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Use of isozymes as chromosome markers in the isolation and characterization of wheat-barley chromosome addition lines

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SUMMARY

The alcohol dehydrogenase (ADH), glutamic oxaloacetic transaminase (GOT), aminopeptidase (AMP), endopeptidase (EP), and esterase (EST) zymogram phenotypes of Chinese Spring wheat, Betzes barley, Chinese Spring-Betzes heptaploids, and a number of presumptive Betzes chromosome additions to Chinese Spring were determined. It was found that four disomic chromosome addition lines could be distinguished from one another and from the other three possible lines on the basis of the zymogram phenotypes of these isozymes.

The structural gene *Adh-H1* was located in Betzes chromosome 4, the genes *Got-H2* and *Amp-H1* in chromosome 6, and the gene *Ep-H1* in chromosome 1. These gene locations provide evidence of homoeology between Betzes chromosomes 4, 6, and 1 and the Chinese Spring chromosomes of homoeologous groups 4, 6, and 7, respectively.

1. INTRODUCTION

The ability of hexaploid wheat (*Triticum aestivum*) to tolerate aneuploidy and to hybridize with other Triticeae species has allowed cytogeneticists to construct a considerable number of wheat lines which contain alien genetic material. Lines have been produced which contain added or substituted whole chromosomes, chromosome arms or segments of arms from other species of *Triticum* and from *Secale*, *Agropyron*, *Haynaldia* and *Hordeum* (for a compendium of wheat-alien chromosome lines, see Driscoll, 1975). The production and analysis of these lines is of importance both because of the potential that exists for the improvement of wheat by the incorporation of alien genetic material and because of the utility of the lines for the study of the genetics and evolution of wheat and its relatives (Riley & Kimber, 1966; Sears, 1972; Shepherd, 1973; Hart, 1979).

A systematic study of each of the chromosomes of a given relative of wheat may be conducted when appropriate wheat-alien chromosome lines are available. A major initial requirement for such a study is the isolation and characterization of the possible disomic chromosome addition lines (O'Mara, 1940; Riley & Chapman,

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1958). The identification of addition lines has conventionally been based on plant phenotypes and/or chromosome morphology, on appropriate tests for meiotic chromosome pairing, and, in a few cases, on genetic markers known to be present in alien chromosomes (Sears, 1975). This process (as well as the development of other wheat lines containing alien genetic material) would be considerably facilitated by the ability to readily detect the expression of individual alien genes in such lines. The presence of one or more genetic markers in *each* alien chromosome would be of particular value.

Aneuploid genetic investigations of isozyme variation in hexaploid wheat have identified at least 57 isozyme structural genes and have located one or more of these genes in 18 of the 21 chromosomes of the species. Most of these genes are members of triplicate sets located in homoeologous chromosomes (for a review, see Hart, 1979). Studies of strains of hexaploid wheat that contain alien genetic material have shown that isozymes encoded by alien genes are expressed in the strains and may be readily detected with the zymogram technique (Barber *et al.* 1968, 1969; Upadhya, 1968; Driscoll & Sears, 1971; Irani & Bhatia, 1972; MacDonald & Smith, 1972; Bergman & Maan, 1973; Tang & Hart, 1975; Hart, McMillin & Sears, 1976; Hart, 1978; Wolf & Rimpau, 1977; Cauderon *et al.* 1978; Kobrehel, 1978). Furthermore, a number of isozyme structural genes have been identified and located in specific alien chromosomes in these studies and, for most of the genes identified, homoeology with the members of a triplicate set of hexaploid wheat genes has been demonstrated. These factors suggest that the use of isozyme structural genes as chromosome markers would be a valuable addition to the repertoire of procedures now available for distinguishing and identifying alien chromosomes in derivatives of hybrids between wheat and other Triticeae species.

We report here the results of a zymogram study of several enzymes that was carried out on presumptive chromosome addition lines during the development of the wheat-barley chromosome addition lines produced by Islam, Shepherd & Sparrow (1978 and, in preparation). The principal analyses and findings reported are as follows: (1) A description and an assessment of the value of the use of isozymes as chromosomal markers during the process of isolating and identifying chromosome addition lines; (2) the chromosomal locations of several barley isozyme structural genes; (3) deductions regarding evolutionary relationships among the chromosomes of barley and hexaploid wheat that stem from the gene locations reported. Brief accounts of some of this research were published in an abstract (Hart, Islam & Shepherd, 1977) and in a review (Hart, 1979).

2. MATERIALS AND METHODS

This study was conducted with materials produced by Islam and colleagues during their development of wheat–barley addition lines. A brief account of pertinent aspects of their work is given here in order to identify the materials used in this investigation. A summary of the main findings of Islam *et al.* has been published (1978).

Nineteen F_1 hybrid plants were produced by pollination of *Triticum aestivum* L. cv. Chinese Spring with *Hordeum vulgare* L. cv. Betzes. Only one of these plants appeared to be a true hybrid with 21 wheat and 7 barley chromosomes; the other plants possessed a wide variety of chromosomal constitutions. Among the latter was one plant which regularly exhibited $21' + 1''$ at meiotic metaphase I. The 28-chromosome wheat–barley F_1 hybrid was pollinated with wheat and 49-chromosome heptaploid progeny obtained from this cross were used in further backcrosses with wheat. From these latter backcrosses plants with 43 chromosomes which exhibited $21'' + 1'$ at meiosis and plants with 44 chromosomes which exhibited $21'' + 2'$ at meiosis were obtained. Derivatives of these plants were the principal source of disomic addition lines. One disomic addition line was obtained from the wheat-barley F_1 hybrid that exhibited $21' + 1''$ at meiosis; pollination of the hybrid with wheat resulted in the production of a disomic chromosome addition line in one step.

