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Esterase isozymes in rye – Characterization, genetic control and chromosomal location

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Summary. The zymogram phenotypes that 'Chinese Spring'–'Imperial', 'Holdfast'–'King II' and 'Kharkov'–'Dakold' wheat-rye addition lines presented for esterase isozymes were determined using polyacrylamide gel ectrophoresis. The analyses were carried out with different parts of the dry kernel, namely embryo plus scutellum and endosperm, leaves and roots. In all cases, embryo plus scutellum, endosperm and leaf presented different patterns of esterases. The patterns of leaves and roots were the same. Results indicate that rye esterases exist as monomers and dimers. Dimeric esterases are controlled by one locus located on the 3R chromosomes of 'Imperial', 'King II' and 'Dakold' rye cultivars. Five loci involved in the production of monomeric esterases have been located on the 6R chromosomes of these cultivars, specifically on the long arm of the 'King II' 6R chromosome. On the basis of these results, considerations concerning chromosome homoeology and homology are made.

Key words: *Secale cereale* – Wheat-rye addition lines – Esterases – Chromosomal location – Isozyme structural genes

Introduction

The rye genus *Secale* L. is a member of the subtribe Triticinae which contains different species, the only cultivated one being *S. cereale* L. Although *S. cereale* L. has, as have all species belonging to the Triticinae, a basic genome composed of seven chromosomes, relationships between these chromosomes and the chromosomes of the other genus of the subtribe are difficult to obtain because of the translocations ocurring in the *Secale* L. genome along its evolutive divergence (Price 1955; Riley 1955).

Evidence regarding the relationships among different chromosomes may be obtained by studying the chromosomal location of specific genetic material. Taking advantage of the hexaploid wheat capacity to admit aneuploidy and to hybridize with other species of the Triticinae, a considerable number of wheat lines containing rye genetic material (added or substituted) have been developed (for a review, see Lacadena 1977). Using these lines, it has been possible to locate a considerable number of genes encoding proteins or multiple forms of enzymes on the chromosomes of rye *S. cereale* L. (for a review, see Garcia-Olmedo et al. 1982; Hart and Tuleen 1983; Salinas and Benito 1983, 1984a, b, 1985a, b). This number, however, is small when compared with the number of genes located on the chromosomes of hexaploid wheat (*Triticum aestivum* L.) (for a review, see Garcia-Olmedo et al. 1982; Hart 1983; Benito and Salinas 1983; Benito et al. 1984). On the other hand, the majority of the rye genes have so far been located on the chromosomes of 'Imperial' rye cultivar. As a consequence, studies on chromosome homology and relationships between different rye cultivars have not been carried out.

The present paper reports on the chromosomal location of the structural genes controlling the esterase (EST) E.C. no. 3.1.1.2 isozymes of 'Imperial', 'King II' and 'Dakold' rye cultivars. On the basis of these results, the genetic control and the quaternary structure of these isozymes are discussed. Also, biochemical evidence of

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homoeology among several chromosomes of rye and hexaploid wheat, and of homology among several chromosomes of the different rye cultivars analysed, is presented.

Materials and methods

The genotypes used were *T. aestivum* L. cv. 'Chinese Spring' (CS), *S. cerale* L. cv. 'Imperial' (I) and CS-I disomic addition lines obtained from E. R. Sears (Missouri); CS-I ditelosomic addition lines 2RL, 4RS, 5RS, 7RL and 7RS supplied by F. J. Zeller (München); *T. aestivum* L. cv. 'Holdfast' (H), *S. cereale* L. cv. 'King II' (KII) and H-KII disomic addition lines obtained from J. P. Gustafson (Manitoba); H-KII ditelosomic addition lines excluding 3RL, 3RS and 7RS obtained from C. N. Law (Cambridge); *T. aestivum* L. cv. 'Kharkov' (K), *S. cereale* L. cv. 'Dakold' (D) and K-D disomic addition lines supplied by J. P. Gustafson (Manitoba). The rye chromosomes of addition lines were named according to the classification proposed by Koller and Zeller (1976).

