Theor Appl Genet (1990) 79:157–160

Chromosomal location of adenylate kinase isozymes in Triticeae species

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Received September 13, 1989; Accepted September 18, 1989
Communicated by F. Mechelke

Summary. One system of monomeric adenylate kinase isozymes, designated ADK, was observed in *Triticum aestivum* and in five diploid Triticeae species. The gene loci *Adk-a, Adk-b* and *Adk-d* were located on *7AL, 7BL* and *7DL Triticum aestivum* cv "Chinese Spring" chromosomal arms, respectively. *Adk* gene loci were also located on the *7RL* chromosomal arm of *Secale cereale* cv "Ailés", the *7H* chromosome of *Hordeum vulgare* cv "Betzes", *7X* of *Agropyron intermedium, 7E* of *Elytrigia elongata* and *CSU-E* of *Aegilops umbellulata*. The results suport the notion of the conservation of gene synteny groups within Triticeae species.

Key words: Adenylate kinase – Isozymes – Structural genes – Triticeae – Wheat

Introduction

The genetics of wheat and its domesticated and wild relatives has been intensively investigated in recent years. The importance of producing a comprehensive genetic map of such plants lies in its applications to both conventional plant breeding and to future possibilities of genetic engineering. In the former case, where readily scored loci can be shown to be linked to loci involved with quantitative or environmentally modulated traits, the markers can be used for indirect selection in segregating populations for characters which otherwise would require a degree of replication of both genotype and environment, whereas in the latter case, a detailed knowledge of the genetic map is required to select and characterise transformants. Furthermore, studies of evolutionary relationships within and among chromosomes are provided by knowledge of the chromosomal locations of homologous markers in different genomes, chromosomes and chromosomal segments. Some of the most useful markers control biochemical phentoypes, which can usually be scored on single individuals, often from individual grains, and are generally unaffected by environment.

Adenylatel kinase (EC 2.7.4.3) isozymes catalyse the phosphorylation of Adenosine-5′-monophosphate (AMP), using as phosphate donor Adenosine-5′-triphosphate (ATP). Their structural genes have never been located in Triticeae species. This paper reports the results of studies designed to identify and determine the chromosomal locations of the genes that encode adenylate kinase (ADK) in some Triticeae species.

Materials and methods

The plant materials used in this study were the five available homoeologous chromosome group 7 ditelosomic strains, namely, the *7AS, 7AL, 7BS, 7BL* and *7DS* ditelosomic strains. "Chinese Spring" (CS) aneuploids examined included all available compensating nullisomic-tetrasomic types. Both ditelosomics and nulli-tetrasomic series were supplied by Prof. E. R. Sears.

Euploid *Triticum aestivum* cv "Chinese Spring", *Secale cereale* cv "Ailés", *Hordeum vulgare* cv "Betzes", *Elytrigia elongata, Aegilops umbellulata* and Anfiploid wheat, *Agropyron intermedium* (supplied by Y. Cauderon), were also studied.

The available disomic chromosome addition lines of some wheat-alien species chromosome addition series and a number of other wheat lines containing alien telosomes were analysed. The lines studied and the recipient varieties, donor species and sources are given in Table 1.

For isozyme analyses, seeds were germinated on moist filter paper at 21° ± 2 °C. Crude extracts were obtained by maceration 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* NaOH,

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Table 1. Wheat-alien species chromosome addition lines analysed along with recipient varieties, donors and sources

Recipient variety	Donor species	Lines examined	Original source
"Chinese Spring"	*Secale cereale* cv "Imperial"	Disomic addns. *1R–7R*	E. R. Sears
"Holdfast"	*Secale cereale* cv "King II"	Disomic addns. *1R–7R*	J. P. Gustafson
"Holdfast"	*Secale cereale* cv "King II"	Ditelosomic addn. *7RS*	C. N. Law
"Chinese Spring"	*Hordeum vulgare* cv "Betzes"	Disomic addns. *2H–7H*	A. K. M. R. Islam
"Vilmorin 27"	*Agropyron intermedium*	*L1, L2, L3, L4, L5, L7*	Y. Cauderon
"Chinese Spring"	*Elytrigia elongata*	Disomic addns. *1E–3E, 5E–7E*	J. Dvorak
"Chinese Spring"	*Aegilops umbellulata*	*CSU-A, -B, -C, -D, -E, -F, -G*	G. Kimber

