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Is the *Mnr* locus of Triticeae species the same as the *Ndh* and *Dia* loci?

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Abstract The menadione reductase (MNR), the nicotinamide adenine dinucleotide dehydrogenase (NDH) and diaphorase (DIA) isozymes were studied in the allohexaploid *Triticum aestivum* cv "Chinese Spring" and in five diploid Triticeae species. The *Mnr1*, *Ndh3* and *Dia1* loci were located on the chromosome arms 3AL, 3BL and 3DL of *T. aestivum*, respectively. These loci were also located on the 3H chromosome of *Hordeum vulgare* cv "Betzes", the 3L chromosome of *Aegilops longissima* and the 6RL chromosome arm of *Secale cereale* cv "Imperial". The chromosomal location results together with the segregation studies support a tetrameric behaviour of the MNR1, NDH3 and DIA1 isozymes. The *Ndh1* and *Dia3* loci were located on homoeologous group 4 showing a monomeric behaviour. The chromosomal locations and linkage data of the *Mnr*, *Ndh* and *Dia* loci suggest that *Mnr1=Ndh3=Dia1*; *Ndh1=Dia3* and *Ndh2=Dia2*.

Keywords *Mnr* · *Dia* · *Ndh* · Chromosomal location isozymes · Triticeae

Introduction

The menadione reductase isozymes (MNR, EC 1.6.99.2) oxidize nicotinamide adenine dinucleotide (NADH) in order to reduce menadione or vitamin K_3 in the chloroplasts. Therefore, the MNR isozymes can also be considered as nicotinamide adenine dinucleotide dehydrogenase isozymes (NADH). The NDH (EC 1.6.99.3) and Diaphorase (DIA, EC 1.8.1.4) are NADH isozymes that oxidize NADH and reduce DCPIP (dichlorophenol indophenol).

The MNR isozymes have been studied in several plant and animal species: yeast (Misaka and Nakanishi 1962), *Quercus* (Kim et al. 1993; Zanetto et al. 1993), *Pinus* (Strauss and Conkle 1986), veal (Märki and Martius 1960), ox (Giuditta and Strecker 1960), dog (Wosilait 1960) and octopus (Di Prisco et al. 1967).

The MNR genes have never been located in Triticeae species. However, the chromosomal location and the genetic variability of the NDH and DIA isozymes have been previously studied in wheat, barley, rye and other Triticinae species (Brown and Munday 1982; Hart 1987; Vapa and Hart 1987; Figueiras et al. 1991; Liu and Gale 1991; Wehling 1991; Benito et al. 1994).

This paper reports the chromosomal location of genes governing the menadione reductase isozymes (MNR) in different Triticeae species and the study of the genetic variability and genetic control of MNR in rye. Moreover, we compare this isozymatic system with other NADH isozymes like NDH and DIA, in order to establish whether they codify for the same or different polypeptides.

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Materials and methods

The plant materials used in this study were the six available homoeologous chromosome group-3 ditelosomic strains, namely 3AS, 3AL, 3BS, 3BL, 3DS and 3DL; and the four available ditelosomic strains of homoeologous group 4 (4AL, 4BS, 4DL and 4DS). The 'Chinese Spring' (CS) aneuploids examined included all available compensating nullitetrasomic types. Both ditelosomic and nullitetrasomic series were supplied by Prof. E.R. Sears.

Euploid *Triticum aestivum* cv 'Chinese Spring', *Secale cereale* cv 'Imperial', *Hordeum vulgare* cv 'Betzes', *Elytrigia elongata*, *Aegilops umbellulata*, *Agropyron intermedium* and the available disomic and ditelosomic addition lines, kindly supplied by E.R. Sears, Y. Cauderon, A.K.M.R. Islam, J. Dvorak and G. Kimber, were also studied.

For isozyme analyses, seeds were germinated on moist filter paper at 21°±2°C. Crude extracts were obtained by macerations of 12-day old seedling leaves. Small pieces of filter paper were soaked with the liquid and then inserted into the gel, consisting of a 12% starch slab (14 cm×17 cm×1 cm). The gel buffer was 0.005 M DL-histidine-HCl adjusted to pH 7.0 with 1 N OHNa and the electrode buffer was 0.135 M Tris (hydroximethyl) aminomethane and 0.0435 M citric acid, pH 7.0.

