Theor. Appl. Genet. 62, 317–320 (1982)

Genetic Control of 6-Phosphogluconate Dehydrogenase (6-PGD) Isozymes in Cultivated Wheat and Rye

S. L. K. Hsam and F. J. Zeller

Lehrstuhl für Pflanzenbau und Pflanzenzüchtung, Technische Universität München, Freising-Weihenstephan (Federal Republic of Germany)

W. Huber

Lehrstuhl für Botanik, Technische Universität München, Freising-Weihenstephan (Federal Republic of Germany)

Summary. The 6-phosphogluconate dehydrogenase (6-PGD) zymogram phenotypes of wheat, rye and their aneuploid derivatives were determined. Two genes involved in the production of 6-PGD isozymes were located on chromosome arms CRL (4 RL) and FRL (6 RL) of 'Imperial' rye. On the basis of differential interactions between wheat and rye chromosomes, evidence was obtained that genes located on chromosomes 6 A, 6 BL and 7 BL control 6-PGD isozyme activities in 'Chinese Spring' wheat. The wheat and rye 6-PGD zymogram phenotypes were indicative of homoeologous relationships between rye chromosome 6 RL to wheat chromosomes of group 6, and rye chromosome 4 RL to wheat chromosomes of group 7.

Key words: Wheat – Rye – Phosphogluconate dehydrogenase – Genetic control

Introduction

Isozymes are useful genetic markers for the detection of individual genes. Genetic and biochemical analyses of isozyme variants in wheat provide information for chromosome homoeology (Hart and Langston 1977) and for genetic relationships among species in the *Triticeae* (Tang and Hart 1975; Hart et al. 1980). Hexaploid wheat *Triticum aestivum* L. em Thell (2n = 6x = 42) consists of three genomes, A, B and D, which are related. Depending on the ability of the chromosomes to compensate for each other, the 21 chromosome pairs are assigned to seven homoeologous groups. Each group is composed of three chromosome pairs, one from each of the three genomes (Sears 1952, 1966).

Localization of duplicate and triplicate homoeologous structural genes in wheat have been made possible by studies of compensating nulli-tetrasomic and ditelosomic strains using the zymogram technique (for a review, see Hart 1979). The presence of alien chromosomes in wheat-rye addition, substitution and translocation lines, and in other wheat-alien synthetic amphiploids are also readily detected by studying isozymes encoded by the alien genes (Irani and Bhatia 1972; Tang and Hart 1975; Hart et al. 1976, 1980; Kobrehel 1978).

Rao and Rao (1980), using wheat-rye addition lines, have located two structural genes for 6-phosphogluconate dehydrogenase (6-PGD, E.C.no. 1.1.1.44) on the long arms of chromosomes CR (4 R) and FR (6 R) in 'Imperial' rye.

The present study deals with genetic control of 6-PGD in 'Chinese Spring' wheat, based on differential interactions of wheat and rye chromosomes influencing the expression of isozyme phenotypes.

Materials and Methods

Triticum aestivum var. 'Chinese Spring' (C.S.), *T. turgidum* var. 'durum' cv. 'Agatha', *Secale cereale* cv. 'Imperial', C.S.-Imperial octaploid triticale, C.S.-Imperial disomic additions CR(4R) and FR(6R), Holdfast-King II disomic additions IV(4R) and II(6R) obtained from R. Riley (Cambridge), ditelosomic Imperial addition line CRL, nulli-tetrasomic compensating lines of homoeologous groups 4, 6 and 7, excluding N4AT4B, N4AT4D and N4DT4B, ditelosomic lines excluding 4Aβ, 4BS and 7DL, and nullisomics for group seven derived from 'Chinese Spring' (supplied by E. R. Sears, Missouri) were examined.

Disomic and ditelosomic substitution lines involving 'Chinese Spring' wheat and 'Imperial' rye chromosomes CR(4A), CR(4B), CRL(7A), CRL(7B) and CRL(7D), previously described by Koller and Zeller (1976), translocation lines 4Aα/CRL (Rao and Rao 1982) and 7BL/CRL (Zeller and Koller 1981), ditelosomic substitution lines involving Holdfast wheat and King II rye chromosomes 6RL(6A), 6RL(6B) and 6RL(6D), supplied by T. E. Miller (Cambridge), 6R(6B) substitution and 6BS/6RL translocation lines involving wheat cultivar 'Sturdy' and 'Insave' rye chromosomes (supplied by N. A. Tuleen, Texas), were also analysed.

