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Chromosomal location of isozyme markers in wheat-barley addition lines

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Summary. The peroxidase (CPX, PER), α-amylase (α-AMY), acid and alkaline phosphatase (PHE, PHS) and esterase (EST) zymogram phenotypes of 'Chinese Spring' wheat, 'Betzes' barley and a number of presumptive 'Betzes' chromosome additions to 'Chinese Spring' were determined. It was found that five disomic chromosome addition lines could be distinguished from one another and from the other two possible lines on the basis of the zymogram phenotypes of these isozymes. The structural genes *Cpxe-H1* and *Cpxe-H2* were located in 'Betzes' chromosome 1, the *Perl-H5* and *Perl-H6* in chromosome 2, the *α-Amy-H2* and *α-Amy-H3* in chromosome 7, the *Phs-H5* and *Phs-H4* in chromosomes 1 and 3 respectively, the *Phe-H2, Phe-H3* and *Phe-H4* in chromosome 1, the *Phe-H1* in chromosome 3, the *Ests-H4, Este-H2* and *Ests-H6, Este-H8* in chromosomes 1 and 3 respectively and the *Estl-H10* and *Estl-H2* structural genes were related to chromosomes 3 and 6 respectively. These gene locations provide evidence of homoeology between 'Betzes' chromosomes 1, 2, 3, 6 and 7 and the rye chromosomes 7, 2, 3, 6 and 5, respectively, and also between 'Betzes' chromosomes 1, 2, 3, 6 and 7 and the 'Chinese Spring' homoeologous groups 7, 2, 3, 6 and 5, respectively.

Key words: Chromosomal location – Isozyme markers – *Hordeum vulgare* L.

Introduction

A considerable number of wheat lines which contain alien genetic material (added or substituted) have been produced by cytogeneticists taking advantage of the capacity of hexaploid wheat to admit aneuploidy. Lines have been obtained with whole chromosomes, chromosome arms or segment of arms from other species of Triticeae.

By zymogram analyses of wheat-rye and wheat-barley addition lines and of the parental wheat, rye and barley cultivars, genetic variation between them can be detected, and genes involved in the production of isozymes can be located on specific rye and barley chromosomes. Moreover, by relating the chromosomal constitution and the electrophoretic phenotypes of pertinent aneuploid strains, it is possible to construct logical modes for the genetic control and subunit structure of rye and barley isozymes (for a review, see Hart 1979 and references cited by Hart et al. 1980; Rao and Rao 1981; Chojecki and Gale 1982; Koebner and Shepherd 1982).

Chromosomal location of structural genes for isozymes can provide information for chromosome homology and homoeology as well as genetic relationships among related species (Tang and Hart 1975; Hart and Langston 1977; Hart et al. 1980; Powling et al. 1981; Hart and Tuleen 1983).

The chromosomal location of isozymes in wheat, rye and barley have enabled researchers to obtain biochemical evidence of homoeology among wheat, rye and barley chromosomes (Table 1). It is therefore interesting to have marked all the chromosome arms of rye and barley with as many isozyme structural genes as possible.

The present paper reports the chromosomal location of the structural genes controlling the peroxidases, α-amylases, acid and alkaline phosphatases and esterases in different parts of dry kernels and leaves of 'Betzes' barley. Also, biochemical evidence of homoeology among hexaploid wheat, rye and barley chromosomes are discussed.

Materials and methods

This study was conducted with materials produced by Islam and his colleagues (1978). The materials used were *Triticum aestivum* L. cv. 'Chinese Spring' (CS), *Hordeum vulgare* L. cv.

Table 1. Chromosomal location of isozyme structural genes in hexaploid wheat, rye and barley

Genes	Chromosomal location (chromosome or chromosome arm)			
	T. aestivum L. cv 'Chinese Spring' Genomes A, B and D	*S. cereale* L. cv I, K II, D Genome R	*H. vulgare* L. cv 'Betzes' Genome H	References of chromosomal location in barley
Homoeologous sets				
Pgi-1[a]	1p	1p	5	Powling et al. (1981)
Got-2[a]	6q	6q	6	Hart et al. (1980)
Got-3[a]	3q	3	3	Benito et al. (1984)
Adh-1[a]	4p	4	4	Hart et al. (1980)
Amp-1	6p	–	6	Hart et al. (1980)
Ep-1	7q	–	1	Hart et al.(1980)
Mdh-2	1q	1q, 3	5, 3	Benito et al. (1984)
Pgm-1	4p	4p	4	Benito et al. (1984) Nielsen et al. (1982)
Other genes				
Mdh-1[a]	–	2q	5	Powling et al. (1981)
6-Pgd-2[a]	–	2q	5	Benito et al. (1984)
Acph 2–6 & 8 (Leaf)	4q	7p	4	Powling et al. (1981)
G-6pdh	–	–	2	Nielsen et al. (1982)
Gdh-1	–	2p	5	Benito et al. (1984)
Cat-1	–	–	4	Benito et al. (1984)
Est	3, 6	3, 6	1, 3	Hart et al. (1980)
β-Amy	4q	–	4	Powling et al. (1981)

