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Use of isozymes as chromosome markers in wheat–rye addition lines and in triticale*

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SUMMARY

The alcohol dehydrogenase (ADH), glutamate oxaloacetate transaminase (GOT), acid phosphatase (ACPH), endopeptidase (EP) and aminopeptidase (AMP) zymogram phenotypes of Chinese Spring wheat, Imperial rye, the Chinese Spring–Imperial triticale and the series of seven disomic Imperial chromosome additions to Chinese Spring were determined. It was found that the zymogram phenotypes produced for one or more of the enzymes by each of the Imperial chromosomes 3, 6, C and D differ sufficiently from that of Chinese Spring so as to provide evidence for the presence or absence of each of these chromosomes in addition lines and triticales. The structural genes *Got-R2* and *Got-R3* were located in Imperial chromosomes 6 and 3 respectively and other genes involved in the production of GOT in chromosomes C and D. By analysis of GOT alone, evidence for the presence or absence of Imperial chromosomes 3, 6, C and D in addition lines and triticales can be obtained. *Adh-R1* was located in chromosome C and a gene(s) involved in the production of an ACPH was located in chromosome D.

The linkages obtained for *Got-R2*, *Got-R3* and *Adh-R1* demonstrate homoeology between the Imperial chromosomes 3, 6 and C and the Chinese Spring chromosomes of groups 3, 6 and 4 respectively. The discovery that *Adh-R1* is located in Imperial chromosome C also suggests that the 4R/7R and 7R/4R homoeologous groupings proposed elsewhere for the chromosomes of the rye cultivars Imperial, Dakold, and King II should be reassessed, since they are inconsistent with the known linkages of *Adh-R1* in the three cultivars. The finding in King II of two forms of ADH and of two ADH genes has been reported. The results of our study of Imperial, King II, and Dakold indicate that rye possesses but one ADH and only one ADH structural gene.

1. INTRODUCTION

Techniques which will facilitate the rapid identification of plant chromosomes and parts thereof are needed. The chromosomes of aneuploid derivatives of higher

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plant species have conventionally been identified largely on the basis of variation in plant morphology caused by specific chromosomes and by chromosome morphology. More recently, the ability to identify individual plant chromosomes has been considerably improved by the development of techniques which differentially stain heterochromatic regions at metaphase. In studies of wheat and rye chromosomes, Gill & Kimber (1974*a*, *b*), using a Giemsa C-banding technique, have shown that rye chromosomes can be distinguished from one another and from wheat chromosomes in wheat–rye addition lines, while Darvey & Gustafson (1975), using Leishman staining, identified the rye chromosomes of four addition series and also of six triticale lines.

Clearly the heterochromatic banding techniques greatly improve the ability to identify plant chromosome. However, at least at this time, they do not provide sufficient resolution to identify parts of rye chromosomes or to allow homoeologous relationships between rye and wheat chromosomes or chromosome arms to be established. Furthermore, the techniques are time consuming and, at present, only a few plants can be identified per day (Darvey & Gustafson, 1975).

Genes which determine variation in isozymes may be of considerable value as markers in aneuploid plants, including wheat–rye addition and substitution lines, and triticales. A number of structural genes for isozymes have been linked to specific chromosomes of hexaploid wheat (Barber *et al.* 1968; Hart, 1970, 1973, 1975; Hart & Langston, 1975; Nishikawa & Nobuhara, 1971), and in addition, genetic variation between hexaploid wheat and rye for genes involved in the production of esterases (Barber *et al.* 1968; Bergman & Maan, 1973) and alcohol dehydrogenases (Irani & Bhatia, 1972) has been reported.

By zymogram analysis of wheat–rye addition and substitution lines and of the parental wheat and rye varieties and their amphiploid product, genetic variation between wheat and rye may be detected and genes involved in the production of isozymes localized to specific rye chromosomes. This paper reports the results of a study designed (1) to determine whether there is genetic variability between the hexaploid wheat cultivar Chinese Spring and the rye cultivar Imperial for the zymogram phenotypes of the enzymes alcohol dehydrogenase, acid phosphatase, glutamate oxaloacetate transaminase, aminopeptidase and endopeptidase and (2) to assess the feasibility of using isozymes as an aid in determining the presence or absence and in the identification of rye chromosomes in wheat–rye addition and substitution lines and in triticales.

2. MATERIALS AND METHODS

The series of seven disomic *Secale cereale* L. cultivar Imperial chromosome additions to the *Triticum aestivum* L. cultivar Chinese Spring developed by E. R. Sears of the University of Missouri, the Chinese Spring and Imperial cultivars, and the Chinese Spring–Imperial triticale were examined.

The zymogram phenotype of the enzymes alcohol dehydrogenase (ADH), acid phosphatase (ACPH), aminopeptidase (AMP), endopeptidase (EP), and glutamate oxaloacetate transaminase (GOT) was determined in one or more tissues at one or

more developmental stages in each of the ten lines. Extracts of mature grains were used for the ADH analyses. The ACPH and GOT analyses utilized extracts of the blade of the first foliage leaf of 7-day-old etiolated seedlings grown in moist paper towelling at 23 °C. For the AMP and EP analyses, extracts were obtained from the leaves of 7- to 21-day-old green seedlings, grown first in moist paper towelling at 23 °C for 3 days and thereafter in peat pots in a growth chamber at 23 °C with a 12 h day. Extracts were obtained by maceration of tissue in sand in a mortar with pestle in a pH 7·5 buffer containing 0·1 M Tham, 0·1 M-KCl, 0·005 M EDTA, and 0·4 M 2-mercaptoethanol (Carlson, 1972) and also containing 0·1 M sucrose. A weight:volume ratio of tissue:buffer of 1:5 was used for mature kernels and of 1:2 for leaves.

The slurry obtained by maceration was centrifuged at 10000 ***g*** in an SS-34 rotor in a Sorvall RC2-B centrifuge. The supernatant obtained was used directly for electrophoresis. Extraction and centrifugation were carried out at 2–5 °C. The zymogram phenotype of each enzyme was determined for a minimum of three plants of each line.

