Theor Appl Genet (1992) 85:274–275

Localization of NAD(P)H-bispecific nitrate reductase genes to chromosomes of barley, rye, wheat and *Aegilops umbellulata*

A. Kilian [1,3], A. Kleinhofs [1,2], and R. L. Warner [1]

[1] Departments of Crop and Soil Sciences and [2] Genetics and Cell Biology, Washington State University, Pullman WA 99164-6420, USA
[3] Department of Genetics, Silesian University, 40-032 Katowice, Poland

Received April 8, 1992; Accepted May 20, 1992
Communicated by F. Salamini

Eukaryotic nitrate reductases (NRs) catalyze the reduction of nitrate to nitrite and are classified into three groups based on reduced pyridine nucleotide dependency (Guerrero et al. 1981): NADH-specific (EC 1.6.6.1), NAD(P)H-bispecific (EC 1.6.6.2) and NADPH-specific (EC 1.6.6.3). Both NADH and NAD(P)H-bispecific NRs have been reported in plants (reviewed in Kleinhofs and Warner 1990). Recently, a barley NAD(P)H-bispecific nitrate reductase was cloned and characterized (Miyazaki et al. 1991). The chromosomal location of the genes for this enzyme in barley, rye, wheat and *Aegilops umbellulata* is presented.

Source of the probe

A genomic clone (pMJ4) containing the sequence coding for NAD(P)H-bispecific NR from barley was recovered and characterized (Miyazaki et al. 1991). A *Sph*I to *Sac*II fragment (approximately 400 bp), containing the 5′ region overlapping the ATG translation start site, which shows low homology to NADH NR was used as a probe for mapping.

Polymorphism in barley

Polymorphism for the NAD(P)H-bispecific NR probe was analyzed for two sets of barley cultivars. In the first set three genotypes were tested using eight restriction enzymes and in the second set ten genotypes with three enzymes. The comparison of three genotypes revealed at least two alleles for six of the eight enzymes. Three alleles were detected for *Eco*RI and *Xba*I and two alleles for *Eco*RV among ten barley cultivars tested in the second set.

Correspondence to: A. Kleinhofs

Chromosomal localization and mapping

The NAD(P)H-bispecific NR was mapped to barley chromosome 6 using wheat-barley addition lines (Fig. 1). Although the band for barley cv. Betzes is approximately the same size as one of the wheat bands, the dosage effect is apparent for the chromosome 6 addition. The probe was subsequently used to map the NAD(P)H NR using the North American Barley Genome Mapping Project (NABGMP) Steptoe by Morex mapping population. The segregation was consistent with the expected 1:1 ratio and the NR gene was localized to the long arm of the chromosome 6, 10.1 cM proximal to *Amy1* (Kleinhofs 1992). The locus for that probe has been designated *Nar7* on the barley map.

The hybridization pattern obtained for wheat-rye addition lines indicates that the sequence homologous to the probe is localized on chromosome 6 in Imperial rye. There is no band corresponding to the *Ae. umbellulata* NR in a set of lines carrying six individual chromosomes from this species in a wheat genetic background. This suggests that the sequence for the NAD(P)H-bispecific NR may be located on chromosome 3U in *Ae. umbellulata* since this chromosome addition line was not available. Alternatively, the chromosome 6 addition line may have lost all or part of 6U. This possibility is partly eliminated since the same addition line DNA was hybridized with the NADH NR probe and a positive signal observed indicating the presence of the short arm of 6U (Kleinhofs et al. 1988). The specific loss of the long arm of 6U from this addition line has not been examined. Hybridization to wheat nullisomic-tetrasomic lines allowed us to assign individual bands detected by the probe to chromosomes 6A, 6B, and 6D. The pattern obtained for ditelosomic lines indicated that the genes for NAD(P)H-bispecific NR are located on the long arms of