The zymogram phenotype of the enzymes alcohol dehydrogenase (ADH), glutamic oxaloacetic transaminase (GOT), aminopeptidase (AMP), endopeptidase (EP), and esterase (EST) was determined for each line examined in this study. Presumptive addition lines were intially classified into groups on the basis of morphological differences. We examined most of the presumptive disomic chromosome addition lines contained in each of the morphological groups, a number of other presumptive whole chromosome and/or telosome addition lines, heptaploids possessing 42 wheat and 7 barley chromosomes, and the cultivars Betzes and Chinese Spring.

Extracts of scutella obtained from grains germinated for 16 h on moist filter paper in Petri dishes in an incubator at 23 °C in the dark were used for the ADH analyses. The GOT, AMP, and EP analyses utilized extracts of shoots of 7-day-old etiolated seedlings grown in an incubator at 23 °C, first in Petri dishes for 2 days and then in moist cotton in 25×150 mm culture tubes. The EST analyses were performed with extracts of 7-day-old green shoots grown in an incubator at 23 °C in Petri dishes in the dark for 2 days and then in moist cotton in culture tubes with continuous light. Extracts were obtained by maceration of tissue with sand in a mortar with pestle in a pH 7·5 buffer containing 0·1 M-tris (hydroxymethyl)aminomethane-HCl, 0·1 M-KCl, 0·005 M-EDTA, 0·04 M-2-mercaptoethanol, and 0·1 M-sucrose. Each scutellum used for ADH analysis was macerated in 125 μl of the extraction buffer. A weight:volume ratio of tissue:buffer of 1:2 was used for AMP, EP, and EST and of 1:6 for GOT. The slurry obtained by maceration was centrifuged at $30000 \times g$ for 20 min. The supernatant obtained was used directly for electrophoresis. Extraction, centrifugation, and electrophoresis were carried out at 2–5 °C. The zymogram phenotype of each enzyme was determined for a minimum of three grains (ADH) or plants of each chromosomal type.

Extracts were electrophoresed for the determination of the ADH, GOT, and EST zymogram phenotypes in disc acrylamide gels (100 μl of supernatant/gel for ADH, 50 μl for GOT, and 200 μl for EST) using procedures described by Hart (1975). Gels were stained for ADH as described by Hart (1970), for GOT as

described by Yang (1971), and for EST as described by Kahler & Allard (1970). The AMP and EP zymogram phenotypes were determined in horizontal starch gels (Electrostarch, 11%, w/v), using methods previously described (Hart, 1973). Because of the relatively small differences in electrophoretic mobility among the AMP and the EP isozymes, electrophoresis was continued until the most anodal AMP isozyme had migrated 18–20 cm and the most anodal EP isozyme 20–22 cm from the sample slots. AMP and EP were stained as described by Hart (1973) and Tang & Hart (1975).

Each disomic addition line was arbitrarily designated with a capital letter when isolated. Subsequent to the completion of this study Islam (1980) assigned standard barley designations to the chromosomes contained in the lines. This was accomplished by relating the N-banding patterns of the barley chromosomes contained in disomic addition lines to the banding patterns of barley trisomics.

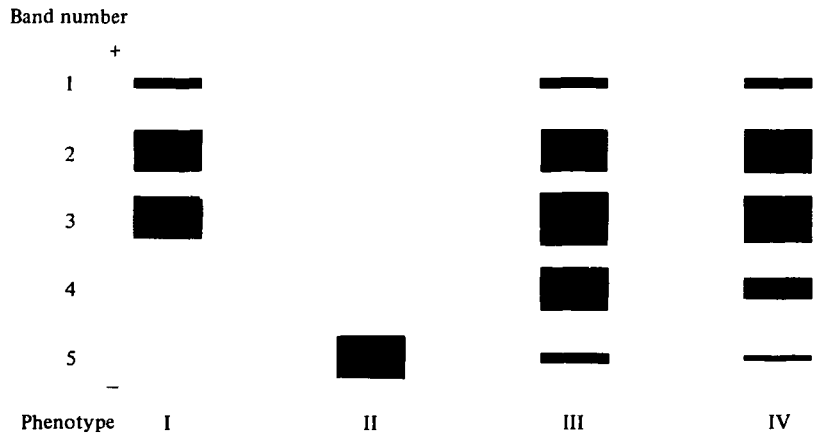
3. RESULTS

(i) *Alcohol Dehydrogenase*

Diagrams of the ADH zymogram phenotypes produced by Chinese Spring wheat, Betzes barley, Chinese Spring-Betzes heptaploids, and the presumptive chromosome addition lines that were examined are shown in Text-fig. 1. The three Chinese Spring isozymes (I, Text-fig. 1) have been designated, in order of decreasing electrophoretic mobility, as ADH-1, ADH-2, and ADH-3 (Hart, 1970). Betzes scutella express one major ADH isoyzme (II, Text-fig. 1) and two minor forms (not shown in Text-fig. 1) that can be detected only after prolonged staining. The major form, which we designate as barley ADH-3, migrates to a position cathodal to Chinese Spring ADH-3. Barley ADH-2, an interlocus heterodimer (A. H. D. Brown, personal communication), has an electrophoretic mobility intermediate between barley ADH-1 and ADH-3 and coincident with Chinese Spring ADH-1. Brown *et al.* (1978) have shown that anaerobicity induces ADH-1 and ADH-2 in barley roots.

Chinese Spring-Betzes heptaploids express five ADH isozymes (IV, Text-fig. 1). The more anodal three isozymes correspond in mobility to the Chinese Spring isozymes and the most cathodal isozyme to Betzes ADH-3. The fifth form, not expressed by either Chinese Spring or Betzes, is intermediate in mobility between Chinese Spring ADH-3 and Betzes ADH-3. Several presumptive disomic chromosome addition lines expressed a phenotype (III, Text-fig. 1) indistinguishable from that of the heptaploids except for slight differences in the relative staining intensities of the isozymes, namely, greater relative expression of the two most cathodal isozymes by the presumptive disomic addition lines than by the heptaploids. All of the other presumptive disomic chromosome addition lines expressed a phenotype indistinguishable from that of Chinese Spring. These findings are similar to those of Irani & Bhatia (1972) and of Tang & Hart (1975) in their analyses of the ADH phenotypes of a series of wheat-rye addition lines and amphiploids and are subject to a similar interpretation.

