Chromosomal location of α-amylase structural genes in rye (*Secale cereale* L.)

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Summary. Rye (*Secale cereale* L.) α-amylase isozymes are controlled by at least four loci located on the 5R (three) and 7R (one) chromosomes. In the case of 'Imperial' and 'King II' cultivars, two of the three 5R chromosome loci could be specifically located on the 5RL chromosome arm; the other one was located on the 5RS. The locus of 7R chromosome was located on the 7RL chromosome arm of 'Imperial' rye.

Key words. Secale cereale L.; wheat-rye addition lines, isozyme structural genes; α-amylases; chromosomal location.

During germination, cereal seeds produce and secrete starch-hydrolyzing enzymes which digest the starch reserves of the storage tissues. This fact means that the amylases play a role in development and differentiation which is worthy of investigation. The two enzymes which have been best characterized are β and α-amylases[1]. α-Amylase isozymes have been studied in various higher plants[2-4]; all cases have been described as being under nuclear control and following mendelian inheritance. Moreover, α-amylases appear to be controlled by polymorphic loci which could express different isozymes or none (null allele). In maize, α-amylase isozymes behave as dimers[3]; but in barley and wheat as monomers[2,4]. So far, the α-amylase isozymes of rye have not been studied.

The present work reports the chromosomal location of the structural genes controlling the rye (*Secale cereale* L.) α-amylase isozymes. On the basis of these results, the genetic control and subunit structure of these isozymes are discussed. Also, the existence of homology among chromosomes or chromosome arms of different rye cultivars and of homoeology among chromosomes or chromosome arms of rye and hexaploid wheat is suggested.

Materials and methods. The genotypes used were: *Triticum aestivum* L. cv. 'Chinese Spring' (CS), *Secale cereale* L. cv. 'Imperial' (I), 'Chinese Spring-Imperial' (CS-I) disomic addition lines obtained from E.R. Sears (Missouri), CS-I ditelosomic addition lines 2RL, 4RL, 4RS, 5RS, 7RL and 7RS (L = long arm, S = short arm) supplied by F.J. Zeller (Munchen); *T. aestivum* L. cv. 'Holdfast' (H), *S. cereale* L. cv. 'King II' (KII), H-KII disomic addition lines (with the 42 wheat chromosomes and two homologous rye chromosomes) obtained from J.P. Gustafson (Manitoba), H-KII ditelosomic addition lines (with the 42 wheat chromosomes and two homologous rye chromosome arms) excluding 3RL, 3RS, 7RL 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).

Analyses were carried out with the individual extracts obtained from the endosperms of five-day germinating kernels, which were crushed in 0.05 M tris-HCl pH 8.6 and heated at 60°C for 15 min. α-Amylase isozymes were resolved by means of horizontal 10% polyacrylamide gel electrophoresis using 0.2 M tris-glycine as gel and bridge buffer. Electrophoresis was carried out at 2-4°C for 16 h in the anodic direction with a constant voltage of 120 V. After electrophoresis, gels were stained as previously described[4].

The chromosomal contents of the analyzed lines were verified by C-banding[5]. All addition lines had the 42 wheat chromosomes and the two suitable chromosomes or chromosome arms of rye; only H-KII-2R addition lines, as previously related[6], showed a deletion in the short arms of 2R chromosomes. The rye chromosome nomenclature used was that proposed by Sybenga[7]. The CS-I-3R disomic addition line contained the 42 wheat chromosomes of 'Chinese Spring' and the two chromosomes 3R of 'Imperial' rye.

Results. Wheat cultivars presented a pattern composed of ten isozymes named from α-AMY-W1 (W = wheat) to α-AMY-W10 (figs 1 and 2). The amylase zymograms of 'King II' (KII) and 'Dakold' (D) cultivars revealed seven isozymes (from α-AMY-R1 to α-AMY-R7). The 'Imperial' (I) cultivar did not show the α-AMY-R7 isozyme (R = rye). Rye α-amylase isozymes, excluding α-AMY-R4, had electrophoretic mobilities similar to those of wheat isozymes, but generally a greater intensity; α-AMY-R1 could be expressed with different intensities (figs 1 and 2). All addition lines exhibited the wheat phenotype but CS-I-3R, CS-I-5R, CS-I-5RS, CS-I-7R, CS-I-7RL, H-KII-5R, H-KII-5RS, H-KII-5RL, H-KII-7R, K-D-5R and K-D-7R expressed at least one isozyme with greater intensity than the corresponding isozyme in wheat (figs 1 and 2). The table summarizes the rye chromosomes or chromosome arms related to the different rye α-amylases. The results obtained suggest that the rye α-amylases are related to the 5R and 7R chromosomes.