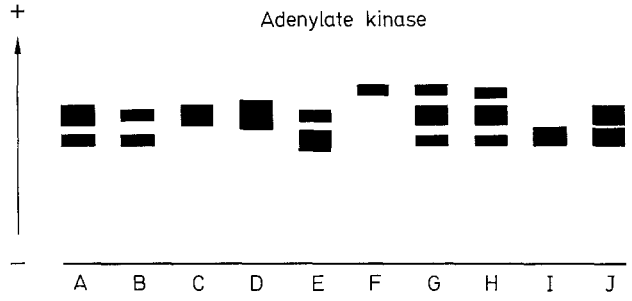

Fig. 1 A–J. Diagrams of the ADK zymogram phenotypes produced by **A** – euploid CS, ditelo-*7AL*, ditelo-*7BL*, nulli-*7A* tetra-*7B*, nulli-*7B* tetra-*7A*, nulli-tetrasomic lines from other homoeology groups, *1R, 2R, 3R, 4R, 5R* and *6R* wheat-rye addition lines, *7RS* wheat-rye ditelosomic addition line, *2H, 3H, 4H, 5H* and *6H* wheat-barley addition lines, *CSU-A, -B, -C, -D, -F* and *-G* wheat-*Aegilops umbellulata* addition lines, *1E, 2E, 3E, 5E* and *6E* wheat-*Elytrigia elongata* addition lines and *L2, L3, L4, L5* and *L7* wheat-*Agropyron intermedium* addition lines; **B** – ditelo-*7AS* and ditelo-*7BS*; **C** – ditelo-*7DS*; **D** – nulli-*7D* tetra-*7A* and nulli-*7D* tetra-*7B*; **E** – nulli-*7A* tetra-*7D* and nulli-*7B* tetra-*7D*; **F** – *Secale cereale* cv "Ailes"; **G** – *7R* wheat-rye addition line; **H** – octoploid (wheat × *Agropyron*) and *L1* (*7X*) wheat-*Agropyron* addition line; **I** – *Hordeum vulgare* cv "Betzes", *Elytrigia elongate, Aegilops umbellulata* and **J** – *7H* wheat-barley addition line, *CSU-E* wheat-*Aegilops umbellulata* addition line and *7E* wheat-*Elytrigia elongata* addition line

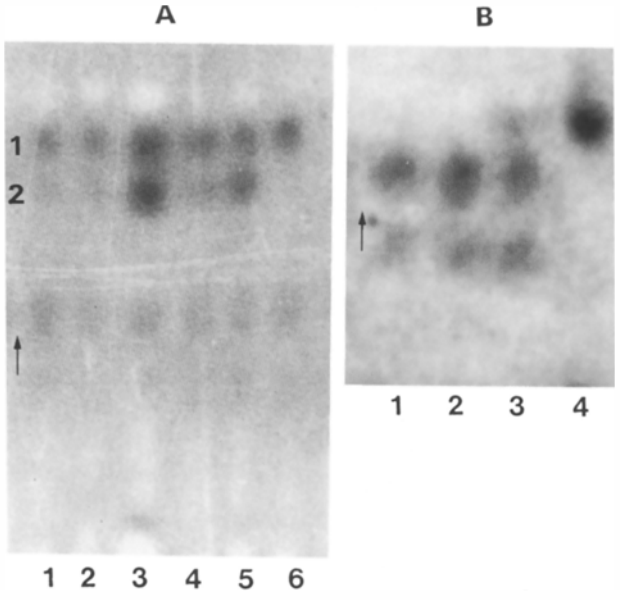

Fig. 2 A and B. Photograph of adenylate kinase zymogram phenotypes produced by "Chinese Spring" (1A, 1B), nulli-*7A* tetra-*7B* (2A), nulli-*7A* tetra-*7D* (3A), nulli-*7B* tetra-*7A* (4A), nulli-*7B* tetra-*7D* (5A), nulli-*7D* tetra-*7A* (6A), wheat-rye addition line *6R* (2B), wheat-rye addition line *7R* (3B) and rye (4B). The *arrow* shows direction of isozyme migration

and the electrode buffer was 0.135 *M* TRIS (hydroxymethyl) aminomethane 0.0435 *M* citric acid, pH 7.0.