The structural genes which encode the promoters which compose Chinese Spring ADH-1, -2, and -3 are located in chromosomes 4A, 4B, and 4D (Hart, 1970; Hart & Langston, 1977). The genes are designated *Adh-A1*, *Adh-B1*, and *Adh-D1*, respectively, and the protomers which they encode as α, β, and δ, respectively. The available evidence indicates that the protomers associate in all possible combinations to produce the six possible types of dimeric enzymes which are expressed as ADH-1 ($\alpha\alpha$ dimers), ADH-2 ($\alpha\beta$ and $\alpha\delta$), and ADH-3 ($\beta\beta$, $\delta\delta$, and $\beta\delta$). The simplest hypothesis which is consistent with the results of this study is that the five isozymes expressed by the heptaploids and by the presumptive chromosome addition



Text-fig. 1. Diagrams of ADH zymogram phenotypes observed. (I) Chinese Spring, (II) Betzes, (III) disomic chromosome addition line 4, and (IV) Chinese Spring-Betzes heptaploids.

lines are composed of all possible dimeric combinations of four protomers, namely, α, β, and δ produced by wheat and an additional protomer, designated θ, which is encoded by a barley gene, designated *Adh-H1*. Consequently, this hypothesis suggests that the several presumptive disomic addition lines which expressed phenotype III (see Text-fig. 1) each possessed the same barley chromosome. Originally designated A, this chromosome has since been shown by Islam (1980) to be barley chromosome 4.

A schematic model for the subunit composition of the three ADH isozymes of Chinese Spring, for ADH-3 of Betzes, and for the five isozymes observed to be expressed by heptaploids and by disomic chromosome addition line 4 is shown in Table 1. Three dimeric forms not found in either parent are assumed to be produced by heptaploids and the addition line. ADH-4 is composed of $\beta\theta$ and $\delta\theta$ dimers while the $\alpha\theta$ dimers have a mobility coincident with Chinese Spring ADH-3.

If the four protomers are produced in equal quantities and associate randomly to produce active dimeric molecules, the expected distribution of the 10 dimeric types contained in heptaploids and in addition line 4 will be based on $(p+q+r+s)^2$, where p, q, r, and s represent the frequencies of α, β, δ, and θ, respectively. In the disomic addition line, $p = q = r = s = \frac{1}{4}$ while in the heptaploids $p = q = r = \frac{2}{7}$

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and $s = \frac{1}{7}$. The expected distributions of the isozymes are shown in Table 1. The observed relative staining intensities of the five zymogram bands are in good accord with these distributions.

(ii) *Glutamic Oxaloacetic Transaminase*

The GOT isozymes contained in the shoots of 7-day-old etiolated seedlings of Chinese Spring are resolved by the electrophoretic procedures described herein into three major zones. The isozymes of the two more cathodal zones (zones 2 and 3) 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 structural genes (Hart, 1975).

The zone 2 GOT zymogram phenotypes observed in this study are shown in Text-fig. 2. The electrophoretically fast, intermediate, and slow forms produced by Chinese Spring are designated GOT-2a, -2b, and -2c, respectively (Hart, 1975). Betzes expresses one isozyme in zone 2, of lesser electrophoretic mobility than the Chinese Spring isozymes. Several of the presumptive disomic chromosome addition lines expressed a phenotype consisting of six isozymes (III, Text-fig. 2), four of which correspond in electrophoretic mobility to the forms produced by Chinese Spring and Betzes. The two new isozymes migrate to positions slightly cathodal to the Chinese Spring isozymes. The phenotype of other presumptive addition lines was indistinguishable from that of Chinese Spring. Six isozymes with mobilities coincident with those of phenotype III were also expressed by Chinese Spring-Betzes heptaploids.

These results are consistent with an interpretation similar to that proposed for the ADH findings presented above. The structural genes which encode the protomers which compose Chinese Spring GOT-2a, -2b, and -2c are located in the three chromosomes of homoeologous group 6 (Hart, 1975). Designated *Got-A2*, *Got-B2*, and *Got-D2*, these genes encode protomers which have been designated α^2, β^2, and δ^2, respectively. The available evidence indicates that these protomers associate to produce six types of dimers which are expressed as 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 $\alpha^2\beta^2$). The presence in the presumptive addition lines which express phenotype III of a Betzes chromosome which carries the structural gene for the Betzes GOT-2 protomer generates expectations consistent with the findings reported here. We designate the structural gene as *Got-H2* and the protomer encoded as θ^2. We propose that the association in all possible dimeric combinations of θ^2 and the three Chinese Spring GOT-2 protomers produces the six heptaploid and addition line GOT-2 isozymes. The chromosome involved, originally designated C, has since been shown by Islam (1980) to be barley chromosome 6.