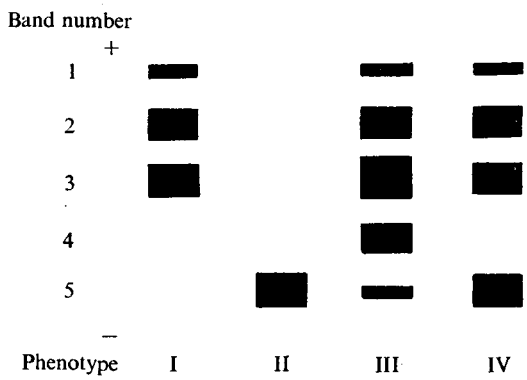
Extracts were electrophoresed for the determination of the ADH, ACPH, AMP and EP zymogram phenotypes in vertical starch gels (Electrostarch, Lot no. 371, 11%, w/v), using methods previously described (Hart, 1973). Gels were stained for ADH activity as described by Hart (1970). Prior to staining for AMP, EP and ACPH, gels were soaked at 2–5 °C in several changes of stain buffer. AMP was stained at 37 °C and ACPH at room temperature as described previously (Hart, 1973). EP was stained at 37 °C using 50 ml of Tris-maleate-NaOH buffer, pH 5·8, containing 20 mg of Black K and 20 mg of *α-N*-benzoyl-D*α*-arginine-*α*-napthylamide-HCl per slice. The dye and substrate were dissolved in 3 ml dimethylsulphoxide prior to addition of the buffer. The GOT zymogram phenotypes were determined using acrylamide gel disk electrophoresis and staining procedures previously described (Hart, 1975). The AMP and EP zymogram phenotype of each line was also determined by this method of electrophoresis, in addition to the determination by starch gel electrophoresis as described above. The GOT staining solution degrades rapidly. It was prepared immediately prior to use by addition of the buffer to the other reagents, followed by rapid mixing and filtering. Staining was visible within 2 min and was usually of desired intensity within 5 min after immersion of gels in the solution. Acrylamide gels were stained for AMP and EP as described above, following incubation of each gel for 3 h at room temperature in 50 ml of the pH 5·8 stain buffer.

The number of chromosomes in putative addition lines was confirmed by counts made in root tips of 3-day-old seedlings. The root tips were pretreated in saturated monobromonapthalene for 4 h, fixed in 3:1 ethanol:acetic acid overnight, hydrolysed in 1 N-HCl at 60 °C for 6 min, and stained in Feulgen for one or more hours. In order to determine the chromosome number of kernels used for ADH analyses, a portion of the endosperm was removed and used for electrophoresis, with the remainder of the kernel germinated and used for the root-tip analysis.

3. RESULTS

(i) *Alcohol dehydrogenase*

Chinese Spring wheat, Imperial rye, the Chinese Spring–Imperial amphiploid, and the seven addition lines produce a total of three distinct zymogram phenotypes which differ in terms of the relative staining intensities and/or the presence or absence of bands.



Text-fig. 1. Diagrams of the ADH zymogram phenotypes observed. (I) Chinese Spring and the addition lines possessing chromosomes 1R, 2R, 3R, 5R, 6R and DR, (II) Imperial, (III) the Chinese Spring–Imperial amphiploid and the addition line possessing chromosome CR, and (IV) a mixture of Chinese Spring and Imperial tissue extracts.

Chinese Spring expresses three ADH isozymes (I, Text-fig. 1), the fast, intermediate and slow forms having been designated ADH-1, ADH-2 and ADH-3 respectively (Hart, 1970). Imperial expresses only one ADH isozyme, of slower electrophoretic mobility than the Chinese Spring isozymes (II, Text-fig. 1). ADH-3 of Chinese Spring is intermediate in mobility between the Imperial enzyme and ADH-1 of Chinese Spring. The zymogram phenotype produced by a mixture composed of equal quantities of wheat and rye tissue extracts consists of four bands – bands 1, 2 and 3 (the sites of wheat ADH-1, ADH-2 and ADH-3 respectively) and 5 (the site of the Imperial enzyme) (IV, Text-fig. 1).

The zymogram phenotype of the Chinese Spring–Imperial amphiploid consists of five bands (III, Text-fig. 1). The three more anodal bands are electrophoretically homologous with the three bands produced by Chinese Spring. The cathodal band is homologous to the band produced by Imperial. A band not produced by either parent, band 4, of mobility intermediate to bands 3 and 5, is produced by the amphiploid. Band 3 stains with the greatest intensity while bands 2 and 4 are of equal but lesser intensity and bands 1 and 5 are of equal and least intensity.

Six of the seven disomic addition lines produce a zymogram phenotype indistinguishable from that of Chinese Spring. Each displays three bands, the electrophoretic mobilities and relative staining intensities of which cannot be distinguished from that of Chinese Spring. However, the addition line which pos-

sesses two doses of chromosome C of Imperial shows an ADH zymogram phenotype indistinguishable from that of the amphiploid (III, Text-fig. 1).

The ADH structural genes of Chinese Spring are located in chromosomes 4A, 4B and 4D (Hart, 1970, 1973). Designated *Adh-A1*, *Adh-B1* and *Adh-D1*, these structural genes code subunits which have been designated α, β and δ respectively. It has been proposed that random association of these subunits results in the production of six types of dimers, which are expressed as the isozymes designated ADH-1 ($\alpha\alpha$ dimers), ADH-2 ($\alpha\beta$, $\alpha\delta$) and ADH-3 ($\beta\beta$, $\delta\delta$ and $\beta\delta$).

The data presented herein demonstrate that there is genetic variability between Chinese Spring and Imperial for a gene (or genes) involved in the production of ADH. The ADH of Imperial possesses an electrophoretic mobility which differs from that of the Chinese Spring isozymes. The amphiploid expresses each of the isozymes of the parental types and an additional form, as does the addition line which possesses chromosome C of Imperial, while the other six addition lines express only the isozymes of Chinese Spring. It may thus be inferred that chromosome C of Imperial possesses a gene (or genes) involved in the production of ADH which confers upon the active rye enzyme a mobility which differs from that of the wheat enzymes. The simplest hypothesis which is in full agreement with the observed phenotypes is that chromosome C of Imperial possesses a structural gene which codes an ADH subunit, that in the amphiploid and in the C chromosome addition line the active dimeric enzymes are produced by the random association of the four types of subunits, and that the Imperial ADH structural gene produces a quantity of ADH subunit approximately equal to that of each individual Chinese Spring ADH structural gene. The ADH structural gene of rye is designated *Adh-R1* and the subunit which it codes as ρ.