[a] Dimeric isozymes; I = Imperial, K II = King II, D = Dakold
p = short arm, q = long arm

'Betzes' (B) and the CS-B disomic addition lines excluding 5H line.

The analyses were carried out with parts of individual kernels, specifically the embryo plus scutellum (S) and endosperm (E), and also with 12 day-old seedling leaves (L). The zymogram phenotype of the enzyme peroxidases (CPXS, CPXE, PERL), α-amylases (α-AMY), acid and alkaline phosphatases (PHS, PHE) and esterases (ESTS, ESTE, ESTL) was determined for each line examined in this study.

The information on both the cathodal peroxidases CPXS and CPXE and the phosphatases was obtained using polyacrylamide gel slabs (10%). The protocols described by Benito and Pérez de la Vega (1979) and Salinas and Benito (1984b), respectively, were followed.

The PERL isozymes were processed under the same conditions as those used for the phosphatases but they were electrophoresed in starch gels (12%) and stained following the method described by Salinas and Benito (1984a).

The α-amylase isozymes were obtained from five day-old germinating endosperm. The extracts were electrophoresed at 4 °C and 16 h at a constant voltage of 100 V in horizontal polyacrylamide gel slabs (10%) with trisglycine (0.066 M Tris, 0.19 M glycine, pH 8.7) as the electrode and gel buffer. They were stained by the method described by Scandalios (1969).

The esterases were seperated as described by Poulik (1957) using both polyacrylamide gel slabs (10%) and starch gels (12%). They were stained by the method of Kahler and Allard (1970).

In addition lines, all the enzyme systems studied showed the wheat isozymes and, sometimes, also the specific barley isozymes.

The nomenclature used for peroxidases and phosphatases has been previously described by Salinas and Benito (1984a).

Results (Fig. 1)

1 Peroxidases

CS-wheat showed three CPXS isozymes, designated CPX-W3, CPXS-W4 and CPXS-W5. B-barley also showed three isozymes in the S of dry kernels with the same migration as those described in CS and designated CPXS-H3, CPXS-H4 and CPXS-H5. All the addition lines showed the same pattern as that found in CS-wheat. Due to this reason, the B-barley isozymes have not been related to any particular chromosome.

In the E of CS-wheat four CPXE isozymes, CPXE-W2 to CPXE-W5, were observed. In B-barley two CPXE isozymes (with a slower cathodic migration than wheat CPXE), designated CPXE-H1 and CPXE-H2, were seen. The wheat-barley addition line with 1H chromosome (CS-B-1H) had the CPXE-H1 and CPXE-H2 of barley.

The CS leaves presented seven PERL isozymes, named from PERL-W1 to PERL-W7. In the B leaves seven PERL isozymes, designated PERL-H1 to PERL-