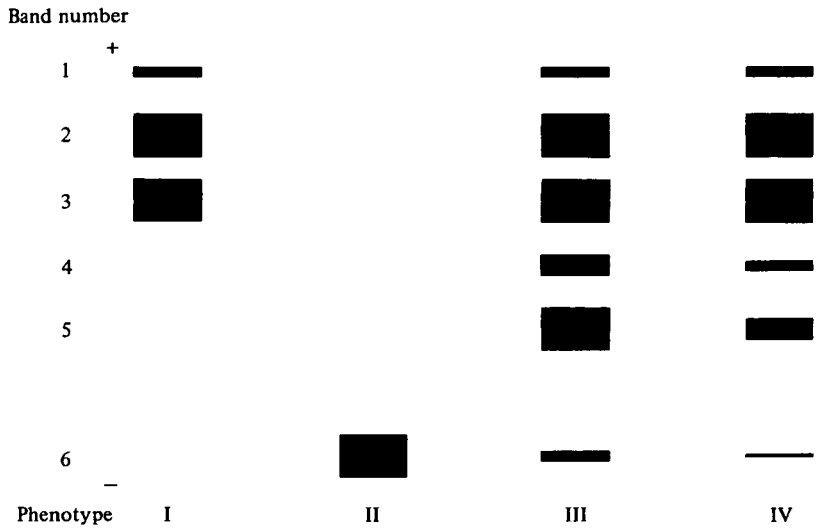
A schematic model for the subunit composition of the GOT-2 isozymes of Chinese Spring, Betzes, Chinese Spring-Betzes heptaploids, and addition line 6 is shown in Table 2. The model proposes that GOT-2a, -2b, and -2c of the addition line and heptaploids consist entirely of Chinese Spring protomers, GOT-2f of

Table 1. *Schematic model for the subunit composition of the ADH isozymes produced by Chinese Spring, Betzes, Chinese Spring-Betzes heptaploids, and disomic chromosome addition line 4.*

(ADH-1 and ADH-2 of Betzes are not shown. The expected quantitative distribution of the isozymes is indicated by the ratios preceeding the dimers).

Chinese Spring		Betzes		Addition Line 4		Heptaploid	
Isozymes	Subunit composition	Isozymes	Subunit composition	Isozymes	Subunit composition	Isozymes	Subunit composition
ADH-1	1/9 $\alpha\alpha$	—	—	ADH-1	1/16 $\alpha\alpha$	ADH-1	4/49 $\alpha\alpha$
ADH-2	4/9 $\alpha\beta$, $\alpha\delta$	—	—	ADH-2	4/16 $\alpha\beta$, $\alpha\delta$	ADH-2	16/49 $\alpha\beta$, $\alpha\delta$
ADH-3	4/9 $\beta\beta$, $\delta\delta$, $\beta\delta$	—	—	ADH-3	6/16 $\beta\beta$, $\delta\delta$, $\beta\delta$, $\alpha\theta$	ADH-3	20/49 $\beta\beta$, $\delta\delta$, $\beta\delta$, $\alpha\theta$
—	—	—	—	ADH-4	4/16 $\beta\theta$, $\delta\theta$	ADH-4	8/49 $\beta\theta$, $\delta\theta$
—	—	ADH-3	$\theta\theta$	ADH-5	1/16 $\theta\theta$	ADH-5	1/49 $\theta\theta$

dimers composed of the θ^2 protomer encoded by *Got-H2*, and GOT-2d and -2c of heterodimers each composed of one Chinese Spring and one Betzes protomer. As shown, the mobility of $\delta^2\theta^2$ heterodimers would be expected to be intermediate between the homodimeric forms $\delta^2\delta^2$ and $\theta^2\theta^2$ and the mobility of the $\alpha^2\theta^2$ and $\beta^2\theta^2$ dimers intermediate between the homodimers in which these protomers are contained.



Text-fig. 2. Diagrams of GOT-2 zymogram phenotypes observed. (I) Chinese Spring, (II) Betzes, (III) disomic chromosome addition line 6, and (IV) Chinese Spring-Betzes heptaploids.

The expected distribution of the possible GOT-2 dimeric forms in addition line 6 and in heptaploids, given production of the protomers in equal quantities and their random association into active dimeric molecules, can be calculated, as for ADH above, by expansion of $(p+q+r+s)^2$, where p, q, r, and s represent, respectively, the frequencies of α^2, β^2, δ^2, and θ^2. In the disomic addition line, $p = q = r = s = \frac{1}{4}$ while in heptaploids $p = q = r = \frac{2}{7}$ and $s = \frac{1}{7}$. The expected distributions are shown in Table 2. These expectations are in good accord with the relative staining intensities observed. It may be noted that the expected relative distribution of the five more anodal isozymes, in order of decreasing electrophoretic mobility, is 1:4:4:2:4 for addition line 6 and 1:4:4:1:2 for the heptaploid. Addition line 6 and heptaploid zymograms can usually be distinguished by this difference. The most cathodal isozyme is too faint in intensity to be useful for this purpose.

Betzes expresses four other GOT isozymes in addition to GOT-2. Three of these have electrophoretic mobilities approximately the same as the zone 1 isozymes of Chinese Spring. Presumably they are the products of three independent structural genes. These three isozymes could not be resolved from the zone 1 Chinese Spring isozymes on zymograms made with heptaploid tissue extracts (see Hart, 1975 and Tang & Hart, 1975). Betzes GOT-3 is coincident in mobility with GOT-3a of Chinese Spring. In this initial study we chose to analyse only isozymes which differ

Table 2. *Schematic model for the subunit composition of the GOT-2 isozymes produced by Chinese Spring, Betzes, Chinese Spring-Betzes heptaploids and disomic chromosome addition line 6.*

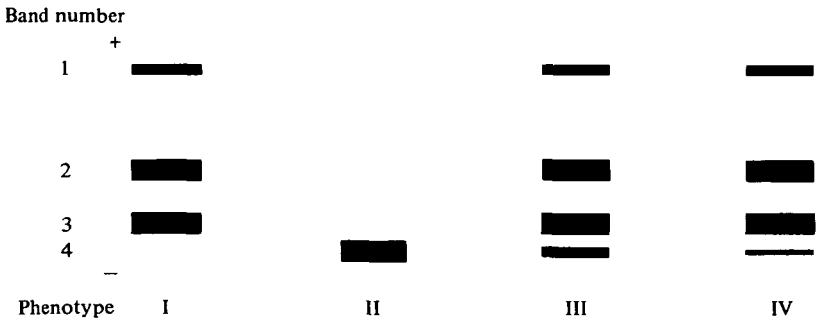
(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers. The superscript '2', which distinguishes GOT-2 subunits from those of other GOT systems, has been omitted from each subunit designation to simplify the Table).

