, except for the line carrying chromosome $4S^1$ $S/7S^1L$, which displayed a third band corresponding to the expression of the *Ae. longissima* parent (Figs. 1L and 3Q). Due to homoeologous relationships between these two species, the *Ndh-1* locus could be located on the short arm ($4S^1S$) of this chromosome.

In wheat, only one band decreases in intensity or disappears altogether when a chromosome is absent. Moreover, only individuals showing one (homozygous) or two bands (heterozygous) were found in the six rye cultivars studied. New bands with an intermediate migration were not observed in the addition lines 4R (rye) or $4S^1$ $S/7S^1L$ (*Ae. longissima*). These results support the hypothesis of the monomeric behavior of NDH-1 isozymes in the Triticeae.

The phenotypic pattern of *Elytrigia elongata* showed only one band with the same migration as band 2 of euploid CS (Fig. 1G). All of the disomic addition lines

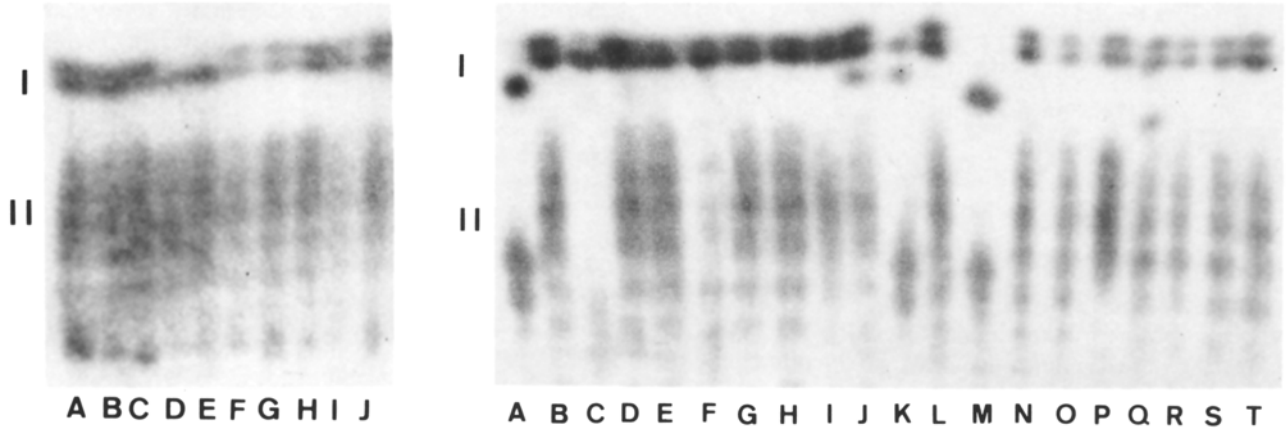

Fig. 2. NDH zymogram phenotypes. *A* and *J*: "Chinese Spring"; *B*: *N4D-T4B*; *C*: *N4D-T4A*; *D*: *N4A-T4B*; *E*: *N4A-T4D*; *F*: ditelo 4DL; *G*: ditelo 4DS; *H*: ditelo 4AL; *I*: ditelo 4Bα

Fig. 3. NDH zymogram phenotypes. *A*, *C*, and *K*: "Imperial"; *B*, *L*, and *T*: "Chinese Spring"; *D*, *E*, *F*, *G*, *H*, and *I*: 1R, 2R, 3R, 5R, 6R, and 7R CS-I disomic addition lines, respectively; *J*: 4R CS-I disomic addition line; *M*: *Ae. longissima*; *N*, *O*, *P*, *R*, and *S*: $1S^l$, $2S^l$, $3S^l$, and $5S^l$ *Ae. longissima* disomic addition lines, respectively; *Q*: $4S^l$ $S/75^l$ L *Ae. longissima* disomic addition line

Table 2. Two-point linkage analyses between *Pgm-1*, *Ndh-1*, and the interchange (I)

Loci	Parental genotypes ♀ ♂	Progeny genotypes				χ^2 linkage	Distance (cM)
		11 11	11 12	12 11	12 12		
Pgm-1, *Ndh-1*	(12, 12) × (11, 11)	75	11	20	67	74.04*	17.91 ± 2.91
		7^{II}		$1^{IV}+5^{II}$			
		11	12	11	12		
Pgm-1, I	(12, $1^{IV}+5^{II}$) × (11, 7^{II})	62	14	19	70	59.50***	20.00 ± 3.11
Ndh-1, I	(12, $1^{IV}+5^{II}$) × (11, 7^{II})	57	19	34	55	22.44*	32.12 ± 3.62

