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Phosphogluco mutase – a biochemical marker for group 4 chromosomes in the Triticinae

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Summary. Structural genes for the isozymes of phosphogluco mutase (PGM) (EC 2.7.5.1) have been located on chromosome arms 4Aα, 4BL and 4DS of hexaploid wheat. These results support the homoeologies observed among these chromosome arms and also support the notion of conservation of gene synteny groups within the Triticinae.

Key words: Phosphogluco mutase – Hexaploid wheat – Chromosomal location – Homoeologous group 4

Introduction

Using nulli-tetrasomic and ditelosomic strains of hexaploid wheat it is possible to relate the absence or presence of a specific isozyme with the absence or presence of a particular chromosome.

The genes controlling isozyme phenotypes in hexaploid wheat and its relatives have proved to be valuable markers in genetic and evolutionary studies with these species. So far, at least 59 structural genes have been assigned to chromosomes and/or chromosome arms of wheat by application of zymogram techniques to aneuploid stocks (Hart et al. 1980; Koebner and Shepherd 1982; Benito and Salinas 1983). These genes are distributed over 20 of the 21 different wheat chromosomes, and in many cases it has been demonstrated that the genes occur as homoalleles in the three genomes. To date the only wheat chromosomes lacking such markers are those of homoeologous group 2.

Phosphogluco mutase isozymes have been studied in different cultivars of hexaploid wheat *Triticum aestivum* L., and no variation in the PGM patterns has been observed (Salinas et al. 1982). Also, the PGM isozymes of barley (Nielsen et al. 1982; Benito et al. 1984) and rye (Salinas and Benito, unpublished) have been related with 4H and 4R chromosomes respectively.

In this paper, we report our results with phosphogluco mutase, which provides a new marker gene for group 4 chromosomes of hexaploid wheat and also which extends the information of gene synteny relationships in the Triticineae.

Material and methods

The following plant materials were used: 36 nullisomic-tetrasomic combinations of wheat (supplied by E. R. Sears), including at least one nullisomic for each wheat chromosome except 2A and 4A, and ditelocentric stocks involving 4Aα, 4BL, 4DL and 4DS chromosomes.

For isozyme analysis, different parts of individual kernels – specifically the embryo plus scutellum (E + S) and endosperm, seven day-old seedling leaves and roots, and adult plant leaves – were studied. Crude extracts were obtained by macerating the tissue in 0.1 M sodium acetate (pH 7.2) for 90 min at 2 °C. Small pieces of filter paper (Whatman 3 MM) were submerged into this solution and then inserted into the gels.

The gel consisted of a 12% starch slab (14 cm × 17 cm × 1 cm). The gel buffer was Tris-citric acid (0.015 M, pH 7.75) and the electrode buffer was NaOH boric acid (0.3 M, pH 8.6). Electrophoresis was carried out at a constant voltage of 280 V for 3 h, and the isozyme migration was from the cathodic to the anodic site. The gels were stained using the method for phosphogluco mutase described by Brewer and Sing (1970). Gels were fixed in ethanol : water (1 : 1) after staining.

Results and discussion

The phosphogluco mutase (PGM) (E.C. 2.7.5.1) phenotype of euploid 'Chinese Spring' consists of two distinct bands, the slower band (band 3) being somewhat thicker and more intense than the faster band 1 (Fig. 1). Occasionally, an additional third band (band 2), faster than band 3 and slower than band 1, is observed, but because of inconsistency in the euploid control, it is thought to be an artefact of the electrophoresis or possibly due to a conformer molecule, and has thus been excluded from the analyses.