Chinese Spring		Betzes		Addition Line 6		Heptaploid	
Isozymes	Subunit composition	Isozymes	Subunit composition	Isozymes	Subunit composition	Isozymes	Subunit composition
GOT-2a	1/9 $\delta\delta$	—	—	GOT-2a	1/16 $\delta\delta$	GOT-2a	4/49 $\delta\delta$
GOT-2b	4/9 $\alpha\delta$, $\beta\delta$	—	—	GOT-2b	4/16 $\alpha\delta$, $\beta\delta$	GOT-2b	16/49 $\alpha\delta$, $\beta\delta$
GOT-2c	4/9 $\alpha\alpha$, $\beta\beta$, $\alpha\beta$	—	—	GOT-2c	4/16 $\alpha\alpha$, $\beta\beta$, $\alpha\beta$	GOT-2c	16/49 $\alpha\alpha$, $\beta\beta$, $\alpha\beta$
—	—	—	—	GOT-2d	2/16 $\delta\theta$	GOT-2d	4/49 $\delta\theta$
—	—	—	—	GOT-2e	4/16 $\alpha\theta$, $\beta\theta$	GOT-2e	8/49 $\alpha\theta$, $\beta\theta$
—	—	—	—	—	—	—	—
—	—	GOT-2	$\theta\theta$	GOT-2f	1/16 $\theta\theta$	GOT-2f	1/49 $\theta\theta$

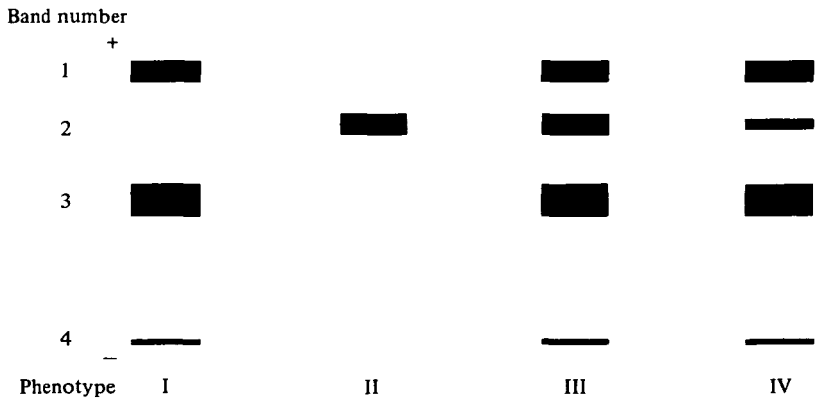
in mobility between Chinese Spring and Betzes so as to avoid attempting to identify alien chromosomes on the basis of dosage effects alone. Consequently, we did not analyse GOT-3.

(iii) *Aminopeptidase and Endopeptidase*

The AMP and EP isozymes of Chinese Spring which have been studied genetically (Hart, 1973; Hart & Langston, 1977) behave as monomeric enzymes. Consequently, the interpretation of the zymogram phenotypes observed for these enzymes is less complex than the interpretation of the ADH and GOT-2 phenotypes.



Text-fig. 3. Diagrams of AMP zymogram phenotypes observed. (I) Chinese Spring, (II) Betzes, (III) disomic chromosome addition line 6, and (IV) Chinese Spring-Betzes heptaploids.



Text-fig. 4. Diagrams of EP zymogram phenotypes observed. (I) Chinese Spring, (II) Betzes, (III) disomic chromosome addition line 1, and (IV) Chinese Spring-Betzes heptaploids.

The AMP zymogram phenotypes observed are diagrammed in Text-fig. 3 and the EP phenotypes in Text-fig. 4. One AMP and one EP isozyme is expressed by Betzes in the tissue analysed. The Betzes AMP was expressed by heptaploids and as well by each of the several presumptive addition lines which expressed GOT-2, that is, by chromosome addition line 6. The other presumptive addition lines expressed only the Chinese Spring isozymes. Consequently, we conclude that

Betzes chromosome 6 carries the structural gene for the Betzes AMP. The three Chinese Spring AMPs are the products of a triplicate set of genes located in chromosomes 6A, 6B and 6D. The genes are designated *Amp-A1*, *Amp-B1*, and *Amp-D1*, respectively. Since it is probable that the Betzes AMP gene is homoeologous to the Chinese Spring set, we designate it as *Amp-H1*.

The Betzes EP was expressed by heptaploids and by several presumptive addition lines, but not by lines 4 and 6. The lines which did not express the Betzes EP produced only the Chinese Spring isozymes. As with the other enzymes considered to this point, the simplest hypothesis is that the presumptive addition lines which expressed the Betzes EP each carry the same Betzes chromosomes, a chromosome which carries the EP structural gene. Evidence has been presented that the EP isozymes of Chinese Spring are the products of four structural genes located in the chromosomes of homoeologous group 7. Three of the genes, *Ep-A1*, *Ep-B1*, and *Ep-D1*, appear to compose a triplicate set. The fourth gene, *Ep1*, has been found to exhibit tissue variation in expression not observed for the other three genes. We tentatively designate the Betzes EP gene as *Ep-H1*. The chromosome which carries the gene, originally designated D, has been shown by Islam (1980) to be barley chromosome 1.

(iv) *Esterases*

The disc acrylamide gel electrophoresis procedures which we have used to study ESTs resolve a large number of isozymes of both wheat and barley. Chinese Spring-Betzes heptaploids express most of the major forms of the two parents (C, Fig. 5, Plate 1). The presumptive addition lines examined could be classified into three groups on the basis of their expression of Betzes ESTs. The lines which carry barley chromosome 1 and express Betzes EP also express two major Betzes EST isozymes (D, Fig. 5, Plate 1). We designate these as EST-1 and -2, in order of decreasing electrophoretic mobility. Several other presumptive addition lines expressed two Betzes EST isozymes of lesser electrophoretic mobility (E, Fig. 5, Plate 1) which we designate as EST-3 and -4. The remainder of the lines did not express any detectable Betzes ESTs.