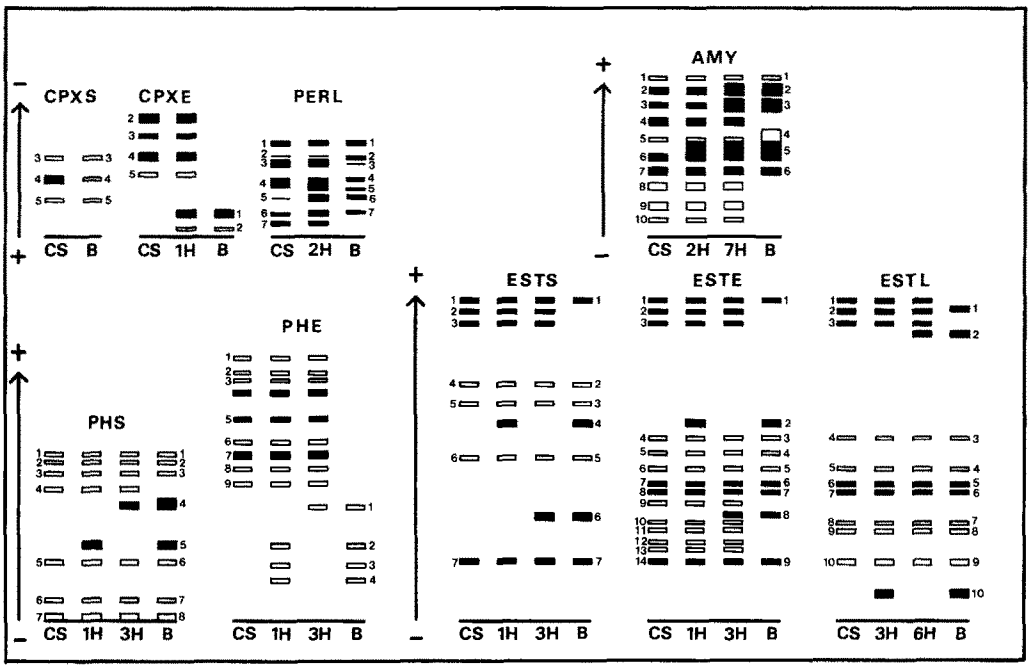

Fig. 1. Diagram of the different isozyme patterns of the 'Chinese Spring' (CS) hexaploid wheat, 'Betzes' (B) barley and the significant 'Chinese Spring-Betzes' addition lines. The addition lines not indicated in the figure showed the hexaploid wheat pattern (the addition line with 5H barley chromosome was not available). CPXS: embryo plus scutellum peroxidases, CPXE: endosperm peroxidases, PERL: leaf peroxidases, AMY: α-amylases, PHS: embryo plus scutellum acid phosphatases, PHE: endosperm acid and alkaline phosphatases, ESTS: embryo plus scutellum esterases, ESTE: endosperm esterases, ESTL: leaf esterases. CPXS, CPXE, PHS, PHE, ESTS and ESTE were electrophoresed using 10% polyacrylamide gel slabs, and PERL and ESTL using 12% starch gels. The numbers on the *left* indicate the hexaploid wheat isozymes and the numbers on the *right* indicate the barley isozymes

H7, were observed. The CS-B-2H addition line had the PERL-H5 and PERL-H6 isozymes of barley.

2 α-amylases

The endosperm of CS showed ten α-AMY isozymes, names from α-AMY-W1 to α-AMY-W10, and B-barley showed six α-AMY isozymes designated from α-AMY-H1 to α-AMY-H6. The CS-B-2H line showed the α-AMY-H5 B-barley isozyme. In the CS-B-7H line the α-AMY-H2, α-AMY-H3 as well as the α-AMY-H5 isozymes of barley were observed.

3 Acid and alkaline phosphatases

The endosperm of CS showed the same isozymatic pattern for acid and alkaline phosphatases (PHE), but in the S of the dry kernel activity of this last-mentioned isozyme was not observed. These results were also observed in B-barley.

The PHS (embryo plus scutellum phosphatases) of CS-wheat are seven isozymes, PHS-W1 to PHS-W7. In the S of B-barley eight PHS isozymes, PHS-H1 to PHS-H8, were observed. The CS-B-1H and CS-B-3H lines showed the PHS-H5 and PHS-H4 B-barley phosphatases, respectively.

The endosperm of CS showed nine PHE isozymes, PHE-W1 to PHE-W9. In the E of B-barley were seen four PHE, PHE-H1 to PHE-H4, all with a slower anodic migration than wheat PHE. The CS-B-1H line showed the PHE-H2, PHE-H3 and PHE-H4 isozymes and the CS-B-3H line the PHE-H1 isozyme of barley.

4 Esterases

The S of CS-wheat showed seven ESTS isozymes, ESTS-W1 to ESTS-W7 and the S of barley presented seven isozymes, ESTS-H1 to ESTS-H7. The CS-B-1H and CS-B-3H addition lines exhibited the ESTS-H4 and ESTS-H6 B-barley isozymes, respectively.

In the endosperm of CS 14 ESTE isozymes, ESTE-W1 to ESTE-W14, were observed and in B-barley nine ESTE isozymes ESTE-H1 to ESTE-H9, were seen. The CS-B-1H and CS-B-3H addition lines showed the ESTE-H2 and ESTE-H8 barley esterases, respectively.