* ($P<0.05$); *** ($P<0.001$)

presented the same pattern as CS, with the exception of line 4E, which showed band 2 more relatively stained as a consequence of overlapping between *Elytrigia* and wheat bands (Fig. 1 M). These results are in agreement with existing knowledge regarding homoeologous relationships among chromosomes 4E, 4R, and wheat homoeology group 4 (Hart and Tuleen 1983).

Furthermore, the electrophoretic pattern of *Aegilops umbellulata* consists of one band with the same migration as the euploid CS band 2 (Fig. 1G). The only disomic addition line that showed more relative staining intensity in this band was line A (Fig. 1 M). This is in contradiction to the expected chromosome location of other isozyme markers on *Ae. umbellulata*. The *Pgm-1* locus, located in homoeology group 4, was associated to addition line B (Benito et al. 1987), where the *Pgi-1* locus was also located related with the homoeology group 1 (Benito et al. 1987). The authors explain these results by inferring that disomic addition line B carries a chromosome partially homoeologous to the 4S and 1S wheat chromosome arms. Since the *Pgd-2* locus (marker of homoeology group 1) was also located (Benito et al. 1987) in addition line A, we postulate that this line carries a chromosome partially homoeologous to the 1L an 4S wheat chromosome arms.

The location of NDH isozymes could not be carried out in the addition lines of *Agropyron intermedium*, because they showed only one band with the same mobility and staining as their parental species (Fig. 1 N).

A map of 4RS chromosome arm of rye

In order to map the *Ndh-1* locus, the progeny of the test-cross previously mentioned were analyzed. The offspring genotypes for this locus showed only one or two bands. This fact reinforces the hypothesis on the monomeric behavior of NDH-1 isozymes in rye.

The only locus linked to *Ndh-1* was *Pgm-1* (17.91 cM) (Table 2). This last locus was located on the 4RS chromosome arm by Salinas and Benito (1985), therefore this

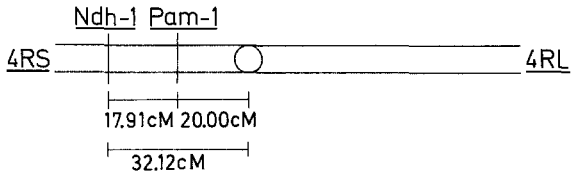

Fig. 4. Genetic map of the *4RS* chromosome arm

linkage relationship is in agreement with the previous location data on the 4R chromosome. Moreover, these two loci appeared linked to the 4RL/5RL interchange, estimating break-point distance of 20.00 cM and 32.12 cM for *Pgm-1* and *Ndh-1*, respectively (Table 2). Most probably, these distances are actually between the loci and the centromere, since no chiasmata at the interstitial segments were observed. The map distances indicate that the *Ndh-1* locus is also located on the *4RS* chromosome arm, and agree with the hypothesis regarding the chromosomal location of locus *Ndh-1* on this arm. The map obtained with these data is shown in Fig. 4.

Coincidence coefficient (c) and chromosomal interference (I) were calculated estimating values of $c=0.67$ and $I=0.33$. The loci mapped on the nontranslocated segments usually present positive interference values, as expected for loci located within nontranslocated arms.

Nielsen and Hejgaard (1986) obtained similar results in barley. They realized a map of the 4HS chromosome arm estimating a distance of 27 cM between loci *Pgm-H1* and *Ndh-H1*. These results agree with the homoeologous relationships previously established between the 4RS and 4HS chromosome arms of rye and barley (Hart et al. 1980).

The data obtained in this work concerning the location of *Ndh-1* loci in some Triticeae species support the hypothesis of conservation of gene synteny groups inherited from the common ancestor of the Triticeae, as proposed by Hart et al. (1980).

Homoeology group 4 chromosomes have only a few isozyme markers. Particularly in rye, the isozyme loci *Adh-1, Pgm-1, E-Per,* and *Amp-2* are located on chromosome 4R. In this paper, we have obtained a new marker (locus *Ndh-1*) for this chromosome, increasing the number of its isozyme markers.

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