We interpret these findings as indicating that Betzes chromosome 1 carries the structural gene(s) for EST-1 and -2 in addition to the EP gene and that another Betzes chromosome carries the structural gene(s) for EST-3 and -4. This latter chromosome, originally designated F, has been shown to be barley chromosome 3 (Islam, 1980). The structural genes for EST-3 and/or -4 may be among the group of three esterase genes shown by Kahler & Allard (1970) to be very tightly linked to each other and by Nielsen & Frydenberg (1971) to be located in chromosome 3.

The genetics of wheat and barley esterases is complex. A number of wheat esterase structural genes have been identified and located in specific chromosomes (Barber *et al.* 1968; Bergman, 1972; May, Vickery & Driscoll, 1973; Nakai, 1976) but the specific genetic basis of most wheat esterases is as yet unknown. Ten barley esterase loci have been identified, three of which, as noted above, compose a tight linkage group in chromosome 3 (Kahler & Allard, 1970; Nielsen & Frydenberg,

1971; Hvid & Nielsen, 1977; Kahler, Heath-Pagliuso & Allard, personal communication). The chromosomal locations of the other seven genes have not been reported.

Establishment of homoeologies between wheat and barley esterases, as described above for the other enzymes investigated, is desirable but cannot be accomplished with the information currently available. Consequently, our report here on esterases is confined to their use as aids in defining addition lines. We consider the designations assigned to the four barley esterases expressed in the addition lines to be temporary, for the purpose of identification in this paper, and have not assigned symbols to the genes which encode these isozymes.

4. DISCUSSION

Six of the seven possible addition lines having individual pairs of barley chromosomes added to the chromosome complement of wheat have been produced (Islam *et al.*, 1978). It is unlikely that the seventh line will be obtained as the chromosome involved, barley chromosome 5, causes meiotic disturbances and self-sterility when added to wheat. The five enzymes analysed in this study characterize four of the six available addition lines, namely, lines 1, 3, 4 and 6. Chromosome addition line 2 was initially identified on the basis of plant morphology and N-banding and addition line 7 by plant and chromosome morphology (barley chromosome 7 is a nucleolar organizer chromosome) (Islam, Shepherd & Sparrow, personal communication). Islam (1980) confirmed the identity of the six disomic addition lines by demonstrating that each of them contains an added pair of barley chromosomes with a distinctive N-banding pattern. Confirmation was also obtained in the observation of meiotic chromosome configurations of $21'' + 1'$ and $21'' + 2'$ in the progeny of crosses of each addition line with wheat and the progeny of intercrosses among the different addition lines respectively (Islam *et al.* 1978 and, in preparation).

It is clear that isozymes have considerable value as a means of distinguishing different addition lines and identifying the chromosomes contained in the lines. Detection of barley isozymes and/or protomers in addition lines and identification of the genes which encode them is easily accomplished. Consequently, isozymes are excellent markers for specific barley chromosomes. In contrast, no obvious morphological characteristics of barley have been detected in the addition lines. Furthermore, seasonal and other commonly encountered environmental differences, e.g. in day-length, light intensity, nutrient and moisture availability, etc. may cause variation in morphological characteristics sufficient to make morphological classification difficult. N-banding identifies each of the seven Betzes chromosomes (Islam, 1980) while the five enzymes examined in this study identify only four chromosomes. However, barley prolamin (hordeins) identifies a fifth chromosome (chromosome number 5, the chromosome that causes meiotic disturbances and self-sterility when added to wheat) (Islam *et al.* 1978; Islam & Shepherd, 1980) and it is probable that analyses of additional isozymes would have led to the location of isozyme genes in one or both of the other barley chromosomes since one or more sets of homoeologous genes have been located in six of the seven

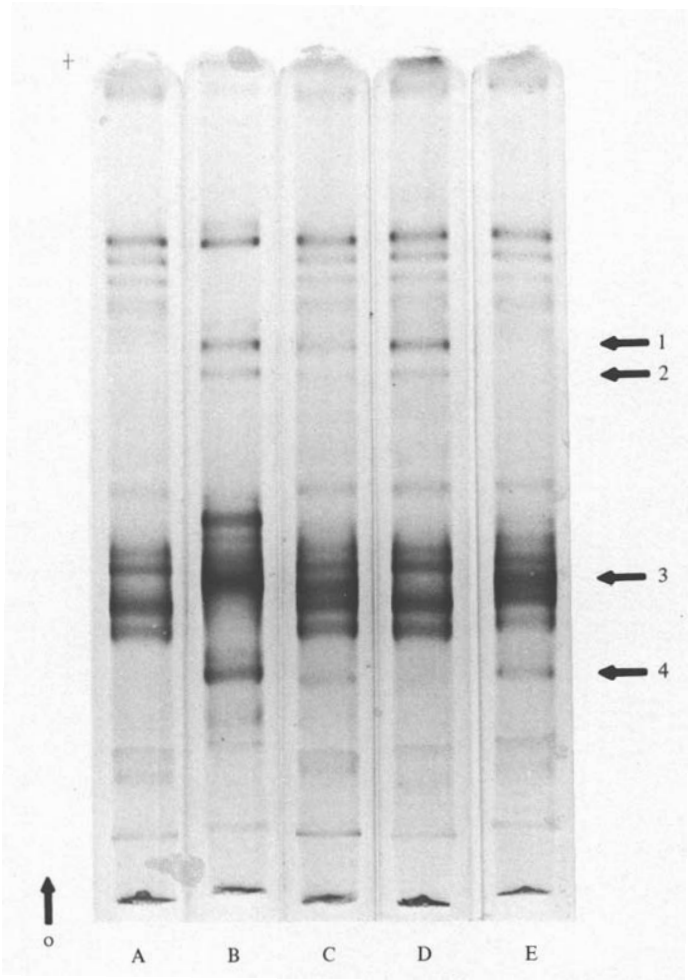


Fig. 5, Plate 1. EST zymogram phenotypes observed. (A) Chinese Spring, (B) Betzes, (C) Chinese Spring-Betzes heptaploids, (D) disomic chromosome addition line 1, and (E) disomic chromosome addition line 3. The numbers to the right identify four Betzes ESTs that are expressed by heptaploids and by either chromosome addition line 1 (EST-1 and -2) or chromosome addition line 3 (EST-3 and -4).

homoeologous chromosome groups of hexaploid wheat (see Hart, 1979). A major advantage of isozyme studies over chromosome banding procedures is that they are technically less complex and less time consuming. A much larger number of plants can be analysed per unit time with the zymogram technique than with a chromosome banding procedure.