The leaves of CS exhibited ten ESTL isozymes, designated ESTL-W1 to ESTL-W10, and the leaves of B-barley also showed ten ESTL, ESTL-H1 to ESTL-H10. The B esterases ESTL-H10 and ESTL-H2 were located in the CS-B-3H and CS-B-6H addition lines, respectively.

Discussion

1 The results obtained for peroxidases of B-barley indicate that the CPXE-H1 and CPXE-H2 are related with the 1H chromosome and the PERL-H5 and PERL-H6 have been associated with 2H chromosome.

The information for endosperm peroxidases of hexaploid wheat have been located in the 4BL, 7AS and 7DS chromosome arms (Kobrehel and Feillet 1975; Kobrehel 1978; Benito and Pérez de la Vega 1979) and these isozymes in rye are related with the 4RL and 7RS chromosome arms (Salinas and Benito 1984a). In hexaploid wheat a translocation between 4BL and 7BS chromosome arms has been proposed (Kobrehel and Feillet 1975) and in rye cytological and biochemical evidence of the translocation between 4RL and 7RS chromosome arms has also been obtained (Koller and Zeller 1976; Hart 1978; Salinas and Benito 1984a, b). The chromosomal location of *Cpxe-H1* and *Cpxe-H2* structural genes of barley on the 1H chromosome constitutes biochemical evidence of homoeology between the 1H barley chromosome, 4RL/7RS rye chromosome and the short arms of the chromosomes of homoeologous group 7 (4BL/7BS, 7AS, 7DS) of hexaploid wheat.

The PERL of hexaploid wheat have been related with the 6BS chromosome arm (MacDonald and Smith 1972), with the 1B and 1D chromosomes (May et al. 1973) and recently, in our laboratory, with the 2B and 2D chromosomes (Bosch et al., in preparation). These different results could be explained by the different buffer systems and staining methods used by these authors. In our case, we have always used the same method to locate the structural genes for leaf peroxidases in hexaploid wheat, rye and barley. In rye, the PERL have been associated with the 2RS chromosome arm (Salinas and Benito 1984a). The chromosomal location of the *Perl-H5* and *Perl-H6* structural genes in the 2H chromosome constitutes biochemical evidence of homoeology among the 2H and 2R and homoeologous group 2 (2B and 2D) of hexaploid wheat (aneuploid strains without the 2A chromosome are not available).

Finally, the CPXS of hexaploid wheat have been associated with chromosomes of homoeologous group 3 (Benito and Pérez de la Vega 1979) and, in rye, with the 6RL chromosome arms (Salinas and Benito 1984a).

Peroxidases are usually characterized by a monogenic control, monomeric behaviour and the presence of null alleles (Brown and Allard 1969; Marshall and Allard 1969; Clegg and Allard 1973; Hoess et al. 1974; Felder 1976; Yen and Sadanaga 1977; Rick et al. 1979, Benito et al. 1980; Garcia et al. 1982). The chromosomal location results obtained in barley for these isozymes also support their monomeric behaviour.

2 Information for the α-AMY-H2 and α-AMY-H3 of B-barley have been located on 7H chromosome, and the α-AMY-H5 has been related to both the 2H and 7H chromosomes.

The *T. aestivum* L. cv. 'Chinese Spring' the α-amylase isozymes are controlled by structural genes located on the long arms of homoeologous groups 6 and 7 (Nisikawa and Nobuhara 1971; Gale et al. 1983). Moreoever, results indicating that α-amylase activity during wheat grain germination can also be related to the chromosomes of homoeologous group 5 have been obtained (Gale et al. 1981). In rye, the α-amylase isozymes of group I (with a faster anodic migration than that of the isozymes of group II) have been related to the 5R chromosome (Salinas and Benito, in preparation).

Therefore, the chromosomal location of α-AMY-H2 and α-AMY-H3 (with a similar migration to the rye group I amylases) isozymes of the barley group I on the 7H chromosome could constitute biochemical evidence of homoeology among chromosomes 7H, 5R and homoeologous group 5 of hexaploid wheat.

The long arms of group 5 chromosomes of hexaploid wheat carry two sets of triplicate isozyme genes coding for lipoxigenase-2 and alcohol dehydrogenase-2 (Hart 1979) while the short arms of this group carry information for a set of triplicate genes coding for shikimate dehydrogenase (Koebner and Shepherd 1982). The chromosome 7H has been tentatively assigned to homoeologous group 5 based on gross plant morphology (Islam and Shepherd 1982) and, based on the separation by starch gel electrophoresis (Koebner and Shepherd 1982), A.D.H. Brown believes this chromosome to carry the shikimate dehydrogenase gene. The location of *α-Amy-H2* and *α-Amy-H3* genes in the 7H chromosome agree with the results obtained by these authors, and support the hypothesis that the 7H chromosome is homoeologous with the 5R and also with homoeologous group 5 of hexaploid wheat.

