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Shikimate dehydrogenase – a biochemical marker for group 5 chromosomes in the Triticinae

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SUMMARY

Structural genes for the isozymes of shikimate dehydrogenase (*E.C.* 1.1.1.25) have been located on chromosome arms 5AS, 5BS and 5DS of wheat, 5RS of cereal rye and on chromosome $5C^u$ of *Aegilops umbellulata* by electrophoretic techniques. This character provides a useful genetic marker for these previously unmarked chromosome arms, and the results support the notion of the conservation of gene synteny groups within the Triticinae.

1. INTRODUCTION

The genes controlling isozyme phenotypes in hexaploid wheat and its relatives have proved to be valuable markers in genetic and evolutionary studies with these species. So far, at least 57 structural genes have been assigned to chromosomes and/or chromosome arms of wheat by application of zymogram techniques to aneuploid stocks (Hart, Islam & Shepherd, 1980). These genes are distributed over 18 of the 21 different wheat chromosomes, and in many cases it has been demonstrated that the genes occur as homoeoalleles in the three genomes. To date the only wheat chromosomes lacking such markers are those of homoeologous group 2.

When these isozyme studies were extended to other genomes occurring in the relatives of wheat, additional homoeoalleles were recognized, and it was concluded that there was much conservation of gene synteny within the Triticinae (Hart, 1979*a*). The marked success of these isozyme studies has stimulated us to search for other enzyme systems which could be used to supply new genetic markers for wheat chromosomes and to extend the information on gene synteny relationships in the Triticinae.

In this paper, we report our results with shikimate dehydrogenase, which provides a new marker gene for group 5 chromosomes of wheat and some other species in the Tribe Triticeae.

2. MATERIALS AND METHODS

The following plant materials were used:

Twenty-two nullisomic-tetrasomic combinations of wheat (Sears, 1966), including at least one nullisomic for each wheat chromosome except 2A and 4A; ditelocentric stocks involving 2AL, 4Aα and the group 5 chromosomes (Sears, 1954).

The amphiploid of wheat and cereal rye (cv. Imperial) together with the set of seven derived addition lines (Driscoll & Sears, 1971) and the ditelocentric addition of 5RL (Chang, Kimber & Sears, 1973).

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Translocation lines involving 4Aα-5RL (Driscoll & Sears, 1965) and 5BL-5RL (Sears, 1967).

The amphiploid of wheat and *Ae. umbellulata*, the five addition lines isolated by Kimber (1967) and addition 'F' from seed originally produced by Riley and Chapman. A set of lines having $5C^u$ substituted for the group 5 chromosomes of wheat (Chapman & Riley, 1970).

Six of the seven possible addition lines of barley (cv. Betzes) chromosomes to wheat (Islam, Shepherd & Sparrow, 1981).

All the above lines are derived from wheat cv. Chinese Spring and have been maintained in this laboratory.

For isozyme analyses, seed was germinated on moist filter paper at 23 °C and kept in the dark to provide etiolated coleoptiles. Crude extracts were obtained by maceration of tissue (etiolated coleoptiles after 5 days growth, embryos after 40 h imbibition) in a Labconic 0·4 ml centrifuge tube, containing 50 μl of a buffer (pH 7·5) consisting of 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. The extracts were centrifuged at 30000 ***g*** for 15 min and 30 μl of the supernatant was loaded into the gels. Centrifugation and electrophoresis were carried out at 2–5 °C.

The gel consisted of a polyacrylamide slab (14 cm × 9 cm × 2 mm) made up of a 1 cm stacking gel (2·5 % acrylamide, 0·6 % bis, pH 6·9) and an 8 cm running gel (7 % acrylamide, 0·2 % bis, pH 8·9). The buffer at anode and cathode was 4 mM-Tris-HCl, 40 mM-glycine at pH 8·5. Electrophoresis was carried out at a constant current of 1·2 mA/cm for $1\frac{1}{2}$ h, 2 mA/cm for 1 h and finally 2·5 mA/cm for $2\frac{1}{2}$ h. Gels were stained in a mixture of 24 mg Tris, 0·1 mg NADP, 0·4 mg $MgCl_2$, 0·25 mg NBT, 0·04 mg PMS and 0·5 mg shikimic acid per ml of solution brought to pH 8·0 with HCl (adapted from Brown & Munday, 1982). Gels were fixed in 7 % acetic acid after staining.