Three to five mature kernels were soaked for 16–20 h and then macerated in 0.1 M Tris-HCl buffer, pH 7.5, containing

0040-5752/82/0062/0317/$ 1.00

0.01 M-KCl, 0.005 M EDTA and 0.004 M 2-mercaptoethanol (Carlson 1972), maintaining a ratio of 0.3 ml buffer per kernel. The slurry obtained by maceration was centrifuged at 17,000 g for 20 min in a SS-34 rotor in a Sorvall RC 2-B refrigerated centrifuge maintained at 4 °C. The supernatant obtained was used directly for electrophoresis. An aliquot of 100 µl was placed over each gel tube (diameter 0.6 cm, length 9 cm), and separation was performed using standard polyacrylamide gels (2.5% spacer gel, 7.5% running gel) at 6–8 °C, following the method described by Davis (1964). Electrophoresis was stopped when the bromphenol blue front marker had reached the end of the gel.

The gels were stained in a solution containing 40 mM Tris-HCl, pH 7.1, with 4.8 mM-$MgCl_2$, 0.053 mM NADP, 0.12 mM nitro blue tetrazolium, 0.07 mM phenazine methosulphate and 0.30 mM 6-phosphogluconate as described by Rao and Rao (1980). A 7% solution of acetic acid was used for the fixation of the zymograms. At least three replicate extractions and electrophoreses were carried out for each line of the materials analysed.

Results

'Chinese Spring' (C.S.) wheat, 'Imperial' rye, C.S.-Imperial octaploid triticale and the wheat-rye addition lines produced four distinct zymogram phenotypes of 6-phosphogluconate dehydrogenase. C.S.-Imperial triticale expressed three 6-PGD isozymes. The fast, intermediate and slow forms have been designated as 6-PGD-1, 6-PGD-2 and 6-PGD-3 by Rao and Rao (1980). Both 'Chinese Spring' and 'durum' wheat possessed one isozyme band which corresponded to 6-PGD-3 in C.S.-Imperial triticale. 'Imperial' rye also had one band that exhibited a mobility similar to the anodal isozyme band (6-PGD-1) in C.S.-Imperial triticale. Wheat-rye additions 4R and 6R from both C.S.-Imperial and Holdfast-King II lines expressed three 6-PGD isozymes which possessed the same mobility as C.S.-Imperial triticale isozymes. Relative intensities of the three isozyme bands in wheat-rye addition lines were similar to a 4:4:1 proportion. However, in C.S.-Imperial triticale the anodal band (6-PGD-1) stained more intensively than in wheat-rye addition lines (Figs. 1, 2). The 6-PGD zymogram of ditelosomic addition CRL was identical to the zymogram phenotypes of the wheat-rye addition lines.

The analysed nulli-tetrasomic compensating lines, the ditelosomic lines and the nullisomic lines produced only one isozyme band which corresponded to the wheat 6-PGD-3 isozyme. Wheat-rye disomic substitutions, ditelosomic substitution and translocation lines expressed three discrete zymogram phenotypes. Disomic substitution lines CR(4A), CR(4B), and trans-

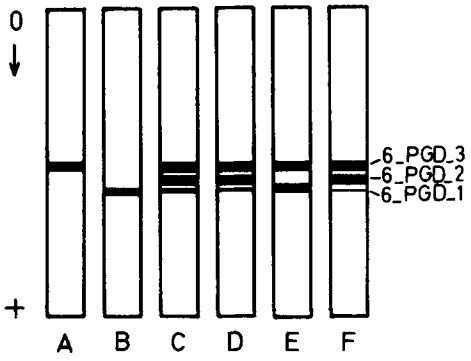

Fig. 1. Diagrams of 6-PGD zymogram phenotypes observed. **A** 'Chinese Spring' (C.S.) wheat, C.S. nulli-tetrasomic and ditelosomic lines of homoeologous groups 4, 6 and 7, nullisomic lines of group 7, 'durum' wheat, **B** 'Imperial' rye, **C** C.S.-Imperial octaploid triticale, **D** Wheat-rye addition lines 4R (CR and IV) and 6R (FR and II), ditelosomic addition line C.S. + CRL, disomic substitution lines CR(4A), CR(4B), translocation line 4Aα/CRL, **E** Disomic substitution line 6R(6B), ditelosomic substitution lines 6RL(6A), 6RL(6B), CRL(7B), translocation line 6BS/6RL, co-electrophoresis of 'Chinese Spring' wheat and 'Imperial' rye kernel extracts, **F** Ditelosomic substitution lines 6RL(6D), CRL(7A), CRL(7D) and translocation line 7BL/CRL

