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SHORT PAPER

Evidence for duplicate genes coding for 6-phosphogluconate dehydrogenase in rye

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SUMMARY

The 6-phosphogluconate dehydrogenase (6-PGD) E.C. no. 1.1.1.44, zymogram phenotypes of wheat–rye addition lines and triticale were determined. Genes involved in the production of 6-PGD were located on the long arms of C and F chromosomes of Imperial Rye and the long arms of II and IV chromosomes of King II rye. The results indicate that this enzyme is a dimer.

1. INTRODUCTION

In genetic studies, enzyme markers for chromosomes facilitate the detection of the presence of the specific chromosome or chromosome segments. Such markers are also useful in mapping of chromosomes and in the identification of chromosomal translocations. Therefore there is considerable interest in identifying enzyme markers for the chromosomes of wheat, rye and related species (see Hart, 1970; Irani & Bhatia, 1972).

This paper reports the location of the structural gene(s) for 6-phosphogluconate dehydrogenase (6-PGD) E.C. no. 1.1.1.44 on the long arms of chromosomes C and F of Imperial rye, and on the long arms of chromosomes II and IV of King II rye using the complete chromosome addition lines and ditelosomic addition lines of Chinese Spring–Imperial rye and Holdfast–King II rye.

2. MATERIALS AND METHODS

Hexaploid wheat variety Chinese Spring, rye (*Secale cereale* L.) variety Imperial and the hexaploid and octaploid triticales (*Triticum turgido-secale* MacKey and *Triticum aestivo-secale* MacKey) stocks were used in the initial investigations. Chinese Spring–Imperial rye disomic addition lines for the chromosomes A, B, C, D, E, F and G were used for locating the structural gene(s) on the chromosome. Based on these results, Holdfast–King II rye ditelosomic addition lines for chromosomes II and IV (after Riley) and the Chinese Spring–Imperial rye ditelosomic addition lines for the chromosomes C and F were examined. Foundation seed of Imperial rye addition lines was obtained from Dr E. R. Sears, Columbia, Missouri, USA and Holdfast–King II ditelosomic addition lines from Dr C. N. Law, Plant Breeding Institute, Cambridge, England. The Chinese Spring–Imperial rye ditelosomic additions for the long arm and the short arm of chromosome F were supplied by Dr F. J. Zeller, Technische Universitat Munchen, 8050 Freising-Weihenstephan.

Mature kernels were soaked in distilled water for 24 h and then homogenized in 0·1 M

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Tris-HCl buffer, pH 7·5, containing 0·01 M-KCl, 0·005 M EDTA and 0·004 M 2-mercaptoethanol, maintaining a ratio of 0·2 ml buffer per kernel. The homogenate was centrifuged at 21000 g for 30 min in Sorvall RC-2B refrigerated centrifuge maintained at 0–4 °C. The supernatant (0·2 ml) was placed over each gel tube for electrophoresis which was carried out on standard polyacrylamide gels (2·5 % spacer gel, 7·5 % running gel) for 3 h at 6–8 °C, essentially following the method described by Davis (1964).

6-PGD activity was visualised by incubating the gels at 37 °C for 30 min 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. Control gels incubated for the same duration in the above mixture without the substrate (6-phosphogluconate) did not show any bands. Also, the band patterns and their respective intensities were the same when dry seeds or the 7-day-old germinated seedlings were used instead of 24 h soaked seeds. At least four replicate extractions and electrophoreses were carried out for each stock.

3. RESULTS

6-PGD zymograms for the different stocks examined are given in Fig. 1 and 2. Chinese Spring (Fig. 1*a*) had one band (6-PGD-3). Three varieties of rye (Imperial, King II and Russian) also had only one band (6-PGD-1) that migrated faster than the wheat band. Co-electrophoresis of Chinese Spring wheat and Imperial rye showed two distinct bands corresponding to the rye and wheat bands. The hexaploid triticale had three bands, two of which corresponded to the rye (6-PGD-1) and wheat (6-PGD-3) bands. In addition, it showed a band of intermediate mobility (6-PGD-2). The intensities of 6-PGD bands 1, 2 and 3 were respectively 1:2:1, suggesting that the enzyme in rye and wheat is a dimer,