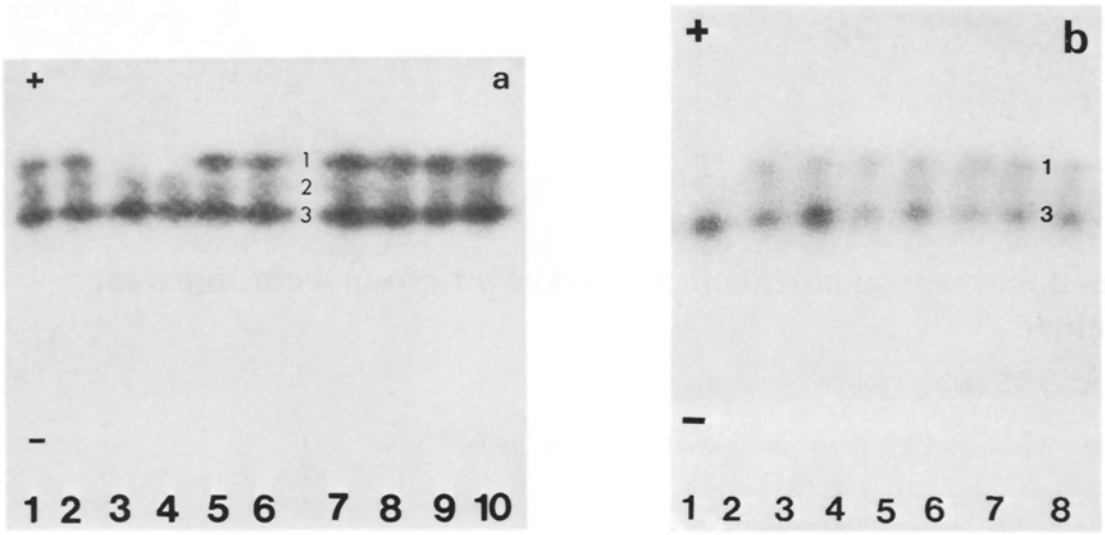

Fig. 1. **a** Phosphogluco mutase (PGM) zymogram phenotypes of leaves: *1* and *2* euploid 'Chinese Spring' wheat; *3* nulli 4B tetra 4A; *4* nulli 4B tetra 4D; *5* nulli 4D tetra 4A; *6* nulli 3A tetra 3B; *7* nulli 3A tetra 3D, *8* nulli 3B tetra 3A; *9* nulli 3B tetra 3D; *10* nulli 3D tetra 3A. **b** Phosphogluco mutase (PGM) zymogram phenotypes of endosperms: *1* nulli 4B tetra 4A; *2* ditelo 4BL (4Bp); *3* nulli 4D tetra 4A; *4* ditelo 4DL (4Dq); *5* ditelo 4DS (4Dp); *6* ditelo 4DL (4Dq); *7* ditelo 4DS (4Dp); *8* ditelo 4A (4Ap)

All the tissues analysed in the euploid 'Chinese Spring' showed the same PGM phenotype.

All the nullisomic-tetrasomic and ditelocentric stocks of 'Chinese Spring' examined, except those involving group 4 chromosomes, gave an identical phenotype to that of the euploid. The absence of chromosome 4B (nulli 4B tetra 4A and nulli 4B tetra 4D) caused the loss of band 1 (Fig. 1) but the band 1 is present in ditelocentric 4BL. These results indicate that this band is coded by a gene on chromosome arm 4BL. Although band 3 does not completely disappear upon the removal of any group 4 chromosome pair from the wheat genome, its relative staining intensity is decreased when the sum of the doses of chromosome arms 4DS and 4Aα is reduced below the four doses present in euploid 'Chinese Spring'. Thus, it is more lightly stained than the euploid in ditelocentric stock 4DL (Fig. 1), but not in nulli 4D tetra 4A, ditelo 4DS and ditelo 4Aα. It is thus concluded that chromosome arm 4DS carries a gene coding for an isozyme with the same mobility of band 3.

The genes controlling isozyme phenotypes in hexaploid wheat are, in many cases, triplicate genes that occur as homoalleles in the three genomes (Hart 1979a, b). The location of one member of each of two triplicate sets of homoeologous genes (the *Adh-1* and *Lpx-1* sets) in the α arm of chromosome 4A (the arms of chromosome 4A are indistinguishable in length), the long arm of chromosome 4B and the short arm of 4D and 2 *Acph* genes in each of the arms 4Aβ, 4BS and 4DL (Hart 1979a; Hart and Langston 1977; Salinas et al. 1981) is evidence that these sets of arms are homoeologous. A test conducted recently of the ability of the chromosome arms to pair (Sear and Sears 1979) confirmed the homoeologies among 4Aα, 4BL, and 4DS (now designated 4Ap, 4Bp and 4Dp, respectively) and among 4Aβ, 4BS and 4DL (now designated 4Aq, 4Bq and 4Dq, respectively) (Hart 1979a).