In order to facilitate their study, rye α-amylases can be divided into two groups: group I composed of the α-AMY-R1,α-AMY-R2, α-AMY-R3 and α-AMY-R4 isozymes, and group II composed of the α-AMY-R5, α-AMY-R6 and α-AMY-R7 isozymes. In the three rye cultivars analyzed (I, KII and D), all the isozymes of the first group, except α-AMY-R1, are related to the 5R chromosome (fig. 1, table). In I and KII cultivars,α-AMY-

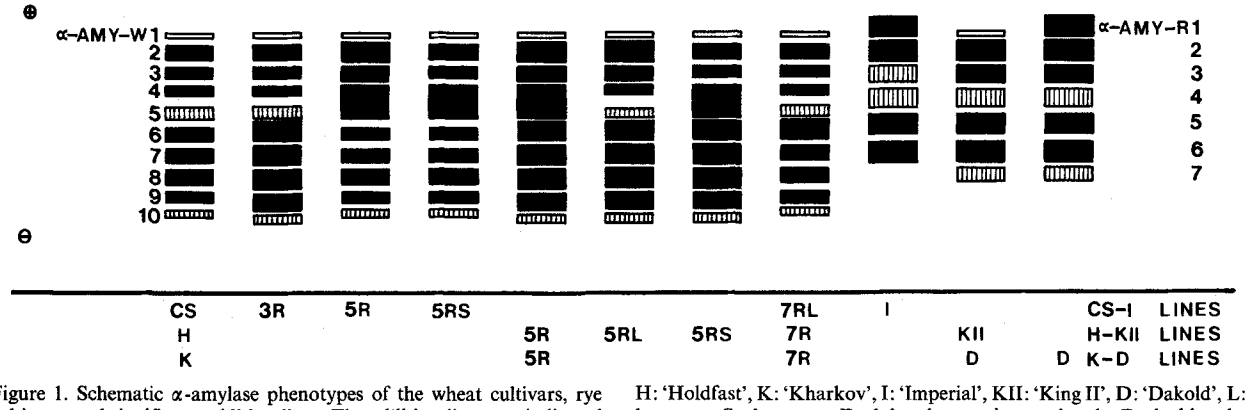

Figure 1. Schematic α-amylase phenotypes of the wheat cultivars, rye cultivars, and significant addition lines. The addition lines not indicated in the figure showed the hexaploid wheat pattern. CS: 'Chinese Spring', H: 'Holdfast', K: 'Kharkov', I: 'Imperial', KII: 'King II', D: 'Dakold', L: long arm, S: short arm. Dark bands; very intense bands: Dashed bands; intermediate intensity bands: White bands; slight intensity bands.

R2 and α-AMY-R3 were related specifically to the long arm of the 5R chromosome (5RL) and α-AMY-R4 to the short arm (5RS). The α-AMY-R1 isozyme has not been related to any rye chromosome (figs 1 and 2). The α-amylases of group II are difficult to associate with the rye chromosomes because of the patterns shown by CS-I-3R, H-KII-5R and K-D-5R addition lines. These patterns express some isozymes (from α-AMY-W6 to α-AMY-W10) with greater intensity than the patterns of wheat cultivars and the patterns of the rest of the addition lines (figs 1 and 2). However, the α-AMY-R6 and α-AMY-R7 rye isozymes could be tentatively associated with the 3R chromosome of 'Imperial' rye and with the 5R chromosome of 'King II' and 'Dakold' cultivars (table).

Discussion. The association of the α-AMY-R6 and α-AMY-R7 rye isozymes with the 3R chromosome of I rye and also with the 5R chromosome of KII and D ryes could suggest the existence of a translocation between 5R and 3R chromosomes. The low intensity observed in the group II wheat α-amylases, when the CS-I-3R, H-KII-5R and K-D-5R addition lines are analyzed, could be an effect (quantitative or qualitative) of the added rye chromosomes on the wheat α-amylase activity. H-KII-5RS and H-KII-5RL ditelosomic addition lines exhibited the same pattern as that of the H-KII-5R addition line so, in the case of KII, both the long and the short arms of chromosome 5R appear to influence the 'Holdfast' α-amylase activity. In consequence, only the α-AMY-R5 isozyme has been related to the rye chromosomes, concretely to the 7R chromosome of all cultivars (long arm in I and KII) (figs 1 and 2). The rest of group II rye α-amylases (α-AMY-R6 and α-AMY-R7) could associate with the rye chromosomes which seem to modify the α-amylase activity of wheat (3R in I and 5R in KII and D). Modifications of wheat isozymatic activity by added chromosomes have been described in various wheat addition lines: Cauderon et al.[8] report the modification of wheat peroxidase activity by a pair of *Agropyron intermedium* chromosomes; the modification of wheat peroxidase activity in the presence of the 2R rye chromosomes has also been described[9]. Moreover, it has been reported[10] that the wheat α-amylase activity is enhanced by the two arms of different wheat chromosomes.