Different parts of individual kernels, namely embryo plus scutellum and endosperm, and seven-day-old seedling leaves and roots were analysed. In all cases, individual samples were crushed and immersed in 0.1 M sodium acetate, pH 7.2. Small pieces of filter paper (Whatmann 3MM) were soaked with the crude extracts and inserted into polyacrylamide horizontal gels (polyacrylamide 10% w/v; 95 : 5 acrylamide : bisacrylamide w/w). Electrophoresis was carried out as related by Kahler and Allard (1970) and EST isozymes were visualized following the method of Bergman and Maan (1973).

The chromosomal control of the addition lines was carried out by C-banding as described by Giraldez and Orellana (1979).

Results

All addition lines had the 42 wheat chromosomes and the two suitable chromosomes or chromosome arms of rye. In the H-KII-2R addition lines, a deletion in the short arm of 2R rye chromosomes was detected. Cytological evidence for this deletion has been reported by Singh and Röbbelen (1976).

In all cases, embryo plus scutellum, endosperm and seven-day-old seedling leaves presented different patterns of esterases. The patterns of leaves and roots were the same. Identical patterns for leaf and root esterases have been described by Bergman and Maan (1973) in wheat and rye.

The leaves and roots of wheat cultivars showed an esterase zymogram composed by 17 isozymes named from EST-W1 to EST-W17 (Fig. 1). Rye cultivars revealed different phenotypes depending up on which of the 18 possibles isozymes (from EST-R1 to Est-R18) were expressed (Fig. 1). When the addition lines were electrophoresed, CS-I-3R, H-KII-3R and K-D-3R leaves and roots exhibited the presence of the 17 wheat esterases, the EST-R1 rye isozyme and a new isozyme (EST- W3′) which had an intermediate mobility between EST- W3 and EST-R1. On the other hand, the leaves and roots of CS-I-6R and K-D-6R addition lines indicated the presence of the wheat isozymes plus the EST-R6, EST-R7, EST-R8 and EST-R9 rye esterases; H-KII-6R and H-KII-6RL leaves and roots displayed the wheat esterase zymogram plus the EST-R7, EST-R8, EST-R9 and EST-R10 rye isozymes. The rest of the addition lines had the same esterase pattern as that of wheat cultivars (Fig. 1).

The pattern of esterases showed by the wheat endosperms was similar to that showed by the wheat leaves and roots although the EST-W4, EST-W5, EST-W6, EST-W7 and EST-W8 esterases were not expressed (Fig. 2). Rye endosperms showed the presence of the isozymes EST-R1, EST-R6, EST-R7, EST-R8, EST-R9 and the isozymes from EST-R11 to EST-R18 (Fig. 2). The endosperms of CS-I-3R, H-KII-3R and K-D-3R addition lines revealed the esterases of wheat endosperm, the EST-R1 rye isozyme and the EST-W3′ isozyme.

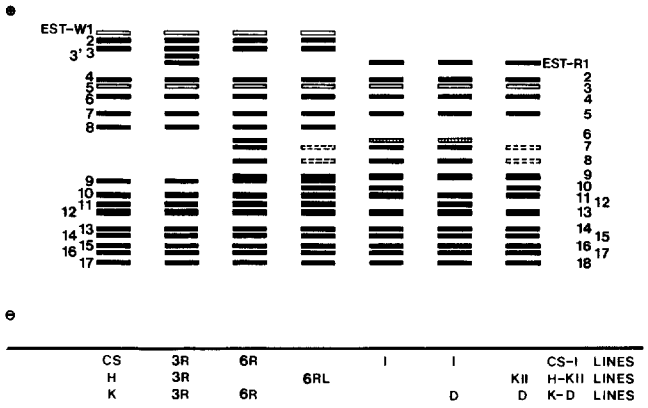

Fig. 1. Esterase phenotypes that wheat cultivars, rye cultivars, and critical addition lines presented when leaves and roots were analysed. The addition lines not indicated in the figure showed the wheat pattern