The nulli 4A tetra 4B, nulli 4A tetra 4D and ditelo 4Aβ stocks are not available, but on the basis of the homoeologies observed among chromosome arms 4Aα, 4BL and 4DS, and taking into account that PGM band 3 does not completely disappear, we have tentatively assigned to chromosome arm 4Aα a gene coding for an isozyme with the same mobility of band 3. Therefore, the chromosome arms 4DS and 4Aα each carry a gene coding for an isozyme with the same mobility which together constitute band 3.

The sharing of genes controlling PGM amongst the short arms (4Ap, 4Bp and 4Dp) of the chromosomes of the homoeologous group 4 suggests that this homoeology can be extended to the whole chromosome, and supports the hypothesis that the chromosome arms 4Aα, 4BL and 4DL are homoeologous. The genes have been assigned the gene symbols *Pgm-Al, Pgm-Bl* and *Pgm-Dl.*

Using addition lines of barley chromosomes (cv. 'Betzes') to wheat and ditelocentric strains Nielsen et al. (1982) and Benito et al. (1984) have concluded that the 4H barley chromosome carries a gene coding for the PGM-Hl isozyme. The failure to identify any hybrid band in the phenotype of the wheat-barley addition line with the chromosome 4H indicated that PGM is probably a monomeric enzyme (Benito et al. 1984). On the other hand, Salinas and Benito (unpublished), using wheat-rye addition lines, have pointed out that chromosome arm 4RS carries a gene coding for the PGM-R1 isozyme.

The location of the genes controlling PGM isozymes to the short arms of the group 4 (4Ap, 4Bp and

4Dp), the presence of Pgm-Rl on 4RS and Pgm-Hl on 4H is evidence that these sets of chromosome arms are homoeologous and lends further support to the notion of the conservation of gene synteny groups inherited from the common ancestor of the Triticinae, as proposed by Hart et al. (1980).

References

Benito C, Salinas J (1983) The chromosomal location of malate dehydrogenase isozymes in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 64:255–258

Benito C, Figueiras AM, Gozalez-Jaen MT, Salinas J (1984) Biochemical evidence of homoeology between wheat and barley chromosomes. Z Pflanzenzücht (in press)

Brewer GJ, Sing CF (1970) An introduction to isozyme techniques. Academic Press, London New York

Hart GE (1979a) Genetical and chromosomal relationships among the wheat and their relatives. Stadler Genet Symp 11:9–29

Hart GE (1979b) Evidence for a triplicate sets of glucosephosphate isomerase structural genes in hexaploid wheat. Biochem Genet 17:585–598

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

Hart GE, Islam AKMR, Shepherd KW (1980) Use of isozymes as chromosome markers in the isolation of wheat-barley chromosome addition lines. Genet Res 36:311–325

Koebner MD, Shepherd KW (1982) Shikimate dehydrogenase – a biochemical marker for group 5 chromosome in the Triticinae. Genet Res 41:209–213

Nielsen G, Johansen H, Hygoard J (1982) Chromosome localisation of loci for isoenzymes on some major lysine-rich endosperm protein in barley. In: 4th Int Congr Isozymes, Austin Tex

Salinas J, Perez de la Vega M, Benito C (1981) The chromosomal location of phosphatase isozymes of the wheat endosperm. Experientia 37:557–558

Salinas J, Perez de la Vega M, Benito C (1982) Identification of hexaploid wheat cultivars based on isozyme patterns. J Sci Food Agric 33:221–226

Sears ER, Sears LMS (1979) The telocentric chromosomes of common wheat. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp. Indian Soc Genet Plant Breed, New Delhi, pp 90–95