For each of the isozyme systems analysed in this study, genetic variation exists between Chinese Spring and Betzes which causes the isozymes of the two cultivars to differ in mobility. Dosage effects can provide evidence for the presence of an alien gene even when its product is coincident in mobility with an isozyme of the recipient variety (Tang & Hart, 1975; Hart *et al.* 1976). However, mobility differences are desirable for variation in relative intensities between isozymes is not as readily detected as is variation in the presence or absence of isozymes. (See discussion of GOT-3 in RESULTS above).

We have identified and determined the chromosomal location of four Betzes isozyme structural genes. There can be little doubt that *Adh-H1* and *Got-H2* are homoeologous to the *Adh-1* and *Got-2* sets, respectively, of Chinese Spring, since the evidence presented here indicates that the products of these genes associate with the products of the respective wheat genes to form active enzymes. Homoeology of *Ep-H1* and *Amp-H1* with the *Ep-1* and *Amp-1* sets of Chinese Spring, respectively, is also likely, based on their similar developmental and tissue specificity. (For a discussion of the types of investigations that can be used to assess homoeologies between isozymes, see Hart, 1979).

The degree of similarity between *Adh-H1* and the *Adh-1* set of Chinese Spring and between *Got-H2* and the *Got-2* set is striking. In those properties observable with the zymogram technique, there has been no greater divergence between the products of the barley gene and the products of the wheat homoeoalleles than there has been between the three products of the hexaploid wheat homoeoallelic sets. The latter have been observed to have diverged only in that one protomer produced by each set has a different electrophoretic mobility than the other two protomers. The available evidence indicates that in heptaploids and chromosome addition lines the barley and wheat structural genes each produce approximately the same quantity of protomer, that the four protomers associate approximately randomly to produce the active dimeric molecules, and that each of the dimeric types produced is approximately equally active. This suggests not only that there has been little divergence in the genes which encode the protomers, but as well that there has been little divergence in the genes which regulate the ADH and GOT-2 isozymes. Essentially identical findings have been reported for four homoeologous *Secale cereale-T. aestivium* structural gene sets and one *Agropyron elongatum-T. aestivium* set. This includes the sets which encode the GOT-2 and GOT-3 isozymes (Tang & Hart, 1975), alcohol dehydrogenase (Irani & Bhatia, 1972; Tang & Hart, 1975), and an esterase system (Barber *et al.*, 1968, 1969) of the former species pair and the GOT-3 set of the latter species pair (Hart *et al.*, 1976). *Secale*, *Agropyron*, and *Triticum* are members of the subtribe Triticinae and *Hordeum* is a member of the subtribe Hordeinae of the tribe Triticeae.

The isozyme gene locations that have been determined in Betzes provide evidence

for at least partial homoeology between three Betzes chromosomes and three Chinese Spring chromosome sets. The Chinese Spring *Adh-1* set is located in the chromosomes of homoeologous group 4, the *Got-2* and *Amp-1* sets in group 6, and the *Ep-1* set in group 7 (see Hart, 1979, for references). The ADH and EP genes of Betzes are located in chromosomes 4 and 1, respectively, and the GOT-2 and AMP genes in chromosome 6. The location of Betzes *Got-H2* and *Amp-H1* in the same chromosome is of particular interest, for *Got-2* and *Amp-1* genes are syntenic in Chinese Spring wheat (Hart & Langston, 1977), Imperial rye (Hart, in preparation) and *Agropyron elongatum* (Hart & Tuleen, in preparation).

The isozyme gene locations determined thus far in Betzes are fully consistent with the gene synteny groups of Chinese Spring and provide evidence for homoeology of Betzes chromosomes 4, 6, and 1 with the chromosomes of Chinese Spring homoeologous groups 4, 6, and 7, respectively. A large number of gene locations must be determined and chromosome substitution studies conducted before the homoeologous relationships between the chromosomes of these two species may be fully assessed. However, the results obtained to date are consistent with conservation of the gene synteny groups which they inherited from their common ancestor.

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5. REFERENCES

Barber, H. N., Driscoll, C. J., Long, P. M. & Vickery, R. S. (1968). Protein genetics of wheat and homoeologous relationships of chromosomes. *Nature* **218**, 450-452.

Barber, H. N., Driscoll, C. J., Long, P. M. & Vickery, R. S. (1969). Gene similarity of the *Triticinae* and the study of segmental interchanges. *Nature* **222**, 897–898.

Bergman, J. W. (1972). Chromosome locations of genes controlling esterase and malate dehydrogenase isozymes in *Triticum*. Ph.D. dissertation, North Dakota State University, U.S.A.

Bergman, J. W. & Maan, S. S. (1973). Genetic control of isozymes in wheat-rye addition lines with rye or wheat cytoplasm. *Proceedings of the Fourth International Wheat Genetics Symposium*, pp. 329–335.

Brown, A. H. D., Nevo, E., Zohary, D. & Dagan, O. (1978). Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). *Genetica* **49**, 97–108.