This hypothesis predicts that the amphiploid and the addition line which carries CR in the disomic condition each possess four ADH structural genes, namely *Adh-A1*, *Adh-B1*, *Adh-D1* and *Adh-R1*, which code four subunits (each in approximately equal quantity), α, β, δ and ρ respectively, which randomly associate to produce the dimers $\alpha\alpha$, $\beta\beta$, $\delta\delta$, $\rho\rho$, $\alpha\beta$, $\alpha\delta$, $\alpha\rho$, $\beta\delta$, $\beta\rho$ and $\delta\rho$.

A schematic model for the subunit composition of the ADH isozymes of Chinese Spring, Imperial, the amphiploid and the CR addition line based on this hypothesis is shown in Table 1. The five isozymes of the triticale and of the CR addition line are designated ADH-1, 2, 3, 4 and 5. Three of these, ADH-1, 2 and 5, are identical in subunit composition to the electrophoretically homologous isozymes of wheat and rye. The molecules of ADH-4 are each composed of one wheat and of one rye subunit ($\beta\rho$ and $\delta\rho$ dimers) and consequently are not found in either parental type. ADH-3 of the triticale and the CR addition line consists of three forms found in Chinese Spring ($\beta\beta$, $\delta\delta$ and $\beta\delta$ dimers) and of one additional form, namely $\alpha\rho$ dimers, also not found in either parental type.

Under the given hypothesis, the expected distribution of the possible dimeric molecules of the Chinese Spring–Imperial amphiploid and the CR addition line will be based on $(p+q+r+s)^2$, where p, q, r and s represent the frequencies of the α, β, δ and ρ subunits, respectively. In these two lines, $p = q = r = s = 1/4$ and

the expected proportions of the dimers are $1/16\alpha\alpha$:$1/16\beta\beta$:$1/16\delta\delta$:$1/16\rho\rho$:$2/16\alpha\beta$:$2/16\alpha\delta$:$2/16\beta\delta$:$2/16\alpha\rho$:$2/16\beta\rho$:$2/16\delta\rho$. Combining the proportions for those isozymes which are of coincident electrophoretic mobility, the expected distribution of the isozymes assumed to be responsible for the production of bands 1, 2, 3, 4 and 5 of the triticale and the disomic CR addition line is 1:4:6:4:1. This proportion is consistent with the observed staining intensities of the bands of these two lines (Text-fig. 1).

Table 1. *Schematic model for the subunit composition of the ADH isozymes produced by Chinese Spring, Imperial, Chinese Spring–Imperial amphiploid and the chromosome CR addition line.*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

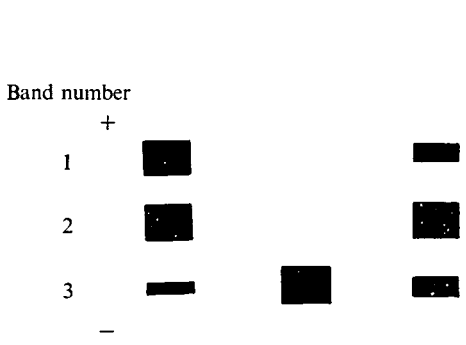
Chinese Spring			Imperial		Triticale and CR addition line		
Isozymes		Subunit composition	Isozymes	Subunit composition	Isozymes		Subunit composition
ADH-1	1/9	$\alpha\alpha$	—	—	ADH-1	1/16	$\alpha\alpha$
ADH-2	4/9	$\alpha\beta$, $\alpha\delta$	—	—	ADH-2	4/16	$\alpha\beta$, $\alpha\delta$
ADH-3	4/9	$\beta\beta$, $\delta\delta$, $\beta\delta$	—	—	ADH-3	6/16	$\beta\beta$, $\delta\delta$, $\beta\delta$, $\alpha\rho$
—		—	—	—	ADH-4	4/16	$\beta\rho$, $\delta\rho$
—		—	ADH-1	$\rho\rho$	ADH-5	1/16	$\rho\rho$

Irani and Bhatia (1972) compared and analysed the ADH zymogram phenotypes of Holdfast wheat, King II rye, the Holdfast–King II triticale, and the disomic additions to Holdfast of King II chromosomes IV (tentatively designated 4R/7R by Darvey, 1973), V (1R), VI (3R), and VII (7R/4R). They also examined the ADH zymogram phenotypes of Chinese Spring and two other accessions of rye and triticale. Their results and interpretation are similar to that included herein with two exceptions. They observed two bands on ADH zymograms of King II and of two other unidentified accessions of rye. One band was reported to be electrophoretically homologous to ADH-3 of Chinese Spring and the second to be of slower mobility. The latter corresponds to the rye ADH reported herein. They concluded that rye produces two ADH isozymes and proposed that rye possesses two ADH genes. We have examined Imperial and the varieties King II and Dakold. We observed in each only one ADH (ADH-1, as defined above). We have found no evidence for an ADH in rye with mobility homologous to that of ADH-3 of wheat. We therefore have found no evidence that rye possesses an ADH structural gene other than *Adh-R1*.

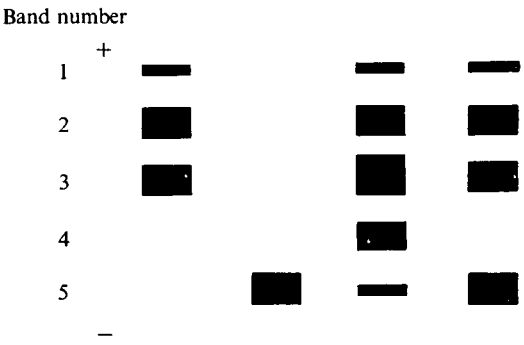
Irani and Bhatia also observed that *Adh-R1* (= *Adh-R2* of Irani and Bhatia) of King II is linked to chromosome IV (= 4R/7R), while we have found that *Adh-R1* is carried in chromosome C of Imperial (= 7R/4R). This finding will be discussed further below.