From the location results, it is possible to propose the existence of at least four loci controlling the rye α-amylase activity: three loci (α-amy-2, α-amy-3, α-amy-4) would be located on the 5R chromosome and one locus (α-amy-5) on the 7R chromosome. In I and KII rye cultivars, α-amy-2 and α-amy-3 would be located on the long arm of the 5R chromosome, and α-amy-4 on the short arm; α-amy-5 locus would be located on the long arm of the 'Imperial' 7R chromosome. Each locus must express at least one allele (α-amy-2a, α-amy-3a, α-amy-4a, α-amy-5a) which would codify for the α-amy-R2, α-amy-R3, α-amy-R4 and α-amy-R5 isozymes, respectively. One quaternary structure for the rye α-amylase isozymes cannot be proposed from the zymograms of addition lines.

In *T. aestivum* cv. 'Chinese Spring', α-amylase isozymes are controlled by structural genes located on the long arms of homoeologous groups 6 and 7 chromosomes[11]. Moreover, results indicating that α-amylase activity during wheat grain germination can also be related with the chromosomes of homoeologous group 5 have been obtained[12]. The location of structural genes coding for rye α-amylases on the 5R and 7R chromosomes, 5RL, 5RS and 7RL chromosome arms in I and KII, provides biochemical evidence of homoeology among the α-amylase structural genes of wheat and rye and, therefore, among the chromosomal fragments that enclose them. Cytological evidence of homoeology between 5R and 7R chromosomes and the corresponding wheat chromosomes has been procured[13,14].

The results obtained in this work corroborate 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 α-amylase structural genes on rye chromosomes provide further evidence for the general conservation of linkage relationships in the members on the Triticinae[15]. Isozyme markers can also be utilized to study homologous genes and to obtain information about chromosome evolution and homology among different cultivars of the same species. In this paper we have demonstrated that α-amylase structural genes are located on the same chromosomes, and on the same chromosome arms when chromosome arm location has been possible, in the three rye cultivars analyzed. These results indicate that 'Imperial', 'King II' and 'Dakold' ryes conserve a certain chromosomal homology.

Rye chromosomes or chromosome arms related to the rye α-amylase isozymes

	Rye α-amylases							Modification of wheat α-amylases from W6 to W10
	R1	R2	R3	R4	R5	R6	R7	
CS-I lines		5R	5R	5RS	7RL	3R	3R	3R
H-KII lines		5RL	5RL	5RS	7R	5R	5R	5R
K-D lines		5R	5R	5R	7R	5R	5R	5R
Critic chromosome or chromosome arm		5RL	5RL	5RS	7RL		3R/5R	3R/5R

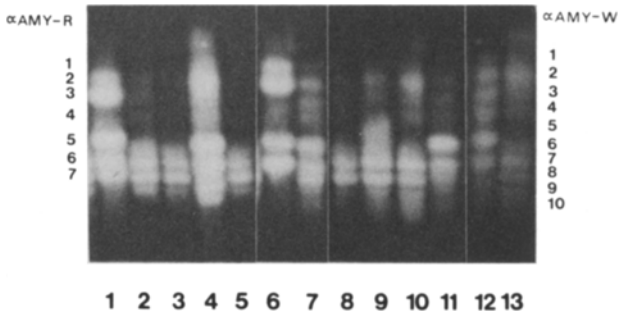

Figure 2. Zymograms of α-amylase. 1. KII and D rye cultivars, 4. H-KII-5R and K-D-5R addition lines (AL), 6. I rye, 7. CS-I-7R, H-KII-7R and K-D-7R AL, 9. CS-I-5RS AL, 11. CS-I-7RL AL, 12. CS-I-3R AL, 13. CS, H and K wheat cultivars. The rest of the patterns are the remainder addition lines. CS: 'Chinese Spring', H: 'Holdfast', K: 'Kharkov', I: 'Imperial', D: 'Dakold', KII: 'King II'. α-AMY-W: wheat α-amylases, α-AMY-R: rye α-amylases.

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