Usually the clearest bands were obtained from the embryo-derived samples.

3. RESULTS AND DISCUSSION

The shikimate dehydrogenase (SKDH) (*E.C.* 1.1.1.25) phenotype of euploid Chinese Spring consists of two distinct bands, the slower band (Band 1) being somewhat thicker and more intense than the faster Band 2 (Fig. 1, Lanes 1, 14). Occasionally an additional third band, faster than Band 2 is observed; but because of its inconsistency in the euploid control, it is thought to be an artifact of electrophoresis or possibly due to a conformer molecule, and has thus been excluded from the analysis.

All of the nullisomic–tetrasomic and ditelocentric stocks of Chinese Spring examined, except those involving group 5 chromosomes gave an identical phenotype to that of the euploid. The absence of chromosome 5A, or its short arm, caused the loss of Band 2 (Fig. 1, Lanes 2, 3, 4). Furthermore, four doses of this chromosome result in a strengthening of Band 2 relative to euploid (Fig. 1, Lanes 5, 8, 11). These results indicate that this band is coded for by a gene on chromosome 5AS. Although Band 1 does not completely disappear upon the removal of any group 5 chromosome pair from the wheat genome, its relative staining intensity is decreased when the sum of the doses of chromosome arms 5BS and 5DS is reduced below the four doses present in euploid wheat. Thus it is lighter staining than euploid in nulli 5B tetra 5A (Fig. 1, Lane 5) and nulli 5D tetra 5A (Fig. 1, Lane 8), but not in nulli 5B tetra 5D (Fig. 1, Lane 6) or nulli 5D tetra 5B (Fig. 1, Lane 9), while ditelo 5BL (Fig. 1, Lane 7) and ditelo 5DL (Fig. 1, Lane 10) are similar to their respective nullisomic phenotypes. It is thus concluded that the chromosome arms 5BS and 5DS each carry a gene coding for an isozyme with the same mobility which together constitute Band 1.

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The long arms of the group 5 chromosomes carry two sets of triplicate isozyme genes, coding for Lipoxygenase-2 and Alcohol Dehydrogenase-2 (see Hart, 1979*a*), and have been shown to be homoeologous in studies of meiotic pairing (Riley & Chapman, 1964). The sharing of genes controlling SKDH amongst the short arms of the chromosomes of this group suggests that this homoeology can be extended to the whole chromosome. The genes have been assigned the gene symbols *Skdh-A1*, *Skdh-B1* and *Skdh-D1*.

Following the successful location of genes controlling SKDH to homoeologous group 5 chromosomes of wheat, these studies were extended to include other genomes within the Triticinae and more widely within the Triticeae. Wheat-alien addition lines involving chromosomes of cereal rye, *Ae. umbellulata* and barley were examined for this purpose.

'Imperial' rye has a single SKDH band of lower mobility than Band 1 of Chinese Spring (Fig. 2, Lane 2). This rye isozyme is expressed in a wheat background, since the phenotype of the Chinese Spring – Imperial amphiploid represents the additive pattern of the two parents (Fig. 2, Lane 3). Six of the seven wheat–rye addition lines possessed a SKDH phenotype similar to that of Chinese Spring. However, addition line 5R gave a phenotype identical to the amphiploid (Fig. 2, Lane 4), indicating the presence of the gene for the rye isozyme on this rye chromosome. The translocation line 4Aα-5RL (Fig. 2, Lane 5) and the ditelocentric addition of 5RL to wheat have an identical phenotype to euploid wheat, suggesting that the gene is located on chromosome arm 5RS. The translocation line 5BL–5RL (Fig. 2, Lane 6) lacks both the rye band and the 5BS band and its phenotype resembles ditelo 5BL as expected.