(ii) *Glutamate oxaloacetate transaminase*

The GOT isozymes contained in the blade of the first foliage leaf of 7-day-old etiolated seedlings of Chinese Spring, Imperial, the Chinese Spring–Imperial amphiploid, and the addition lines are resolved by the electrophoretic procedures described herein into three major zones (Fig. 4, Plate 1). The isozymes of zones 2 and 3 of Chinese Spring have been shown to be the products of two independent sets of triplicate homoeologous structural genes, while the available evidence suggests that the isozymes of zone 1 are the products of three independent genetic systems, each of which may consist of triplicate homoeologous structural genes (Hart, 1975).



Text-fig. 2



Text-fig. 3

Text-fig. 2. Diagram of the zone 3 GOT zymogram phenotypes observed. (I) Chinese Spring and the chromosome 1R, 2R, 5R, 6R, CR and DR addition lines, (II) Imperial, and (III) the Chinese Spring–Imperial amphiploid and the chromosome 3R addition line.

Text-fig. 3. Diagram of the zone 2 GOT zymogram phenotypes observed. (I) Chinese Spring and the chromosome 1R, 2R, 3R, 5R, CR and DR addition lines, (II) Imperial, (III) the Chinese Spring–Imperial amphiploid and the chromosome 6R addition line, and (IV) a mixture of Chinese Spring and Imperial tissue extracts.

The zone 3 GOT zymogram phenotypes of Chinese Spring, Imperial, the Chinese Spring–Imperial amphiploid and the seven addition lines are shown in Text-fig. 2 and Fig. 5, Plate 1. Three distinct phenotypes, differing in terms of the relative staining intensities and/or the presence or absence of bands, are produced by these types. Chinese Spring expresses three zone 3 GOT isozymes (I, Text-fig. 2 and Fig. 5, Plate 1), the fast, intermediate, and slow forms having been designated GOT-3a, GOT-3b and GOT-3c respectively (Hart, 1975). Imperial expresses one isozyme in zone 3, the electrophoretic mobility of which is coincident with that of GOT-3c of Chinese Spring (II, Fig. 5, Plate 1). The Chinese Spring–Imperial amphiploid produces a zymogram phenotype which consists of three bands (III, Text-fig. 2 and Fig. 5, Plate 1) that are electrophoretically homologous to the three Chinese Spring isozymes. The staining intensity of the band of intermediate mobility is about twice that of the other two bands. Six of the seven disomic addition lines produce a zone 3 zymogram phenotype indistinguishable

from that of Chinese Spring. However, the addition line which possesses two doses of chromosome 3 of Imperial shows a zone 3 phenotype indistinguishable from that of the amphiploid.

The triplicate structural genes which produce the isozymes of zone 3 of Chinese Spring are located in chromosomes 3A, 3B and 3D (Hart, 1975). Designated *Got-A3*, *Got-B3* and *Got-D3* respectively, these structural genes code subunits which have been designated α^3, β^3 and δ^3 respectively. It has been proposed that random association of these subunits results in the production of six types of dimers, which are expressed as the isozymes designated GOT-3a ($\beta^3\beta^3$, $\delta^3\delta^3$ and $\beta^3\delta^3$ dimers), GOT-3b ($\alpha^3\beta^3$ and $\alpha^3\delta^3$) and GOT-3c ($\alpha^3\alpha^3$).

Imperial expresses one form of GOT in zone 3, the mobility of which is coincident to that of GOT-3c. Since the addition line which possesses two doses of Imperial chromosome 3 produces a zone 3 phenotype indistinguishable from that of the amphiploid, it is clear that chromosome 3 of Imperial possesses a gene (or genes) involved in the product of the zone 3 Imperial GOT. The simplest hypothesis which is in full agreement with the observed zone 3 phenotypes is that chromosome 3 of Imperial possesses a structural gene which codes a GOT subunit, that in the amphiploid and in the chromosome 3 addition line the active dimeric enzymes are produced by the random association of the four types of subunits, and that the Imperial GOT structural gene produces a quantity of GOT subunit approximately equal to that of each individual Chinese Spring GOT-3 structural gene. This Imperial GOT structural gene is designated *Got-R3* and the subunit which it codes as ρ^3.

This hypothesis predicts that the amphiploid and the addition line which carries 3R in disomic condition each possess four GOT-3 structural genes, namely *Got-A3*, *Got-B3*, *Got-D3* and *Got-R3*, which code four subunits (each in approximately equal quantity), α^3, β^3, δ^3 and ρ^3 respectively, which randomly associate to produce the dimers $\alpha^3\alpha^3$, $\beta^3\beta^3$, $\delta^3\delta^3$, $\rho^3\rho^3$, $\alpha^3\beta^3$, $\alpha^3\delta^3$, $\alpha^3\rho^3$, $\beta^3\delta^3$, $\beta^3\rho^3$ and $\delta^3\rho^3$.

A schematic model for the subunit composition of the zone 3 GOT isozymes of Chinese Spring, Imperial, the amphiploid and the 3R addition line is shown in Table 2. The three isozymes of the triticale and of the 3R addition line are designated GOT-3a, GOT-3b and GOT-3c. GOT-3a is identical in subunit composition to the electrophoretically homologous isozyme of Chinese Spring. GOT-3b is composed of two types of wheat heterodimers, $\alpha^3\beta^3$ and $\alpha^3\delta^3$, and of two types of dimers which each contain one wheat and one rye subunit, $\beta^3\rho^3$ and $\delta^3\rho^3$. GOT-3c consists of one form found in Chinese Spring, the $\alpha^3\alpha^3$ dimers, of one form found in rye, the $\rho^3\rho^3$ dimers, and of one wheat–rye heterodimeric form, the $\alpha^3\rho^3$ dimers.