Electrophoresis was carried out at a constant voltage of 150 V for 4 h 30 min at 2–4°C. The isozyme migration was from the ca-

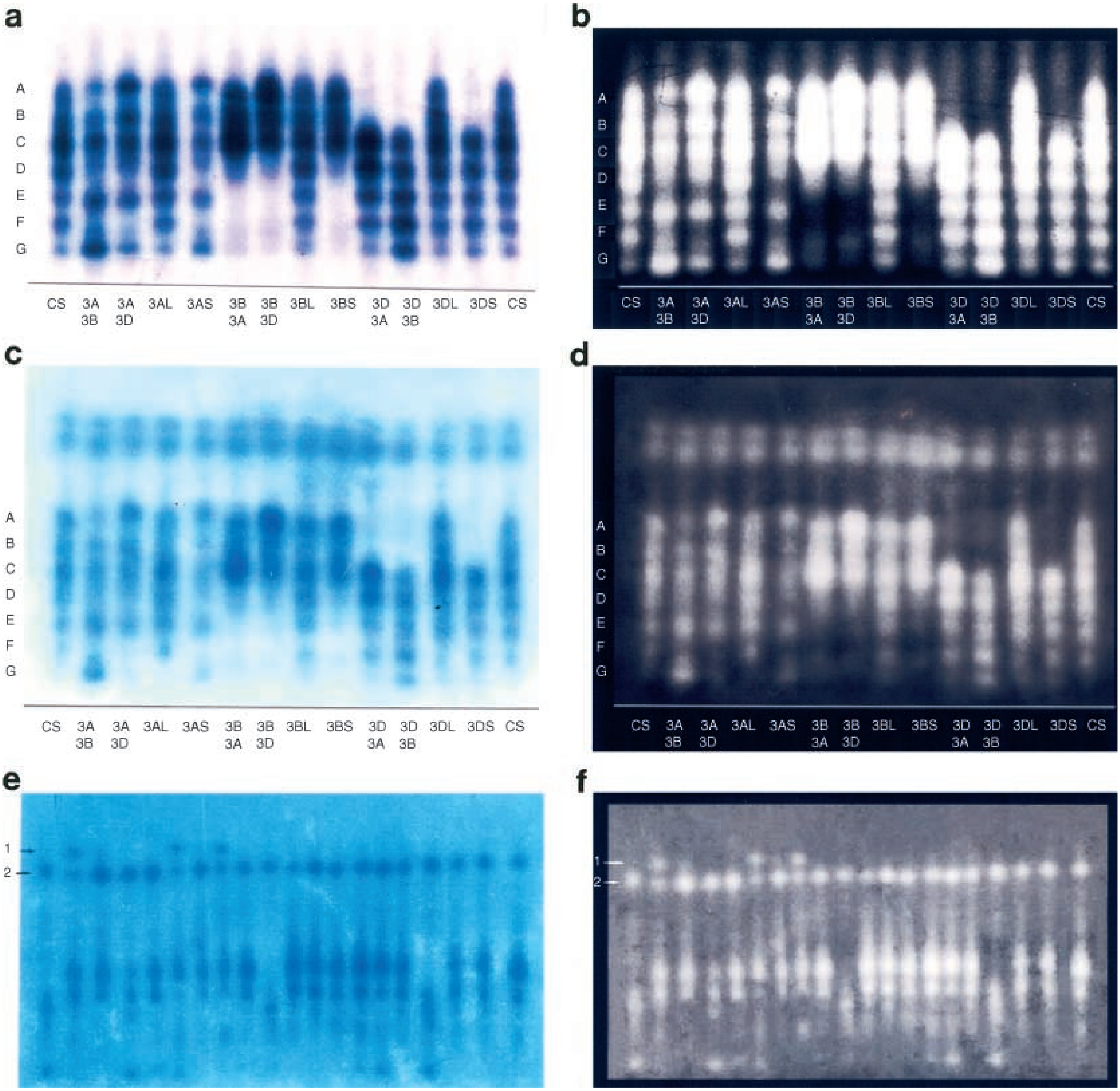

Fig. 1 a–d Zymogram phenotypes of nullitetrasomic (N3AT3B, N3AT3D, N3BT3 A, N3BT3D, N3DT3 A and N3DT3B) and ditelocentric (3AL, 3AS, 3BL, 3BS, 3DL and 3DS) strains of homoeologous group 3 of *T. aestivum* cv "Chinese Spring" (CS). The seven different isozyme bands observed in euploid (*CS*) were named A to G. **a** MNR isozymes. **b** DIA isozymes. **a** and **b** are different slices of the same starch gel. **c** NDH isozymes. **d** DIA isozymes. **c** and **d** are different slices of the same gel. **e** NDH zymogram phenotypes of different plants of rye cv "Ailés". **f** DIA zymogram phenotypes of different plants of rye cv "Ailés". **e** and **f** are different slices of the same gel. The different alleles observed in the fast activity zones of NDH and DIA isozymes were designated *1* and *2*

thodic to the anodic side. The gels were cut horizontally into three slices (2-mm thick), which were stained at 37°C over 1 h using the following three different staining methods. The staining solution for MNR isozymes was prepared by mixing 10 ml of 1 M tris-HCl pH 8.0, 25 mg of NADH, 25 mg of NBT and 30 mg of Menadione with 90 ml of water. The staining solution for DIA isozymes was prepared by mixing 10 ml of 1 M tris-HCl pH 8.0, 20 mg of NADH and 5 mg of DCPIP (previously dissolved with 10 ml of water) with 80 ml of water. The staining solution for NDH isozymes was prepared by mixing 10 ml of 1 M tris-HCl pH 8.0, 20 mg of NADH, 20 mg of MTT and 5 mg of DCPIP (previously dissolved with 10 ml of water) with 80 ml of water.