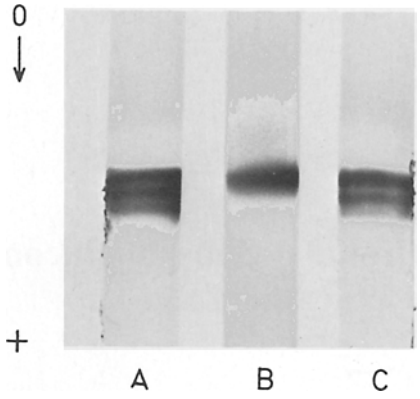

Fig. 2. Zymogram of 6-PGD of wheat, Chinese Spring-Imperial triticale and wheat-rye addition lines. **A** Chinese Spring-Imperial octaploid triticale, **B** C.S. nulli-tetrasomic and ditelosomic lines of groups 4, 6 and 7, **C** Wheat-rye addition lines C.S. + CR, C.S. + CRL, C.S. + FR

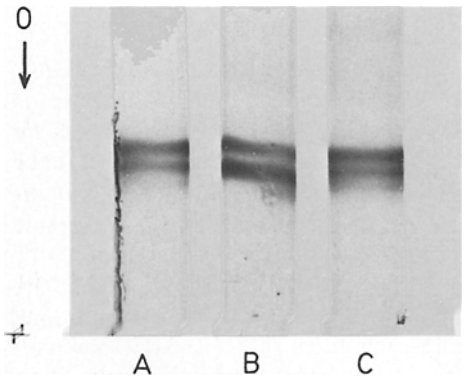

Fig. 3. Zymogram of 6-PGD of wheat-rye aneuploid derivatives. **A** Ditelosomic substitution line 6RL(6D), **B** ditelosomic substitution lines 6RL(6A), 6RL(6B), CRL(7B), translocation line 6BS/6RL, **C** Ditelosomic substitution lines CRL(7A), CRL(7D) and translocation line 7BL/CRL

location line 4Aα/CRL produced three 6-PGD isozymes, which were identical to the zymogram phenotypes expressed by the wheat-rye 4R and 6R addition lines (Figs. 1, 2). Ditelosomic substitution lines CRL(7A), CRL(7D), 6RL(6D), and the translocation line 7BL/CRL also expressed three isozyme bands. However, their zymograms could be readily distinguished from that of wheat-rye addition lines on the basis of the staining intensity of the isozyme bands. The most striking difference was the very weak staining intensity of the anodal band (6-PGD-1). Ditelosomic substitution lines CRL(7B), 6RL(6A), 6RL(6B), and translocation line 6BS/6RL expressed only two 6-PGD bands, similar to the zymogram phenotype produced by the co-electrophoresis of wheat and rye kernel extracts (Figs. 1, 3).

Discussion

These results indicate that isozyme variants of 6-PGD may be readily detected in wheat-rye aneuploid derivatives. Rye produced one isozyme band which migrated faster than the wheat band. A hybrid isozyme molecule is additionally formed only when the long arm of rye chromosomes 4R (CR and IV) and 6R (FR and II) are added to the wheat genomes. However, differential gene interactions are expressed in the absence of specific wheat chromosomes, as evidenced in wheat-rye substitution and translocation lines. In disomic substitution lines 6RL(6A) and 6RL(6B), the hybrid isozyme resulting from the association of wheat and rye subunits were not formed (Fig. 1 E). The same was also observed in the translocation line 6BS/6RL, which indicates that 6BL of wheat is involved in the production of the hybrid molecule. It is also seen that 6A is involved in 6-PGD expression. Designation of the specific chromosome arm will become possible when a translocation line involving an arm of chromosome 6A and 6RL is available. Similarly, the hybrid isozyme molecule was not formed in ditelosomic substitution line CRL(7B). However, the translocation line 7BL/CRL expressed this hybrid isozyme band. Therefore, it may be clearly inferred that genes controlling 6-PGD in wheat are located on 6A, 6BL, and 7BL.

In addition, there are also indications that other chromosomes of homoeologous groups 6 and 7 played some part in 6-PGD expression. The anodal band (6-PGD-1) in ditelosomic substitution lines 6RL(6D), CRL(7A) and CRL(7D) was very weakly stained when compared to the wheat-rye addition lines (Fig. 1 F). It appears that the association of wheat and rye isozyme subunits are also influenced by chromosomes 6D, 7A and 7D. Leibenguth (1977) found that alloploid genome interactions in wheat and triticale affected the random dimerization of alcohol dehydrogenase isozyme subunits. This in turn modified the relative band staining intensities of the isozymes. In the present study, wheat-rye addition lines zymogram phenotypes appeared closely to the relative proportions given by Scandalios (1969) for endosperm tissue. Thus the deeply stained anodal band (6-PGD-1) in C.S.-Imperial triticale suggests that the presence of both rye chromosomes 4R and 6R in the amphiploid genome produces an additive effect on the production of this band.