As with ADH, the expected distribution of the possible dimeric molecules of the Chinese Spring–Imperial amphiploid and of the 3R addition line will be based on $(p+q+r+s)^2$, where p, q, r and s represent the frequencies of the α^3, β^3, δ^3 and ρ^3 subunits respectively. In these two lines $p = q = r = s = 1/4$. When the tetranomial is expanded, and the proportions for those dimers which are of coincident electrophoretic mobility are combined, the expected distribution of the isozymes assumed to be responsible for the production of bands 1, 2 and 3 of the triticale

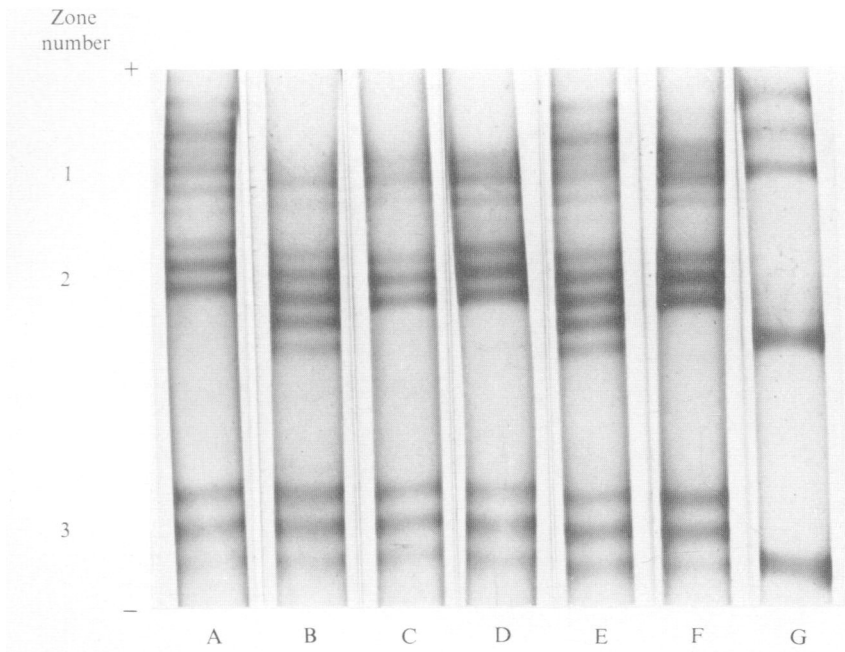


Fig. 4. GOT zymogram phenotypes observed. (A) Chromosome CR addition line, (B) Chromosome 6R addition line, (C) Chinese Spring and chromosome 1R, 2R and 5R addition lines, (D) Chromosome 3R addition line, (E) Chinese Spring–Imperial amphiploid, (F) Chromosome DR addition line, and (G) Imperial.

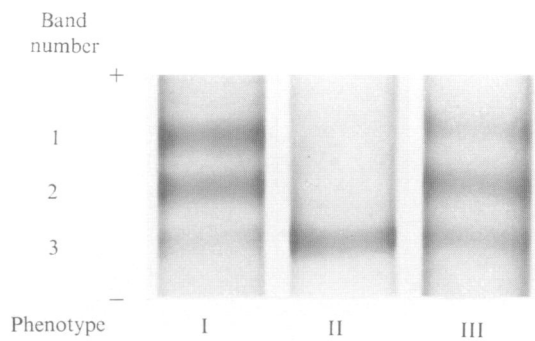


Fig. 5. Zone 3 GOT zymogram phenotypes observed. (I) Chinese Spring and chromosome 1R, 2R, 5R, 6R, CR and DR addition lines, (II) Imperial, and (III) the Chinese Spring–Imperial amphiploid and the chromosome 3R addition line.

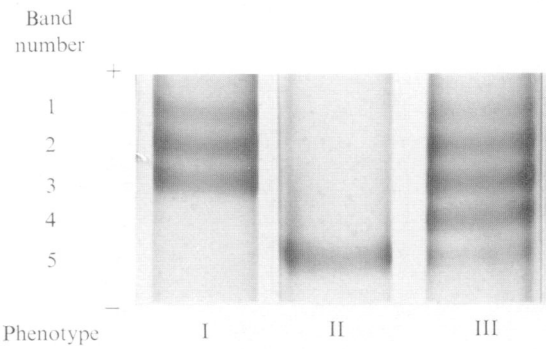


Fig. 6. Zone 2 GOT zymogram phenotypes observed. (I) Chinese Spring and the chromosome 1R, 2R, 3R, 5R, CR and DR addition lines, (II) Imperial, and (III) the Chinese Spring–Imperial amphiploid and the chromosome 6R addition line.

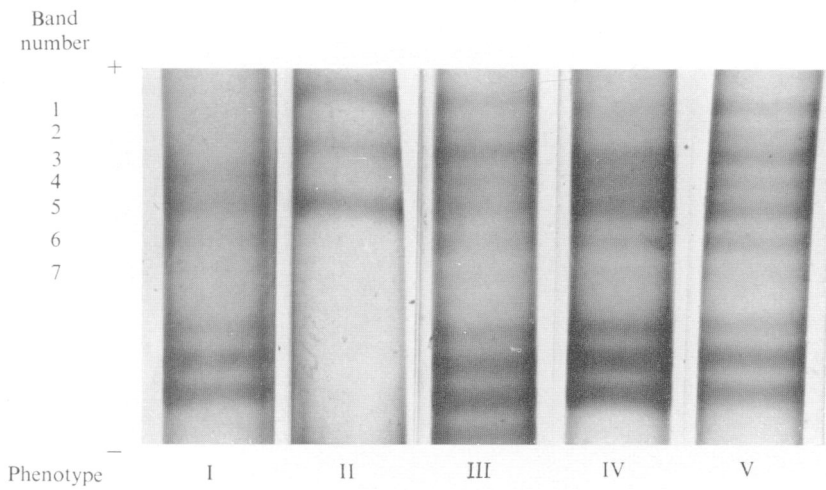


Fig. 7. Zone 1 GOT zymogram phenotypes observed. (Zone 2 is visible in the lower portion of four of the gels.) (I) Chinese Spring and the chromosome 1R, 2R, 3R, 5R and 6R addition lines, (II) Imperial, (III) Chinese Spring–Imperial amphiploid, (IV) the chromosome DR addition line, and (V) the chromosome CR addition line.

and the disomic 3R addition line is 1:2:1. This proportion is in good agreement with the observed staining intensities of these three bands (III, Text-fig. 2 and Fig. 5, Plate 1).