Results

The pattern of MNR isozymes showed one activity zone. The MNR phenotype of euploid Chinese Spring (CS) consists of seven bands with different staining intensities, named from A to G (Fig. 1a). All of the nullisomic and ditelocentric series of CS showed the same pattern as the euploid, except those involving homoeologous group-3 chromosomes. A correlation between the absence of chromosome 3 A or its long arm and the lack of bands F and D was found (N3AT3B, N3AT3D and 3AS series in Fig. 1a). Similarly, the absence of bands D, E, F and G is correlated with the lack of the whole chromosome 3B or its long arm (N3BT3 A, N3BT3D and 3BS series in Fig. 1a). Finally, the lack of chromosome 3D or its long arm (N3DT3 A, N3DT3B and 3DS series) is correlated with the absence of the bands A and B (Fig. 1a). Furthermore, the relative intensities of bands C, G and A, increase, respectively, when chromosomes 3 A, 3B or 3D are in four doses.

Therefore, the results obtained in CS indicated that the information for MNR isozymes is coded by three loci (*Mnr1A*, *Mnr1B* and *Mnr1D*) located in the long arms of group-3 chromosomes (3AL, 3BL and 3DL, respectively). The fact that four bands disappear simultaneously on the absence of one single chromosome arm, indicates that the allozyme structure is multimeric.

The *Mnr1* locus has also been located on the 3H chromosome of *H. vulgare* cv "Betzes", the 3L chromosome of *Aegilops longissima* and the 6RL chromosome arm of *S. cereale* cv "Imperial", using the corresponding wheat-alien addition lines.

The results obtained using the staining methods for the NDH and DIA isozymes were fully coincidental; two different activity zones were observed (Fig. 1c and d); the activity in the fast-moving zone corresponds to genes located on homoeologous group 4 (*Ndh1* and *Dia3*) and the activity in the slow-moving zone to genes located on homoeologous group 3 (*Ndh3* and *Dia1*). Surprisingly, the number of isozymes and their migration in the gel in the slow moving zone is absolutely identical for the three staining methods, MNR, NDH and DIA (compare Fig. 1a and b with Fig. 1c and d).

Also, we have analysed a sample of plants of the highly variable rye cultivar "Ailés", and, in all cases, identical patterns were obtained with the three staining methods (Fig. 1e and f). The *Ndh1* and *Dia3* loci showed two different alleles and monomeric behaviour (heterozygous plants have two isozymes, each codified by one allele), and the *Ndh3*, *Dia1* and *Mnr1* loci presented at least four different alleles and a tetrameric behaviour (five isozymes expected in heterozygous plants).

Discussion

The *Mnr1, Ndh3* and *Dia1* loci

In this work, we have located the *Mnr1, Ndh3 and Dia1* loci in the long arm of chromosomes of homoeologous group 3 of wheat (3AL, 3BL and 3DL) and in the 6RL chromosome arm of rye, in the 3H chromosome of barley and in the 3L chromosome of *A. longissima*. Electrophoretic patterns reveal a tetrameric behaviour of the isozymes controlled by these loci. Their chromosomal location in the 6RL chromosome arm of rye supports the existence of a translocation between the chromosome arms 3RL and 6RL. Evidences of this translocation have been previously obtained using different kind of markers (Miller 1984, Naranjo et al. 1987; Naranjo and Fernández-Rueda 1991; Rognli et al. 1992; Devos et al. 1993). This is also supported by the close linkage (6.38±2.5 cM) between the *Est6* locus, on the 6RL chromosome arm, and *Mnr1* found in a cross between the rye cultivars "Ailés" and "Elbon" (unpublished data).

In the reviews of isozyme loci located in wheat and rye, *Ndh3* and *Dia1* are considered different loci (Schlegel et al. 1986; McIntosh 1988; Melz et al. 1992; Hart et al. 1993). Previous works had identified *Ndh3* as a multimeric locus mapping on homoelogous group 3 of wheat (3AL, 3BL, 3DL) (Liu and Gale 1991), and the *Dia1* as a locus with tetrameric behaviour on the 6RL chromosome arm of rye (Wehling 1991; Wricke 1991). In studies using the isoelectric focusing method performed in both loci, the corresponding coded isozymes presented a very similar pI (Liu and Gale 1991; Wehling 1991).