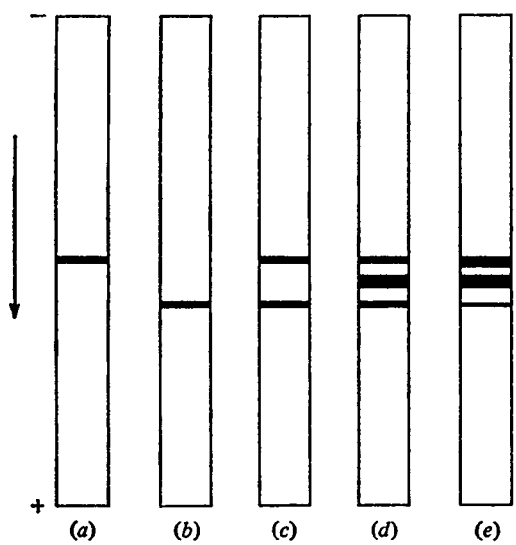

Fig. 1. Zymogram of 6-phosphogluconate dehydrogenase (6-PGD) in wheat, rye, triticale and wheat-rye ditelosomic addition lines. (*a*) Diploid (*T. monococcum*), tetraploid (*T. durum*) and hexaploid (*T. aestivum* var Chinese Spring and Holdfast) wheats. (*b*) *Secale cereale* (2 ×) var Imperial, King II and Russian. (*c*) Co-electrophoresis of Chinese Spring wheat and Imperial rye extracts. (*d*) Hexaploid triticale. (*e*) Chinese Spring–Imperial rye chromosome addition lines CS + C, CS + F and Ditelosomic addition lines CS + C (long) CS + F(long).

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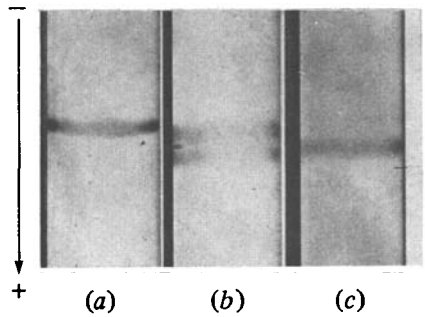

Fig. 2. Zymogram of 6-phosphogluconate dehydrogenase (6-PGD) of wheat, rye and mixture. (*a*) Diploid (*T. monococcum*), tetraploid (*T. durum*) and hexaploid (*T. aestivum* var Chinese Spring and Holdfast) wheats. (*b*) Co-electrophoresis of Chinese Spring wheat and Imperial rye extracts. (*c*) *Secale cereale* (2 ×) var. Imperial, King II and Russian.

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and the middle hand (6-PGD-2) represents a heterodimer formed by the association of rye and wheat monomers. 6-PGD in *Neurospora crassa* was earlier reported to be a dimer (Scott & Abrahamsky, 1973).

4. ADDITION LINES

Disomic addition lines of Imperial rye chromosomes C and F to Chinese Spring wheat showed three bands similar to those observed in triticale. However, the other rye chromosome addition lines had only one band (6-PGD-3) corresponding to that of wheat. Chinese Spring–Imperial rye ditelosomic addition lines for the long arms of chromosomes C or F showed a three band pattern similar to that obtained in the complete chromosome addition lines.

Imperial rye chromosome F corresponds to the King II rye chromosome II (according to Riley's designations) and both fall in the homoeologous group 6 (Darvey, 1973; Koller & Zeller, 1976) and should be referred to as 6R. Imperial rye chromosome C is homologous to King II rye chromosome IV (Koller & Zeller, 1976). Therefore, ditelosomic additions of King II rye chromosomes II and IV (after Riley) were examined. Long arm ditelosomic additions of both chromosomes II and IV showed three bands like the long arm ditelosomic addition lines of Chinese Spring–Imperial rye for chromosomes C and F. The short arm ditelosomic additions had only one band (6-PGD-3) as in wheat.

5. DISCUSSION

The above results clearly show that in diploid rye there are two structural genes coding for the enzyme 6-phosphogluconate dehydrogenase which are located on the long arms of chromosomes II and IV of King II rye (after Riley). The two genes in Imperial rye are located on the long arms of chromosomes C and F. This is probably the first case of duplicate structural genes for an enzyme in rye whose chromosomal location(s) is worked out.

Based on cytological evidence, Koller & Zeller (1976) have suggested that the cultivated rye (*Secale cereale* L) differs from the wild rye (*Secale montanum* Guss) by two interchanges involving chromosomes 4R, 6R and 7R. Duplicate genes for 6-PGD in this study have been located on 4R (long arm) and 6R (long arm) (Koller and Zeller's classification), the two chromosomes involved in the translocations as suggested by Koller and Zeller (1976). It is therefore likely that the observed duplication might have originated during the course of chromosomal rearrangements associated with the evolution of the cultivated rye from the wild rye. A translocation heterozygote usually gives rise to duplication-deficiency gametes (adjacent disjunction of the chromosomes from a ring of four chromosomes). If there is no selection against these gametes they will give rise to duplication-deficiency progeny with respect to certain chromosomal segments. It is possible that during the evolution of the cultivated rye from the wild progenitor, this type of chromosomal alteration might have led to duplication of a certain segment of the 6R chromosome and corresponding deficiency in the Imperial rye chromosome C; this may also be the reason for the observed poor compensating ability of the chromosome C of Imperial rye for any of the wheat chromosomes.

Addition of individual *Secale montanum* Guss chromosomes to wheat would be the easiest way to check if it also carries duplicate genes for 6-PGD on different chromosomes. However, *Secale montanum* addition lines are not available at present.

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