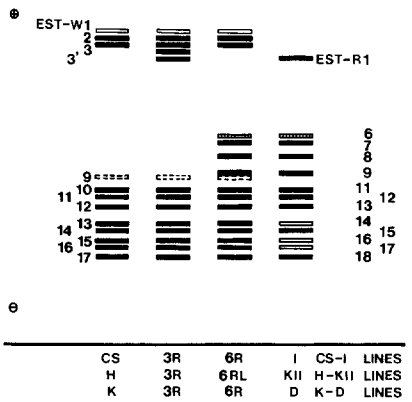

Fig. 2. Esterase phenotypes that wheat cultivars, rye cultivars, and critical addition lines presented when endosperms were analysed. The addition lines not indicated in the figure showed the wheat pattern

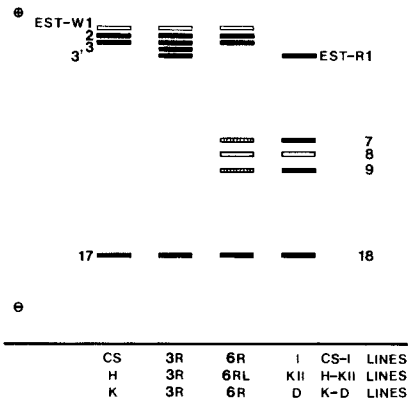

Fig. 3. Esterase phenotypes that wheat cultivars, rye cultivars, and critical addition lines presented when embryo plus scutellum was analysed. The addition lines not indicated in the figure showed the wheat pattern

CS-I-6R, H-KII-6R, H-KII-6RL and K-D-6R addition line endosperms showed the esterases of wheat endosperm plus the EST-R6, EST-R7, EST-R8 and EST-R9 esterases of rye. The endosperms of the remaining addition lines presented an esterase phenotype identical to that of wheat endosperms (Fig. 2).

The embryo plus scutellum of wheat cultivars expressed four esterases: EST-W1, EST-W2, EST-W3 and EST-W17 (Fig. 3). The embryo plus scutellum of rye cultivars expressed five isozymes: EST-R1, EST-R7, EST-R8, EST-R9 and EST-R18 (Fig. 3). CS-I-3R, H-KII-3R and K-D-3R addition line embryo plus scutellum exhibited the four esterases of wheat embryo plus scutellum, the EST-R1 rye isozyme and the EST-W3′ isozyme. In CS-I-6R, H-KII-6R, H-KII-6RL and K-D-6R addition lines, the embryo plus scutellum manifested the four wheat embryo plus scutellum esterases plus the EST-R7, EST-R8 and EST-R9 isozymes of rye. The embryo plus scutellum of the rest of the addition lines showed the same esterase zymogram to that of wheat cultivar (Fig. 3).

Discussion

The results obtained suggest the existence of two well-defined groups of rye esterases. These groups would differ in electrophoretic mobility, substrate specificity, quaternary structure and chromosomal location.

In all the tissues analysed, the rye esterase phenotypes could be divided in two zones. Zone I (the most anodal one) would be composed of one isozyme (EST-R1), zone II would comprise the remainder of the esterase isozymes (Figs. 1–3). EST-R1 isozyme would have a higher specificity for β- than for α-naphthylacetate as substrate; zone II esterases, however, would show more specificity for α- than for β-naphthylacetate (Bergman and Maan 1973).

The patterns of addition lines containing 3R chromosomes always show the presence of corresponding wheat esterases, the EST-R1 rye isozyme and a new isozyme (EST-W3′) of intermediate mobility between the EST-W3 wheat esterase and the EST-R1 esterase of rye. These results indicate that the rye esterase of zone I (EST-R1) shoud be a dimer related to the 3R chromosomes of I, KII and D rye cultivars. The zymograms of the 6R addition lines exhibit the presence of corresponding wheat esterases plus some esterase of rye but no new intermediate bands are detected. These results suggest that EST-R6, EST-R7, EST-R8, EST-R9 and EST-R10 zone II rye esterases are monomers related to the 6R chromosomes of the three rye cultivars studied and specifically to the long arm of the KII 6R chromosome (Figs. 1–3). Similar results have been obtained by Barber et al. (1968) and Bergman and Maan (1973) studying the esterases of 'Imperial' rye.