This coincidence, together with the perfect match we found in the electrophoretic patterns obtained by the three staining methods (MNR, NDH and DIA) in the slow migration activity zone and the correspondence with the previous chromosomal locations, indicates that *Mnr1*, *Ndh3* and *Dia1* are actually the same locus (*Ndh3=Dia1=Mnr1*).

The *Ndh1* and *Dia3* loci

In this work, we have located *Ndh1* and *Dia3* in the chromosomes of homoeologous group 4 of wheat (4AL, 4BS and 4DS), in the 4RS rye chromosome arm, in the 4H chromosome of barley, in the 4 E chromosome of *Elytrigia elongata*, in the $4S^l/7S^l$ of *Ae. longissima* and CSU-A line of *Ae. umbellulata* . The bands corresponding to these isozymes appear in the fast-migration activity zone of the gels, which is not revealed by the MNR staining method. The electrophoretic patterns support a monomeric behaviour for the isozymes controlled by the *Ndh1* and *Dia3* loci.

The *Ndh1* locus had been previously located in the same chromosomes also showing a monomeric behaviour (Brown and Munday 1982; Hart 1987; Vapa and Hart 1987; Figueiras et al. 1991; Liu and Gale 1991; Liu et al. 1992; Benito et al. 1994). In addition, it was also located on chromosome 4 V of *Dasypirum villosum* by Liu and Gale (1991). The *Dia3* locus has a monomeric behaviour in rye (Wehling 1991) showing at least two different alleles.

The *Ndh1* and *Dia3* loci have been studied using isoelectric focusing (Liu and Gale 1991; Wehling 1991) and the isozymes coded by these loci present a very similar pI. Moreover, in our results the fast-activity zone detected in starch gels with the staining methods for NADH and DIA revealed identical isozyme patterns detecting one locus situated in the same chromosome arm and also with a monomeric behaviour. Again, these coincidences indicate that *Ndh1* and *Dia3* are actually the same locus (*Ndh1=Dia3*). Linkage data between the *Ndh1* and *Pgm1* loci confirm the location of the *Ndh1* locus on the short arm of chromosome 4 of barley and rye (Nielsen and Hejgaard 1986; Figueiras et al. 1991; Benito et al. 1994).

The *Ndh2* and *Dia2* loci

In our gels, the NDH2 and DIA2 isozymes cannot be clearly distinguished from those codified by other loci. The *Ndh2* locus has been previously located on 7AS and 7DS in wheat, and on 7RS in rye (Liu and Gale 1991). The *Dia2* locus has been tentatively located on 4R in rye (Wehling 1991) on the basis of the existence of linkage relationships between the *Dia2* and *Got1* loci (13 cM). However, *Got1* and *Got2* are duplicated loci located on 4RL and 7RL respectively, and they codify for isozymes with very similar electrophoretic migrations. This makes difficult the unambiguous location of new loci on either 4RL or 7RL chromosome arms simply on the basis of linkage to *Got* alleles, as the two loci *Got1* and *Got2* can be easily mistaken. The only definite way to solve this uncertainty is to establish linkage with a third locus.

The extensive study of Wehling (1991) is based only on two-point linkage analyses. In the first instance, the locus *Est10* appeared linked to the *Pgm1* locus, unequivocally located on chromosome 4R. In a different offspring, one *Got* locus was linked to *Est10*, thus identified as *Got1* and located also on chromosome 4R. Finally, in a third offspring, *Got* activity appeared linked to the *Dia2* locus. This last Got activity was assumed to be also *Got1*; then the *Dia2* locus was assigned to chromosome 4R.

This conclusion is in disagreement with the perfect paralellism we found between Dia and Ndh activities. In addition, as it happens with the other *Ndh* and *Dia* loci, electrofocusing techniques identify Ndh2 and Dia2 isozymes with a very similar pI (Liu and Gale 1991; Wehling 1991). If, as we propose, Ndh and Dia activities correspond actually to the same loci, one would expect *Dia2* locus to be located on chromosome arm 7RS like *Ndh2* (Liu and Gale 1991).

This conflict would disappear considering that in the offspring where the linkage between *Got* and *Dia2* was analysed, the *Got* segregating locus was *Got2* instead of *Got1*. Then, *Dia2* would be located on chromosome 7R, which is not in disagreement with our hypothesis of identity between *Ndh* and *Dia* loci.

Summing up, based on the coincidence of electrophoretic patterns, isoelectric points (pIs) and chromosomal location, we propose that MNR, NDH and DIA activities correspond to the same loci, with the following correspondence: *Ndh3=Dia1=Mnr1*, *Ndh1=Dia3* and *Ndh2=Dia2*. We suggest to use only the *Ndh* denomination as it is the most general term, including the majority of described loci.

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