The evidence for the formation of wheat–rye ADH heterodimers consists of (1) the finding of a band, no. 4, in the triticale and in the CR addition line that is not produced by either Chinese Spring or Imperial and (2) the agreement between

Table 2. *Schematic model for the subunit composition of the zone* 3 *GOT isozymes produced by Chinese Spring, Imperial, Chinese Spring–Imperial amphiploid and the chromosome* 3*R addition line.*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Chinese Spring			Imperial		Triticale and 3R addition line		
Isozymes	Subunit composition		Isozymes	Subunit composition	Isozymes	Subunit composition	
GOT-3a	4/9	$\beta^3\beta^3$, $\delta^3\delta^3$, $\beta^3\delta^3$	—	—	GOT-3a 1/4	$\beta^3\beta^3$, $\delta^3\delta^3$, $\beta^3\delta^3$	
GOT-3b	4/9	$\alpha^3\beta^3$, $\alpha^3\delta^3$	—	—	GOT-3b 2/4	$\alpha^3\beta^3$, $\alpha^3\delta^3$, $\rho^3\beta^3$,	$\rho^3\delta^3$
GOT-3c	1/9	$\alpha^3\alpha^3$	GOT-3	$\rho^3\rho^3$	GOT-3c 1/4	$\alpha^3\alpha^3$, $\rho^3\rho^3$, $\alpha^3\rho^3$	

the expected and observed staining intensities of the bands of the triticale and the CR addition line. Since the Imperial GOT-3 has coincident mobility with GOT-3c of Chinese Spring, this first type of evidence for the formation of wheat–rye zone 3 GOT heterodimers is not available. However, since in the absence of the formation of wheat–rye zone 3 GOT heterodimers the expected distribution of staining intensities of the 3 bands of the triticale and the disomic 3R addition line is 1:1:1 (assuming an equal quantity of each of the four types of subunits are contained in active enzymes), the observed relative staining intensities of the three bands provide good evidence for the hypothesis described above of random association between each of the types of subunits.

The zone 2 GOT zymogram phenotypes of Chinese Spring, Imperial, the triticale and the seven addition lines are shown in Text-fig. 3 and Fig. 6, Plate 2. Three distinct phenotypes, differing in terms of the presence or absence and/or the relative staining intensities of bands, are produced by these types.

Chinese Spring expresses three zone 2 GOT isozymes (I, Text-fig. 3 and Fig. 6, Plate 2), the fast, intermediate, and slow forms having been designated GOT-2a, GOT-2b and GOT-2c respectively (Hart, 1975). Imperial expresses one zone 2 GOT isozyme, of slower electrophoretic mobility than the Chinese Spring isozymes (II, Text-fig. 3 and Fig. 6, Plate 2). GOT-2c of Chinese Spring is intermediate in mobility between the Imperial isozyme and GOT-2a of Chinese Spring. The zymogram phenotype of the triticale consists of five bands (III, Text-fig. 3 and Fig. 6, Plate 2). The three more anodal bands are electrophoretically homologous with the three bands produced by Chinese Spring. The cathodal band is homologous with the band produced by Imperial. A band not produced by either parent, band 4, is produced by the amphiploid. Band 3 stains with the greatest intensity while bands 2 and 4 are of equal but lesser intensity and bands 1 and 5 are of equal and least intensity.

Six of the seven disomic addition lines produce a zymogram phenotype indistinguishable from that of Chinese Spring. The seventh line, which is disomic for chromosome 6R, shows a zone 2 GOT zymogram phenotype indistinguishable from that of the triticale.

The GOT-2 structural genes of Chinese Spring are located in chromosomes 6A, 6B and 6D (Hart, 1975). Designated *Got-A2*, *Got-B2* and *Got-D2* respectively, the genes code subunits designated α^2, β^2 and δ^2 respectively. It has been proposed that random association of these subunits results in the production of six types of dimers which are expressed as the isozymes designated GOT-2a ($\delta^2\delta^2$ dimers), GOT-2b ($\alpha^2\delta^2$ and $\beta^2\delta^2$) and GOT-2c ($\alpha^2\alpha^2$, $\beta^2\beta^2$ and $\alpha^2\beta^2$).

Table 3. *Schematic model for the subunit composition of the zone 2 GOT isozymes produced by Chinese Spring, Imperial, Chinese Spring–Imperial amphiploid and the chromosome 6R addition line.*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Chinese Spring			Imperial		Triticale and 6R addition line		
Isozymes	Subunit composition		Isozymes	Subunit composition	Isyzomes	Subunit composition	
GOT-2a	1/9	$\delta^2\delta^2$	—	—	GOT-2a	1/16	$\delta^2\delta^2$
GOT-2b	4/9	$\alpha^2\delta^2$, $\beta^2\delta^2$	—	—	GOT-2b	4/16	$\alpha^2\delta^2$, $\beta^2\delta^2$
GOT-2c	4/9	$\alpha^2\alpha^2$, $\beta^2\beta^2$, $\alpha^2\beta^2$	—	—	GOT-2c	6/16	$\alpha^2\alpha^2$, $\beta^2\beta^2$, $\alpha^2\beta^2$, $\delta^2\rho^2$
—		—	—	—	GOT-2d	4/16	$\alpha^2\rho^2$, $\beta^2\rho^2$
—		—	GOT-2	$\rho^2\rho^2$	GOT-2e	1/16	$\rho^2\rho^2$

The zone 2 GOT zymogram phenotypes observed in this study are, in terms of presence or absence, relative electrophoretic mobility, and relative staining intensities of bands, identical to those of ADH described above. Consistent with the discussion of the genetic control of ADH, it is proposed (1) that chromosome 6R of Imperial possesses a GOT structural gene, designated *Got-R2*, which codes a subunit, designated ρ^2, (2) that in the triticale and in the chromosome 6R addition line the active dimeric enzymes are produced by the random association of the four types of subunits, and (3) that *Got-R2* produces a quantity of subunit approximately equal to that of each individual Chinese Spring GOT-2 structural gene.

A schematic model for the subunit composition of the GOT-2 isozymes of Chinese Spring, Imperial, the 6R addition line and the triticale based on this hypothesis is shown in Table 3. The expected distribution of the isozymes which produce the zone 2 bands 1, 2, 3, 4 and 5 of the triticale and the 6R addition line (see the discussion of ADH above) is 1:4:6:4:1. The observed relative staining intensities of the bands of these two strains are in good agreement with this expectation.