Electrophoresis was carried out at a cosntant voltage of 150 V for 5 h at 2°–4 °C. The isozyme migration was from the cathodic to the anodic side. The gels were cut horizontally into four slices (2 mm thick), which were stained at 37 °C over 2 h using the following technique: the staining solution was prepared by mixing 12 ml of 1 *M* TRIS-HCl, pH 8.0, 2 ml of 10% MgCl, 900 mg of glucose, 25 mg of NADP, 20 mg of ADP, 20 mg of NBT and 5 mg of PMS with 80 ml of water. Glucose-6-phosphate dehydrogenase (80 µ) and Hexokinase (160 µ) were also required.

Gels were fixed in ethanol-water (1 : 1) after staining.

Results and discussion

The leaf adenylate kinase isozymes of euploid "Chinese Spring" (CS) consist of two bands, band 1 being more intense and anodal than band 2. Aneuploid analyses indicate that the two bands are produced by one group of isozymes, designated ADK (Figs. 1 A and 2).

All of the nulli-tetrasomic lines of homoeologous chromosome groups 1, 2, 3, 4, 5 and 6 present the same ADK zymogram phenotype as CS (Fig. 1 A). However, two different phenotypes are produced by the nulli-tetrasomic lines belonging the homoeologous group 7 (Fig. 1 D and E). Band 2 is not present on the zymograms of nullisomic strains for chromosome *7D*, whereas band 1 is always present, showing less intensity when nullisomic lines for chromosomes *7A* or *7B* were analysed (Fig. 1 B and C). Moreover, both *7AL* and *7BL* ditelosomic strains presented the same pattern as CS, while *7AS* and *7BS* lines showed less intensity in band 1 than CS. *7DS* had lost band 2 (Fig. 1 A–C).

These results constitute strong evidence that the ADK isozymes are monomers and that they are encoded

by three gene loci, located one each in the *7L* chromosome arms. The adenylate kinase structural gene loci located in *7AL, 7BL* and *7DL* are designated *Adk-a, Adk-b* and *Adk-d,* respectively. Band 1 is produced by *Adk-a* and *Adk-b,* while *Adk-d* encodes the isozyme present in band 2.

The ADK isozyme produced by the rye plants examined presented faster mobility than band 1. When analyses were carried out on wheat-rye addition lines only the *7R* line had the rye band. The *7RS* ditelosomic addition line did not have the rye band. These results confirm the hypothesis that the structural gene encoding adenylate kinase isozyme in rye is located on the *7RL* chromosomal arm. Both "Imperial" and "King II" showed the same results (Figs. 1 F and G and 2).

The zymogram phenotypes observed in the study of the "CS-Betzes" addition lines are shown in Fig. 1 A and J. Strong evidence was obtained that the "Betzes" *Adk* structural gene is located on the *7H* chromosome since, among the "CS-Betzes" addition lines studied, only the *7H* line expressed the "Betzes" ADK isozyme (Fig. 1 I).

Anfiploid wheat, *Agropyron intermedium,* showed three bands; only the most anodal one corresponded to Agropyron (Fig. 1 H). L2, L3, L4, L5 and L7 presented the two wheat characteristic bands (Fig. 1 A), while L1 (*7X*) showed three bands (Fig. 1 H). These results support the hypothesis that the *Adk* structural gene is located on the *7X* chromosome in *Agropyron intermedium.*

Strong evidence was obtained that the *Adk* genes of *Elytrigia elongata* are located in the *7E* chromosome, since the *7E* addition line has the band shown in diploid *Elytrigia elongata* (Fig. 1 I and J), while *1E, 2E, 3E, 5E* and *6E* showed the same pattern as CS (Fig. 1 A).