In maize, α-amylase isozymes behave as dimers (Scandalios 1969) while in barley and wheat they behave as monomers (Frydenberg and Nielsen 1965; Nisikawa and Nobuhara 1971). The results of this work support the monomeric behaviour of the barley α-amylases.

3 The PHS-H5 and PHS-H4 B-barley phosphatases have been associated with the 1H and 3H chromosomes respectively. Similar results have been found when the PHE were analyzed since the *Phe-H2, Phe-H3* and *Phe-H4* structural genes have been located on the 1H chromosome and the *Phe-H1* gene has been related with the 3H chromosome. The PHS-H4 and the PHE-H1 have the same migration in polyacrylamide gel and both are related to the 3H chromosome. Also, the PHS-H5 and the PHE-H2 presented the same migration and both have been associated with the 1H chromosome. Therefore, the information for phosphatase isozymes of S and E is related with the same barley chromosomes and, it is possible that the PHS-H4 and PHE-H1 could be codified by the same structural gene, similarly PHS-H5 and PHE-H2.

In CS wheat, the leaf acid and endosperm phosphatases have been related with the chromosomes of homoeologous group 4 (Hart and Langston 1977; Salinas et al. 1981). The leaf acid phosphatases of rye have been related with the 7RS chromosome arm (Tang and Hart 1975; Hart 1978) and dry kernel and leaf phosphatases with the 7RS and 7RL chromosome arms (Hart et al. 1980; Salinas and Benito 1984b).

Powling et al. (1981) have found that the information for the leaf acid phosphatases of barley is located on the 4H chromosome. The above cited authors and Benito et al. (1984), the latter studying several isozymic systems, have pointed out that 4H and 1H chromosomes shown homoeology with the homoeologous groups 4 and 7, respectively. As we have mentioned already, we have related the information for PHS and PHE with the 1H and 3H barley chromosomes. This discrepancy in the chromosomal location of phosphatases could be explained by means of a reciprocal translocation between the 1H and 4H chromosomes. Koller and Zeller (1976) have proposed that the chromosomes of *Secale cereale* are products of two interchanges between the ancestral chromosome arms 4RL and 7RS and between 6RL and 7RL. Additional evidence of the 4RL/7RS translocation previously cited has been found by Hart (1978) studying phosphatase isozymes of wheat and rye leaves, and by Salinas and Benito (1984a, b) studying endosperm phosphatases of rye. Therefore, the translocation between the 4^{th} and 7^{th} chromosomes (4H and 1H of barley, respectively) seems to occur frequently in the Triticeae (4BL/7BS in hexaploid wheat, 4RL/7RS in rye) and it is possible that this rearrangement would have occurred in the ancestral Triticeae genome. Moreover, the chromosomal location of phosphatases PHS-H5, PHE-H2, PHE-H3 and PHE-H4 in the 1H chromosome and the PHS-H4 and PHE-H1 in the 1H chromosome could be explained by a translocation between the 1H and 3H chromosomes. Phosphatases, commonly classified as acids, have been described as dimeric enzymes in different higher plants (Efron 1973, in *Zea mays;* Grover and Byrne 1975 in *Arabidopsis thaliana;* Endo 1981 in *Oryza sativa*). However, Marshall and Allard (1969) and Powling et al. (1981) found monomeric phosphatases in *Avena barbata* and *Hordeum vulgare* respectively. Further, Brown et al. (1978) in *H. spontaneum* and Brown and Allard in *Zea mays* have described monomeric and dimeric phosphatases at the same time. In *Secale cereale* the phosphatases have been described as monomers and dimers at the same time (Pérez de la Vega and Allard 1984). The results obtained in this work support the hypothesis that the barley PHS and PHE are monomers (new isozymes with a intermediate migration between wheat and barley parental phosphatases were not observed).