Several diploid species have been reported to carry two 6-PGD loci. Brody and Mendlinger (1980) showed that two-week old leaves of diploid wheats, including *Triticum monococcum* L., *Aegilops speltoides* Tausch, *Ae. longissima* Schweinf., *Ae. bicornis* Jaub, and *Ae. squarrosa* L., expressed two discernible 6-PGD gene loci. Stuber and Goodman (1980) showed that 6-PGD isozymes were coded by two unlinked loci in maize. The presence of two 6-phosphogluconate dehydrogenases were also detected in barley seedlings (Kahler et al. 1981), and in cultured tobacco tissue (Al-Quadan et al. 1981). The present results are in agreement with Rao and Rao (1980), that two 6-PGD genes are located on rye chromosomes 4RL and 6RL. From cytogenetic evidence, Koller and Zeller (1976) suggested that chromosomes CR(4R), FR(6R) and DR(7R) in *Secale cereale* are interchanged differentiating cultivated rye from the wild form. Imperial rye chromosomes CR and DR were interpreted as 4R/7R and 4R/7R/6R translocated chromosomes respectively.

The report by Hart (1978) indicated that 'Imperial' chromosome CRS carried genes for alcohol dehydrogenase, and DRS for acid phosphatase isozymes respectively. This constituted evidence that CRS and DRS were homoeologous to wheat chromosomes of group 4. The present findings that CRL(4RL) and 7BL co-determined the association of 6-PGD hybrid isozyme subunits, indicate the homoeology of CRL to wheat chromosome(s) of group 7. In addition, the common causative influence expressed by rye chromosome FRL(6RL) and wheat chromosomes 6A and 6B indicate their homoeologous relationships.

The enzyme 6-phosphogluconate dehydrogenase is one of the controlling enzymes of the pentose phosphate pathway. The increase in glucose oxidation via the pathway was shown to be associated with developmental processes in plants (Fowler and Rees 1970). The existence of homoeologous genes on both groups 6 and 7 chromosomes in wheat is indicative of the significance of 6-PGD in metabolism. However, more information is needed to postulate the functional and regulatory aspects of these genes. Nishikawa and Nobuhara (1971) showed that α-amylase isozymes in wheat were controlled by two triplicate genetic systems on homoeologous groups 6 and 7. These two groups of homoeoallelic genes were later found to possess activities which probably differed in starch degradation (Nishikawa et al. 1978). Wolf et al. (1977) found that structural genes for phosphodiesterase isozymes in hexaploid wheat were located on the three homoeologous chromosomes of group 3. However, dosage de-

pendent "regulatory genes" were also present on group 5 chromosomes which positively controlled the expression of the structural gene on 3D.

Hence, the occurrence of a given isozyme band, or changes in relative intensities, may be due to a particular structural gene(s), or due to interactions within a regulatory genetic system. As only one 6-PGD isozyme band is expressed in wheat, it may be presumed that the subunit molecules produced by genes on both homoeologous groups 6 and 7 possess similar mobilities when separated by polyacrylamide gel electrophoresis. Kazazian (1966) had earlier reported that identical 6-PGD enzyme molecules were produced from both homozygous and heterozygous phenotypes in *Drosophila.* This posed a technical difficulty in using nullitetrasomic or ditelosomic lines for studying the genetic control of 6-PGD isozymes in wheat. Nevertheless, further studies based on interactions of wheat and alien chromosomes similar to the results reported herein, may elucidate more on the activities of the homoeologous 6-PGD genes.

Acknowledgement

The authors thank Frau S. Stallenberger for her technical assistance. S. L. K. Hsam is grateful for a sabbatical leave granted him by the University of Mandalay, Burma, and also for a research fellowship sponsored by the Alexander von Humboldt-Stiftung.