The Imperial zone 1 GOT zymogram phenotype consists of three bands (II, Fig. 7, Plate 2). The most cathodal band (Imperial band 3) is electrophoreticallyhomologous with GOT-1c of Chinese Spring, an isozyme which occurs at the site of

band 3 of Chinese Spring (Hart, 1975). The Imperial band of least intensity (Imperial band 2) has an electrophoretic mobility intermediate between that of the anodal and cathodal bands, a mobility only slightly greater so as to partially overlap that of Chinese Spring GOT-1a. The triticale expresses each of the bands expressed by Chinese Spring and Imperial, plus an additional band intermediate between the two more anodal Imperial bands, a total of seven resolvable bands.

Evidence that a set of triplicate homoeologous GOT structural genes located in the chromosome arms 6Aα, 6BS and 6Dα of Chinese Spring code for isozymes located at the sites of Chinese Spring bands 1, 2 and 3 has been presented (Hart, 1975). The available evidence suggests that the zone 1 GOT isozymes of Chinese Spring are the products of three independent genetic systems, each of which may consist of triplicate structural genes. The finding that Imperial expresses three isozymes in zone 1 is consistent with this suggestion.

Five of the seven addition lines produce zone 1 phenotypes not distinguishable in terms of number of bands from that of Chinese Spring. On zymograms produced by the 6R addition line, band 3 appears to show greater relative staining intensity than does the band 3 of Chinese Spring zymograms. The difference is insufficient to be diagnostic for the 6R addition line. However, this finding is consistent with the possession by 6R of a GOT structural gene, homoeologous to the *Got-1* set of Chinese Spring, which encodes a subunit whose active dimeric product has an electrophoretic mobility equal to that of Chinese Spring GOT-1c.

The zone 1 GOT zymogram phenotypes of the CR and DR addition lines are consistently distinguishable from that of Chinese Spring. The CR addition line phenotype is indistinguishable from that of the triticale. The phenotype of the DR line differs from that of the triticale only in the relative intensity of its bands (IV, Fig. 7, Plate 2), bands 1 and 2 of the DR line being of lesser intensity and band 4 of greater intensity, relative to the other bands which compose the phenotype, than the corresponding bands of triticale.

On the basis of these findings it may be concluded that Imperial chromosomes CR and DR possess genes, probably structural, involved in the production of the zone 1 GOT isozymes. However, since in this zone several isozymes of coincident electrophoretic mobility underlie several bands, it has not been possible to define their genetic control in terms of specific structural genes.

(iii) *Acid phosphatase*

Three ACPH zymogram phenotypes were observed among the strains examined in this study. The Chinese Spring phenotype consists of five bands and the triticale of six. The five more anodal triticale bands have mobilities coincident with the five Chinese Spring bands. The sixth band, the most cathodal, has a mobility coincident with that of a band expressed by Imperial. Six of the seven addition lines possess a phenotype indistinguishable from that of Chinese Spring. The seventh, the DR addition line, shows a phenotype indistinguishable from that of the triticale.

The finding that the triticale and the DR addition line each express an ACPH

not expressed by Chinese Spring or the other addition lines demonstrates that the DR chromosome possesses a gene (or genes) involved in ACPH production. Each of the Chinese Spring group 4 chromosomes has been shown to possess ACPH structural genes (Hart, 1973). Whether these are homoeologously related to each other or to the DR ACPH gene (genes) cannot be determined with the evidence available.

(iv) *Aminopeptidase and endopeptidase*

Chinese Spring expresses three AMP isozymes, the products of structural genes located in chromosomes 6A, 6B and 6D (Hart, 1973). The electrophoretic mobilities of the two cathodal forms (AMP-2 and -3) differ but slightly so that the bands of precipitated dye which they produce on starch gels are almost contiguous. The anodal isozyme (AMP-1) has sufficiently greater electrophoretic mobility so as to be clearly separated from the other two forms.

Imperial expresses two AMP isozymes on zymograms. The anodal form is homologous in mobility to AMP-2 while the cathodal form has a mobility approximately intermediate to that of AMP-2 and AMP-3 of Chinese Spring.

Since the isozymes of ADH, GOT-2 and GOT-3 have been expressed in proportions expected on the basis of the dosage of the structural genes which code their subunits (see above), it is to be expected that the triticale would show for AMP an increase in staining intensity at the site of Chinese Spring band 2 (AMP-2) and in the region intermediate between band 2 and band 3 (AMP-3) relative to that of band 1. Further, it is to be expected either that one of the addition lines will show this same change in relative intensity in both regions or that two different addition lines will each show a change in one of these two regions.

This expectation is difficult to test because band 1 of Chinese Spring stains considerably less intensely than bands 2 and 3 and is considerably removed from these bands in mobility. Thus, it is a poor reference against which to gauge changes in staining intensity in the region of bands 2 and 3. Also, the close juxtaposition of bands 2 and 3 on the gel makes detection of changes in staining intensity either at the site of band 2 or in the region between bands 2 and 3 difficult.

To date, changes in relative staining intensity have not been observed in the 1R, 2R, 3R, 5R and CR addition lines. On one or more zymograms prepared from tissue extracts of the DR and 6R addition lines and the triticale, changes in relative staining intensities have been detected in the band 2 region, but in other preparations no relative staining intensity differences were observed. Due to a shortage of seeds of the DR and 6R lines, these analyses have temporarily been discontinued. Further analyses are needed, but it appears unlikely that the AMP's of Imperial will serve as good chromosome markers.

The EP zymogram phenotype of Chinese Spring consists of three bands. The intermediate band has been shown to be the product of two isozymes. The structural genes for the four Chinese Spring EP's expressed in 7-day-old seedlings are located in the chromosomes of group 7 (Hart & Langston, 1975; and, in preparation).

Imperial expresses two EP isozymes whose mobilities closely approximate two of the Chinese Spring forms. Due to a shortage of seed stocks of certain addition lines, only very limited studies of the EP zymogram phenotypes of the triticale and the addition lines have been conducted. To date, no differences have been observed between the zymogram phenotypes of the triticale and the addition lines and Chinese Spring. As with AMP, it appears unlikely that the Imperial EP's will serve as good chromosome markers.