Cauderon, Y., Autran, J. C., Joudrier, Ph. & Kobrehel, K. (1978). Identification de chromosomes d'*Agropyron intermedium* impliqués dans la synthèse des gliadines, des β-amylases et des peroxydases à l'aide de lignees d'addition Blé × *Agropyron*. *Annales Amélioration Plantes* **28**, 257–267.

Driscoll, C. J. (1975). First compendium of wheat-alien chromosome lines. *Annual Wheat Newsletter* **21**, 16–32.

Driscoll, C. J. & Sears, E. R. (1971). Individual addition of the chromosomes of 'Imperial' rye to wheat. *Agronomy Abstracts*, p. 6.

Hart, G. E. (1970). Evidence for triplicate genes for alcohol dehydrogenase in hexaploid wheat. *Proceedings of the National Academy of Sciences, U.S.A.* **66**, 1136–1141.

Hart, G. E. (1973). Homoeologous gene evolution in hexaploid wheat. *Proceedings of the Fourth International Wheat Genetics Symposium*, pp. 805–810.

Hart, G. E. (1975). Glutamate oxaloacetate transaminase isozymes of *Triticum*: Evidence for multiple systems of triplicate structural genes. In *Isozymes*, vol. III (ed. C. L. Markert), pp. 637–657. Academic Press.

HART, G. E. (1978). Chromosomal arm locations of *Adh-R1* and an acid phosphatase structural gene in Imperial rye. *Cereal Research Communications* **6**, 123–133.

HART, G. E. (1979). Genetical and chromosomal relationships among the wheats and their relatives. *Stadler Genetics Symposium* **11**, 9–29.

HART, G. E., ISLAM, A. K. M. R. & SHEPHERD, K. W. (1977). Isozyme expression in wheat-barley addition lines. *Proceedings of the 24th Annual Meeting of the Genetics Society of Australia* **263** (Abstract).

HART, G. E. & LANGSTON, P. J. (1977). Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. *Heredity* **39**, 263–277.

HART, G. E., MCMILLIN, D. E. & SEARS, E. R. (1976). Determination of the chromosomal location of a glutamate oxaloacetate transaminase structural gene using *Triticum-Agropyron* translocations. *Genetics* **83**, 49–61.

HVID, S. & NIELSEN, G. (1977). Esterase isoenzyme variants in barley. *Hereditas* **87**, 155–162.

IRANI, B. N. & BHATIA, C. R. (1972). Chromosomal location of alcohol dehydrogenase gene(s) in rye, using wheat-rye addition lines. *Genetica* **43**, 195–200.

ISLAM, A. K. M. R. (1980). Identification of wheat-barley addition lines with N-banding of chromosomes. *Chromosoma* **76**, 365–373.

ISLAM, A. K. M. R. & SHEPHERD, K. W. (1980). Cytological abnormalities in wheat:barley hybrids and their derivatives. (Submitted for publication.)

ISLAM, A. K. M. R., SHEPHERD, K. W. & SPARROW, D. H. B. (1978). Production and characterization of wheat-barley addition lines. *Proceedings of the Fifth International Wheat Genetics Symposium*, pp. 365–371.

KAHLER, A. L. & ALLARD, R. W. (1970). Genetics of isozyme variants in barley. I. Esterases. *Crop Science* **10**, 444–448.

KOBREHEL, K. (1978). Identification of chromosome segments controlling the synthesis of peroxidases in wheat seeds and in transfer lines with *Agropyron elongatum*. *Canadian Journal of Botany* **56**, 1091-1094..

MACDONALD, T. & SMITH, H. H. (1972). Variation associated with an *Aegilops umbellulata* chromosome segment incorporated in wheat. II. Peroxidase and leucine aminopeptidase isozymes. *Genetics* **72**, 77–86.

MAY, C. E., VICKERY, R. S. & DRISCOLL, C. J. (1973). Gene control in hexaploid wheat. *Proceedings of the Fourth International Wheat Genetics Symposium*, pp. 843-849.

NAKAI, Y. (1976). Isozyme variations in *Aegilops* and *Triticum*. III. Chromosomal basis of the esterase isozyme production in different organs of Chinese Spring wheat. *Botanical Magazine, Tokyo* **89**, 219–234.

NIELSEN, G. & FRYDENBERG, O. (1971). Chromosome localization of the esterase loci *Est*-1 and *Est*-2 in barley by means of trisomics. *Hereditas* **67**, 152–154.

O'MARA, J. G. (1940). Cytogenetic studies on Triticale. I. A method for determining the effects of individual Secale chromosomes on Triticum. *Genetics* **25**, 401–408.

RILEY, R. & CHAPMAN, V. (1958). The production and phenotypes of wheat–rye chromosome addition lines. *Heredity* **12**, 301-315.

RILEY, R. & KIMBER, G. (1966). The transfer of alien genetic variation to wheat. *Annual Report of Plant Breeding Institute, Cambridge*. 1964–1965, pp. 6–36.

SEARS, E. R. (1972). Chromosome engineering in wheat. *Stadler Genetics Symposium* **4**, 23–38.

SEARS, E. R. (1975). The wheats and their relatives. In *Handbook of Genetics*, vol. II (ed. R. C. King), pp. 59–91. Plenum Press.

SHEPHERD, K. W. (1973). Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. *Proceedings of the Fourth International Wheat Genetics Symposium* pp. 745–760.

TANG, K. W. & HART, G. E. (1975). Use of isozymes as chromosome markers in wheat-rye addition lines and in triticale. *Genetical Research* **26**, 187–201.

UPADHYA, M. D. (1968). Peroxidase isoenzyme associated with an *Aegilops umbellulata* chromosome segment transferred to Chinese Spring (*Triticum aestivum*). *Experientia* **24**, 613–614.

WOLF, G. & RIMPAU, J. (1977). Evidence for cytoplasmic control of gene expression in higher plants. *Nature* **265**, 470–472.

YANG, S. Y. (1971). *Appendix: Studies in Genetics. University of Texas Publications*. 6(7103), 85–90.