The existence of one esterase locus, *Est-1,* located on the 3R chromosomes can be postulated. This locus could express at least one active dimeric subunit (a) that would be codified by the structural gene *Est-1a-* in the dimeric form it would constitute the EST-R1 isozyme. The EST-6R, EST-R7, EST-R8, EST-R9 and EST-R10 isozymes simultaneously appear in the 6R addition lines: they must be controlled by active alleles of five different loci (*EST-6, EST-7, Est-8, Est-9, Est-10*) located on the 6R chromosome (6RL arm in KII cultivar). The *Est-6a, Est-7a, Est-8a, Est-9a,* and *Est-10a* structural genes would codify active monomeric subunits that would constitute the EST-R6, EST-R7, EST-R8, EST-R9 and EST-R10 isozymes, respectively.

The esterase variability that is detected in 6R addition line leaves and roots is a consequence of the existing variability in the leaves and roots of rye cultivars. Recently, Schmidt-Stohn and Wehling (1983), when studing the rye leaf esterases, also found isozymatic variability. They postulate a monomeric structure for rye leaf esterases and at least ten loci encoding for up to five different allelic esterases per locus. That all the leaf esterases detected by these authors behave as monomers could be due to the fact that they only used α-naphthylacetate as the esterase reaction substrate and consequently only the esterases of zone II were revealed. Correlations between the esterase loci characterized by us and those characterized by Schmidt-Stohn and Wehling (1983) is difficult because the methodology used to separate the rye leaf esterase isozymes is very different.

Esterase isozymes from different plants have been described as being monomers (Marshall and Allard 1969 in *Avena;* Lundkvist and Rudin 1977 in *Picea;* Nakagahra 1977 in

Oriza) or dimers (Scandalios 1969 in *Zea mays;* Babbel and Waine 1977 in *Hordeum*). In *Lycopersicon* (Tanksley and Rick 1980) and *T. aestivum* (Barber et al. 1968; May et al. 1973; Jaaska 1980), esterase isozymes exist as monomers and dimers at the same time. Wheat dimeric esterases are more anodal than monomeric ones and structural genes codifying for them have been located on the short arms of the homoeologous group 3 chromosomes (Barber et al. 1968; Jaaska 1970). Wheat monomeric esterases have been related to the long arms of the homoeologous group 6 chromosomes (May et al. 1973; Jaaska 1980). On the other hand, wheat dimeric esterases would have more specificity for β- than for α-naphthylacetate and a substrate while the specificity would be reversed in the case of wheat monomeric esterases (Bergman and Maan 1973).

The chromosomal location of rye esterase structural genes on the 3R and 6R (long arm in KII) chromosomes, together with the identity in substrate specificity, quaternary structure, and relative migration between the wheat and rye esterases of zone I and II, constitutes biochemical evidence of the existing homoeology among the wheat and rye esterase structural genes and therefore, among the chromosomal fragments that enclose them. In addition, this evidence is corroborated by the fact that esterase subunits expressed by zone I wheat and rye structural genes are able to form active heterodimers in 3R addition lines, i.e. EST-W3′ (Figs. 1–3). Cytological evidence of homoeology between 3R and 6R rye chromosomes and corresponding wheat chromosomes (homoeologous group 3 and 6 chromosomes) have been described by Acosta (1968) and Riley (1965), respectively.

The results obtained in this work confirm the usefulness of isozyme markers in studies designed to determine the genetic relationships between chromosomes, chromosome arms or chromosome segments of different genomes: the location of esterase loci on rye chromosomes provides further evidence for the general conservation of linkage relationships in the members of the Triticinae (Hart 1979). Isozyme markers can also be utilised to study homologous genes and to obtain information on chromosome evolution and homology among different cultivars of the same species. In this paper, we have demonstrated that the esterase structural genes we have located are to be found on the same chromosomes in the three rye cultivars analysed. This result indicates that the 3R and 6R chromosomes of I, KII and D ryes are homologous. Chromosome homoeology and homology conclusions support the relation established by Koller and Zeller (1976) between the different chromosomes of 'Imperial', 'King II' and 'Dakold' rye cultivars and their placement into the Triticinae homoeologous groups.

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