4. DISCUSSION

Four of the addition lines, namely those possessing chromosomes 3R, 6R, CR and DR, produce, for one or more of the enzymes studied, a zymogram phenotype which differs from that of Chinese Spring. However, the zymogram phenotypes of the 1R, 2R and 5R addition lines are, for each of the enzymes studied, indistinguishable from Chinese Spring. This latter finding is not unexpected. In earlier genetic studies of Chinese Spring, the structural genes for most of the enzymes under study here were located in specific chromosomes. Association has been made to date with the chromosomes of homoeologous groups 3, 4, 6 and 7 but not with the chromosomes of groups 1, 2 and 5.

GOT is the most useful of the several enzymes for the purpose of detecting the presence or absence of Imperial chromosomes in addition lines and triticales, as four different chromosomes have distinguishable effects on the GOT zymogram phenotype. The production of five bands in GOT zone 2 (as opposed to three bands by Chinese Spring) due to the activity of the structural gene *Got-R2* of chromosome 6R is the most readily observable effect. The change in zone 1 produced by CR is also readily observable, but in poor preparations it can be confused with the effect of DR. Imperial chromosome 3 carries the gene *Got-R3*. Its presence in addition lines and triticales is detectable as a change in the relative staining intensities of the bands of GOT zone 3 as opposed to that of Chinese Spring. Changes in relative intensities of bands are clearly not as useful as changes in number of bands. However, the isozymes of zone 3 are well separated from each other on gels and stain intensely, characteristics which allow relative intensity changes to be readily observed.

One leaf of a seedling of from 7 days to several weeks of age is usable as a source of GOT for zymogram procedures, an additional desirable feature. However, with the starch-gel procedures used in this study, the zone 1 GOT isozymes do not resolve into discrete bands (indeed, resolution of zone 2 bands is poor). Consequently, the effects of Imperial chromosomes C and D on GOT could not be distinguished following starch-gel electrophoresis. Since it is unlikely that starch-gel procedures capable of routinely resolving the GOT isozymes of zone 1 into discrete bands can be devised, it appears probable that it will be necessary to use disk acrylamide gel electrophoresis, or another procedure with equivalent resolving power, to distinguish the effects of each of the Imperial chromosomes 3, 6, C and D on the zymogram phenotype of a line.

The effect of the gene *Adh-R1* of Imperial chromosome C in the addition line and triticale on the ADH zymogram phenotype is as easily distinguishable as is the effect of *Got-R2* on the zone 2 GOT phenotype. This effect is readily observable on both starch and acrylamide gels. A disadvantage is the absence of detectable quantities of ADH in the leaf. The kernel is the best known source of the enzyme, although it appears probable that the roots of seedlings, at least if grown under anaerobic conditions, would also be an acceptable source.

Several ACPH isozymes are contained in the leaves of 7-day-old seedlings. However, all but one of those observable on starch gels have electrophoretic mobilities approximately the same as the ACPH's of Chinese Spring. In 7-day-old seedlings, only Imperial chromosome D produces a phenotype which differs from Chinese Spring, this being due to the presence of an ACPH in the region between Chinese Spring ACPH-6 and the cathode.

Whether EP or AMP will be suitable markers for Imperial chromosomes in addition lines and triticales can only be determined after additional study. However, since the Imperial enzymes have mobilities similar to those of the Chinese Spring forms and dosage effects have been difficult to observe with these enzymes, it appears unlikely that they will give the resolution required for reliable detection of specific chromosome effects.

In this study, Imperial chromosomes 3, 6, C and D have been shown to possess genes involved in the production of one or more forms of the enzymes GOT, ADH and ACPH. Direct evidence for the homoeology of rye chromosomes 3 and 6 with wheat chromosomes 3 and 6 respectively comes from the linkage of *Got-R3* and *Got-R2* to Imperial chromosomes 3 and 6 respectively, since the structural genes for the GOT-3 and -2 systems of hexaploid wheat are located in the chromosomes of groups 3 and 6 respectively of Chinese Spring (Hart, 1975). These findings are consistent with homoeologous relationships between wheat and rye chromosomes suggested by other studies (see Darvey, 1973). However, the linkage of *Adh-R1* to Imperial chromosome C is not consistent with the placement of this chromosome in a homoeologous group with chromosomes III of Dakold and VII of King II, a group designated 7R/4R by Darvey (1973), nor with the placement of Imperial chromosome D in a group with V of Dakold and IV of King II (4R/7R of Darvey). We have shown (unpublished) that *Adh-R1* is located in chromosome V (4R/7R) of Dakold rye and Irani and Bhatia (1972) have placed *Adh-R1* in IV (4R/7R) of King II rye. The structural genes for ADH of Chinese Spring are located in the 4 group (Hart, 1970, 1973). These linkages suggest that Imperial may differ from Dakold, King II and Chinese Spring by a reciprocal translocation involving the chromosomes of groups 4 and 7, such that C and D of Imperial are each partially homoeologous with V and III of Dakold, IV and VII of King II and the 4 and 7 groups of Chinese Spring. Darvey & Gustafson (1975) have made a similar suggestion based on a comparison of heterochromatin banding patterns of wheat–rye addition lines.

This study clearly demonstrates that isozymes can be useful markers for rye chromosomes in wheat–rye addition and substitution lines and in triticales. They

are most useful for this purpose when genetic variation exists which causes the isozymes of the wheat and rye varieties to differ in mobility, as with the ADH and the GOT-2 systems. However, because of dosage effects, variation in mobility of isozymes between the varieties is not a necessity, as seen with the GOT-3 system. This study also demonstrates that definitive evidence for homoeology between wheat and rye chromosomes and parts thereof can be obtained by analysis of the pattern of variation shown by those isozymes whose structural genes have in wheat been linked to specific chromosomes.

These techniques would seem to be limited in utility at this time only because of the limited number of isozyme systems that have been studied genetically in *Triticum*. Since techniques are available for study of a large number of other isozymes, it seems likely that further genetic studies will soon result in the location of structural genes for isozymes in each of the seven homoeologous chromosome groups of hexaploid wheat, and subsequently of useful isozyme markers in each of the seven chromosomes of rye.

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