4 The ESTS and ESTE of dry kernels of B-barley are related to chromosomes 1H (ESTS-H4, ESTE-H2 isozymes) and 3H (ESTS-H6 and ESTE-H8 isozymes). The information for ESTL has been located in chromosomes 3H (ESTL-H10 isozyme) and 6H (ESTL-H2 isozyme). In addition the ESTS-H4 and ESTE-H2 have the same migration, so it is possible that these two isozymes could be codified by the same structural gene. A similar result has been observed for the ESTS-H6 and ESTE-H8.

Hart et al. (1980) have found that the information for leaf esterases of barley is related with the 1H and 3H chromosomes. In hexaploid wheat, the esterases have been related with the chromosomes of the homoeologous groups 3 and 6 (Barber et al. 1968; Bergman 1972; May et al. 1973; Nakai 1976). Also, the rye esterases have been associated with the 3R and 6R chromosomes (Bergman and Maan 1973).

The genetics of wheat and barley esterases is complex and the specific genetic basis of most wheat esterases is as yet unknown. Ten barley esterase loci have been identified, three of which compose a tight linkage group in chromosome 3 (Kahler and Allard 1970; Nielsen and Frydenberg 1971; Hvid and Nielsen 1977) and the other seven genes have not been reported. So, the establishment of homoeologies among wheat, rye and barley esterases is difficult to carry out.

Comparing the results obtained using wheat-rye and wheat-barley addition lines, the leaf esterases of rye with the fastest migration have been related with the 3R chromosome while those of barley with the 6H chromosome. On the other hand, the rye leaf esterases of intermediate migration have been associated with the 6R chromosome, while the barley leaf esterases with the 3H chromosome. In both cases the chromosomes involved in the production of esterases are the same (6 and 3), but the migration of esterases is inverted.

Table 2. Homoelogous relationships among the hexaploid wheat, rye and barley chromosomes based on isozyme markers

Genes	*T. aestivum* L. cv 'Chinese Spring' Genomes A, B and D	*S. cereale* L. cv I, K II, D Genome R	*H. vulgare* L. cv 'Betzes' Genome H	
Ep-1	7q	–	1	
Cpxe[a]	7p	7p/4q	1	
Perl[a]	2	2	2	
Got-3	3q	3	3	
Est[a]	3, 6	3, 6	3, 6, 1 translocation 1/3	
Adh-1	4p	4	4	
Pgm-1	4p	4p	4	
Acph 2–6 & 8	4q	7p	4	translocation 1/4
Phe[a]	4q	7p/4q	1, 3	translocation 1/4
β-Amy	4q	–	4	
Pgi-1	1p	1p	5	
Mdh-2	1q	1q, 3	5, 3	
Got-2	6q	6q	6	
Amp-1	6p	–	6	
α-Amy[a]	5	5	7	
Skdh	5p	5p	7?	

[a] Isozymes studied in this work. I = Imperial, K II = King II, D = Dakold
p = short arm, q = long arm

Summarizing, the information for barley esterases has been related to the 1H, 3H and 6H chromosomes, with the 3R and 6R chromosomes in rye, and with the chromosomes of homoeologous groups 3 and 6 in hexaploid wheat. Therefore, we believe that these results support the homoeology between the 3H and 6H barley chromosomes, 3R and 6R rye chromosomes and the chromosomes of homoeologous groups 3 and 6 of hexaploid wheat. The chromosomal location of the ESTE-H2 and ESTS-H4 in the 1H chromosome could be explained by a translocation between 1H and 3H chromosomes. This translocation could also explain the chromosomal location results obtained for phosphatases.

On the basis of the results obtained in this work it is possible to propose the homoeology of the 1H, 2H, 3H, 6H and 7H barley chromosomes with the 7R, 2R, 3R, 6R and 5R rye chromosomes and the chromosomes of the homoeologous groups 7, 2, 3, 6 and 5, respectively (Table 2). Therefore, taking in account the previous data about chromosomal location in hexaploid wheat, rye and barley, and the results obtained here (Table 2), it can be observed that the gene synteny relationships that occurred in the ancestral Triticeae genome are largely conserved in the actual Triticeae genomes (Hart 1979; Hart et al. 1980; Hart and Tuleen 1983; Powling et al. 1981; Benito et al. 1984; Salinas and Benito 1985).

Finally, the 1H, 2H, 3H, 4H, 5H, 6H and 7H barley chromosomes seem to carry information similar to that carried by the 7R, 2R, 3R, 4R, 1R, 6R and 5R rye chromosomes, and also to that carried by the chromosomes of homoeologous groups 7, 2, 3, 4, 1, 6 and 5 of hexaploid wheat, respectively (Table 2).

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