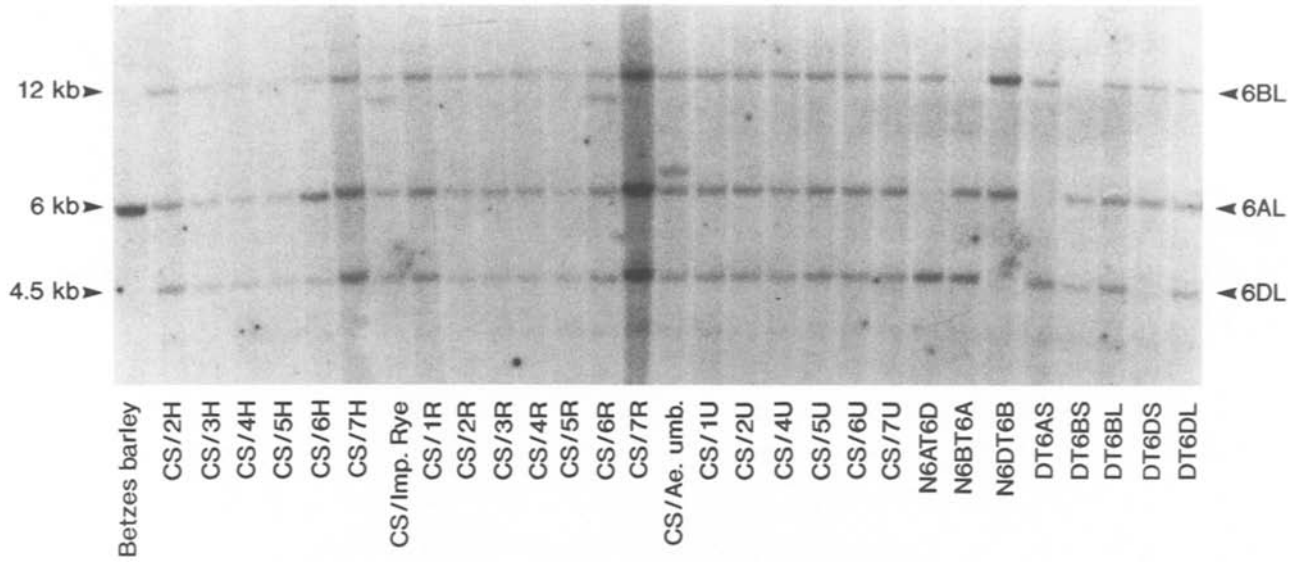

Fig. 1. Hybridization of genomic DNA from various sources with the NAD(P)H-bispecific NR probe. The blots were prepared and hybridized as previously described (Kleinhofs et al. 1988). Barley cv. Betzes (*lane 1*), wheat-barley addition lines (Islam et al. 1981) (*lanes 2–7*), Chinese Spring (CS) wheat/Imperial rye amphiploid (*lane 8*), wheat-rye addition lines (Driscoll and Sears 1971) (*lanes 9–15*), CS wheat/*Ae. umbellulata* amphiploid (*lane 16*), wheat-*Ae. umbellulata* addition lines (Kimber 1967) (*lanes 17–22*), CS nullisomic-tetrasomic lines for group 6 chromosomes (Sears 1966) (*lanes 23–25*), CS ditelosomic lines for group 6 chromosomes (Sears and Sears 1978) (*lanes 26–30*). Bands hybridizing with the NAD(P)H NR probe are observed for barley chromosome 6H and rye chromosome 6R. The *Ae. umbellulata* addition lines do not show a hybridizing band although it is clearly visible in the amphiploid. The absence of a band in the appropriate nullisomic-tetrasomic and ditelosomic lines identifies the location of the band to the wheat genomes and chromosome arms as indicated by the *arrows*

group 6 chromosomes, the same location as in the homoeologous barley chromosome.

In summary, the NAD(P)H-bispecific NR probe is a highly conserved single-copy sequence in barley and its relatives (final wash, 0.5 SSC and 65 C). The chromosomal location is conserved for all genomes studied, with the possible exception of *Ae. umbellulata*. The NAD(P)H-bispecific NR probe detects levels of polymorphism suitable for mapping in barley.

Genetic organization of bispecific NR in four monocot species representing six homoeologous genomes is different from the NADH NR (Kleinhofs et al. 1988). The NADH-specific enzyme has duplicated sequences located on chromosomes 6 and 7 in *Ae. umbellulata* and the wheat genomes A and D. In the B genome the sequence for NADH NR was translocated from chromosome 7 to 4. In rye, three bands homologous to the NADH NR, which may represent one or more loci, were localized to chromosome 4R. The evolutionary processes which resulted in the different genetic organization between the two NRs and among monocot species are currently under investigation.

Probe availability: Contact A. Kleinhofs

Acknowledgements. This research was supported in part by National Science Foundation grant DCB-8904551. This publication is Scientific Paper Number 9201-28, College of Agriculture and Home Economics Research Center, Washington State University, Pullman, WA, USA. Project nos. 0745 and 0223.

References

Driscoll CJ, Sears ER (1971) Individual addition of the chromosomes of "Imperial" rye to wheat. Agron Abstr 1971:6

Guerrero MG, Vega JM, Losada M (1981) The assimilatory nitrate-reducing system and its regulation. Annu Rev Plant Physiol 32:169–204

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley addition lines. Heredity 46:161–174

Kimber G (1967) The addition of the chromosomes of *Aegilops umbellulata* to *Triticum aestivum* (var. "Chinese Spring"). Genet Res 9:111–114

Kleinhofs A (1992) The NABGMP mapping progress report. Barley Genet Newslett (in press)

Kleinhofs A, Warner RL (1990) Advances in nitrate assimilation. In: Stumpf PK, Conn EE (eds) The biochemistry of plants. Miflin BJ, Lea PJ (eds) Intermediary Nitrogen Metabolism, vol 16. Academic Press, New York, pp 89–120

Kleinhofs A, Chao S, Sharp PJ (1988) Mapping of nitrate reductase genes in barley and wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, vol 1. Bath Press, Bath, pp 541–546

Miyazaki J, Juricek M, Angelis K, Schnorr KM, Kleinhofs A, Warner RL (1991) Characterization and sequence of a novel nitrate reductase from barley. Mol Gen Genet 228:329–334

Sears ER (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulations and plant genetics. Oliver and Boyd, Edinburgh, Suppl Heredity 20:29–45

Sears ER, Sears LMS (1978) The telocentric chromosomes of common wheat. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp, Indian Soc Genet and Plant Breed, Delhi, pp 389–407