The zymogram phenotypes observed in the study of the "CSU" addition lines are shown in Fig. 1 I and J. *CSU-A, -B, -C, -D, -F* and *-G* had the same pattern as CS (Fig. 1 A), while *CSU-E* had the pattern shown in Fig. 1 J. These results support the hypothesis that the *Adk* structural gene in *Aegilops umbellulata* is located on the chromosome of line E. Therefore, the chromosome of line E could be homoeologous to the long arm of group 7. Moreover, line E shows *Acph,* a group 7 marker (Benito et al. 1987).

"The chromosomal locations of orthologous gene loci in different Triticeae species provide evidence of homoeology among the chromosomes and chromosomal segments in which the loci are located, just as the chromosomal locations of the members of paralogous sets of gene loci in different genomes within polyploid Triticeae species provide evidence of homoeology among the chromosomes and chromosomal segments in which these loci are located" (Hart 1979). In this paper we present a new molecular marker which can be used as the homoeology group 7 marker of Triticeae. The *Adk* locus is located on the chromosomes of homoeologous group 7. Besides, both in wheat and in rye, the molecular marker is located on the same chromosomal arm (*7AL, 7BL, 7DL* and *7RL,* respectively). Therefore, we think it is possible that the *Adk* structural genes in barley, *Agropyron intermedium* and *Elytrigia elongata* could be located on *7HL, 7XL* and *7EL,* respectively.

The molecular markers that have been identified in the chromosomes of the homoeologous group 7 of wheat, rye and barley, including the *Adk* gene loci identified in this study, are listed in Table 2.

A possible agronomical use of the *Adk* loci as chromosome markers is their putative link to eyespot disease resistance, caused by the fungus *Pseudocercosporella herpotrichoides.* This resistance is linked to the endopeptidase loci located on the long arm of group 7 chromosomes of wheat (Law et al. 1988; Worland et al. 1988; Hart and Langston 1977). In addition, the adenylate ki-

Table 2. Summary of structural genes located on group 7 chromosomes in wheat, rye, barley, *Aegilos umbellulata* and *Agropyron intermedium*; 1 Nishikawa et al. (1981), 2 Nishikawa and Nobuhara (1971), 3 Hart and Langston (1977), 4 Jaaska (1980), 5 Kobrehel (1978), 6 Kobrehel and Feillet (1975), 7 Sanchez et al. (1988), 8 Hart (1975), 9 Brown and Jacobson (1982), 10 Hvid and Nielsen (1977), 11 Salinas and Benito (1984), 12 Benito et al. (1987), 13 Figueiras et al. (1986)

Location	Structural gene	Reference
7AL	α-Amy-A2 (*Amy 7A1*)	1, 2
7BL	α-Amy-B2 (*Amy 7B1*)	1, 2
7DL	α-Amy-D3 (*Amy 7D1*)	1, 2
7AL	*Ep-A1*	3
7BL	*Ep-B1/Ep1*	3
7DL	*Ep-D1*	3
7AL	*Adk-a*	
7BL	*Adk-b*	
7DL	*Adk-d*	
7BS	*Est-B3* (*Est-3B*)	4
7DS	*Est-D3* (*Est-3D*)	4
7AS	*Per3*	5, 6
7DS	*Per1*	5, 6
7BL	*Acph-B*	7
7DL	*Acph-D*	7
7A	*Got 1*	8
7B	α-Amy 3 (*Amy 7B2*)	1
7H	α-Amy 2	9
7H	End	—
7H	*Est 3*	10
7H	*Est 5*	10
7H	*Adk*	
7RL	*Acph*	11
7RL	*Adk*	
CSU-E	*Acph*	12
CSU-E	*Cpxe*	12
CSU-E	*Adk*	
7X	*Acph*	13
7X	*Adk*	

nase isozymes can be directly analysed in the leaves of the resistant plants; this confers to the adenylate kinase systems a considerable methodological advantage over the endopeptidase markers, which are usually analysed in the embryo of the progeny of resistant plants.

Acknowledgements. This work has been supported by a grant of the U.S.-Spain Joint Committee for Scientific and Technological Cooperation.

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