Literature

Al-Quadan, F.; Wender, S.H.; Smith, E.C. (1981): The effect of some phenolic compounds on the activity of 6-phosphogluconate dehydrogenase from tobacco tissue cultures. Phytochemistry **20,** 961–964

Brody, T.; Mendlinger, S. (1980): Species relationships and genetic variation in the diploid wheats (*Triticum, Aegilops*) as revealed by starch gel electrophoresis. Plant Syst. Evol. **136,** 247–258

Carlson, P.S. (1972): Locating genetic loci with aneuploids. Mol. Gen. Genet. **114,** 272

Davies, J. (1964): Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121,** 404–427

Fowler, M.W.; Rees, T. (1970): Carbohydrate oxidation during differentiation in roots of *Pisum sativum.* Biochim. Biophys. Acta **201,** 33–44

Hart, G.E. (1978): Chromosomal arm locations of *Adh-R1* and an acid phosphatase structural gene in Imperial rye. Cereal Res. Commun. **6,** 123–133

Hart, G.E. (1979): Genetical and chromosomal relationships among the wheats and their relatives. Stadler Genet. Symp. **11,** 9–29

Hart, G.E.; Islam, A.K.M.R.; Shepherd, K.W. (1980): Use of isozymes as chromosome markers in the isolation and characterisation of wheat-barley chromosome addition lines. Genet. Res. **36,** 311–325

Hart, G.E.; Langston, P.J. (1977): Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity **39,** 263–277

Hart, G.E.; McMillin, D.E.; Sears, E.R. (1976): Determination of the chromosomal location of a glutamate oxaloacetate transaminase structural gene using *Triticum-Agropyron* translocations. Genetics **83,** 49–61

Irani, B.N.; Bhatia, C.R. (1972): Chromosomal location of alcohol dehydrogenase gene(s) in rye, using wheat-rye addition lins. Genetica **43,** 195–200

Kahler, A.L.; Heath-Pagliuso, S.; Allard, R.W. (1981): Genetics of isozyme variants in barley II. 6-phosphogluconate dehydrogenase, glutamate oxalate transaminase, and acid phosphatase. Crop Sci. **21,** 536–540

Kazazian, H.H. (1966): Molecular size studies on 6-phosphogluconate dehydrogenase. Nature **212,** 197

Kobrehel, K. (1978): Identification of chromosome segments controlling the synthesis of peroxidases in wheat seeds and in transfer lines with *Agropyron elongatum.* Can. J. Bot. **56,** 1091–1094

Koller, O.L.; Zeller, F.J. (1976): The homoeologous relationships of rye chromosomes 4R and 7R with wheat chromosomes. Genet. Res. **28,** 177–188

Leibenguth, F. (1977): Activity tests of alcohol dehydrogenases in wheat, rye and triticale. Experientia **33,** 1434–1436

Nishikawa, K.; Nobuhara, M. (1971): Genetic studies of α-amylase isozymes in wheat I. Location of genes and variation in tetra- and hexaploid wheat. Jpn J. Genet. **46,** 345–353

Nishikawa, K.; Furuta, Y.; Hina, Y.; Fuji, S. (1978): Some differential properties of alpha-amylase isozymes in growing and germinating seed of wheat. Wheat Inf. Ser. **47,** 47–48

Rao, I.N.; Rao, M.V.P. (1980): Evidence for duplicate genes coding for 6-phosphogluconate dehydrogenase in rye. Genet. Res. **35,** 309–312

Rao, I.N.; Rao, M.V.P. (1982): Identification of the chromosomes involved in a wheat-rye translocation using isozyme markers. Genet. Res. **39,** 105–109

Scandalios, J.G. (1969): Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genet. **3,** 37–79

Sears, E.R. (1952). Homoeologous chromosomes in *Triticum aestivum* (Abst.). Genetics. **37,** 624

Sears, E.R. (1966): Nullisomic-tetrasomic combination in hexaploid wheat. In: Chromosome Manipulations and Plant Genetics (eds. Riley, R.; Lewis, K.R.). Suppl. Heredity **20,** 29–45. Edinburgh: Oliver and Boyd

Stuber, C.W.; Goodman, M.M. (1980): Genetics of 6-PGD isozymes in corn. Maize Genet. Coop. Newslett. **54,** 99

Tang, K.S.; Hart, G.E. (1975): Use of isozymes as chromosome markers in wheat-rye addition lines and in triticale. Genet. Res. **26,** 187–201

Wolf, G.; Rimpau, J.; Lelley, T. (1977): Localization of structural and regulatory genes for phosphodiesterase in wheat (*Triticum aestivum*). Genetics **86,** 597–605

Zeller, F.J.; Koller, O.L. (1981): Identification of a 4A/7R and a 7B/4R wheat-rye chromosome translocation. Theor. Appl. Genet. **59,** 33–57

Received April 19, 1982
Communicated by R. Riley

Dr. S. L. K. Hsam
Dr. F. J. Zeller
Lehrstuhl für Pflanzenbau und Pflanzenzüchtung
Dr. W. Huber
Lehrstuhl für Botanik
Technische Universität München
D-8050 Freising-Weihenstephan
(Federal Republic of Germany)