Ae. umbellulata possesses a single SKDH band of similar mobility to Chinese Spring Band 2 (Fig. 3, Lane 11). The amphiploid between *Ae. umbellulata* and Chinese Spring shows a phenotype similar to a tetra-5A stock (Fig. 3, Lane 8), as expected if the parental patterns are additive as in wheat and rye. Of the six addition lines examined, only one – $5C^u$ – gave a pattern resembling the amphiploid (Fig. 3, Lane 7). This chromosome has been substituted for wheat chromosomes of group 5 (Chapman & Riley, 1970), and these stocks were expected to provide further information on the chromosomal control of SKDH in *Ae. umbellulata*. Thus it was predicted that the substitution $5C^u$ (5A) would possess the *umbellulata* band in addition to the single band characteristic of nulli 5A (Fig. 3, Lane 3) and these two bands were observed in this stock (Fig. 3, Lane 2). Furthermore, it was expected that the $5C^u$ (5B) and $5C^u$ (5D) phenotypes would resemble those of nulli 5B tetra 5A and nulli 5D tetra 5A, respectively. Whereas the phenotype of $5C^u$ (5D) (Fig. 3, Lane 6) agreed with this expectation, $5C^u$ (5B), in repeated samples, gave only a single band (Fig. 3, Lane 4). Homoeologous recombination is known to occur in this stock due to the absence of chromosome 5B (Chapman & Riley, 1970); thus it is possible that the seed tested was deficient for genes on chromosome arm 5DS as well as for chromosome 5B caused by homoeologous recombination in an earlier generation.

Barley (cv. Betzes) possesses a doublet SKDH band intermediate in mobility between Chinese Spring Bands 1 and 2 (Fig. 3, Lane 12). The results of analysis of the six addition lines of Betzes chromosomes to Chinese Spring have proved inconclusive. Barley chromosome 7 has been tentatively assigned to homoeologous group 5 based on gross plant morphology (Islam & Shepherd, 1982), and A. H. D. Brown (pers. comm.) believes this chromosome to carry the SKDH gene based on separation by starch gel electrophoresis. In our study, the phenotype of this addition line (Fig. 3, Lane 13) consisted of two bands, as in Chinese Spring. Band 1 was observed to be more heavily stained than in the euploid phenotype in some samples. However, this difference was not sufficiently consistent to assign the locus of the barley SKDH gene to this chromosome. Furthermore, the pattern of this phenotype did not conform to the additive pattern observed in hybrids between wheat and rye or *Ae. umbellulata*.

The failure to identify any hybrid bands in the phenotype of the wheat–rye amphiploid

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indicates that SKDH is probably a monomeric enzyme. Of the 19 isozymes of the Triticeae listed by Hart (1979*a*), 12 are monomeric, while the remainder are dimeric. Within wheat, *Skdh-A1* has the greatest activity (as detected by this assay), followed by *Skdh-D1* and *Skdh-B1*. Differences in activity between isozymes have been noted for phosphoglucose isomerase in wheat (Hart, 1979*b*) and alcohol dehydrogenase in maize (Freeling, 1973).

The location of the genes controlling SKDH to the short arms of the group 5 chromosomes in wheat provides the first genetic marker for these chromosome arms. The presence of *Skdh-R1* on 5RS and *Hp* on 5RL gives useful markers for both arms of this rye chromosome, the SKDH marker having the advantage that it can be detected in seedlings whereas *Hp* is only expressed at heading.

The existence of genes controlling a similar biochemical marker on the group 5 chromosomes of wheat, rye and *Ae. umbellulata* lends further support to the notion of the conservation of gene synteny groups inherited from the common ancestor of the Triticinae, as proposed by Hart, Islam & Shepherd (1980).

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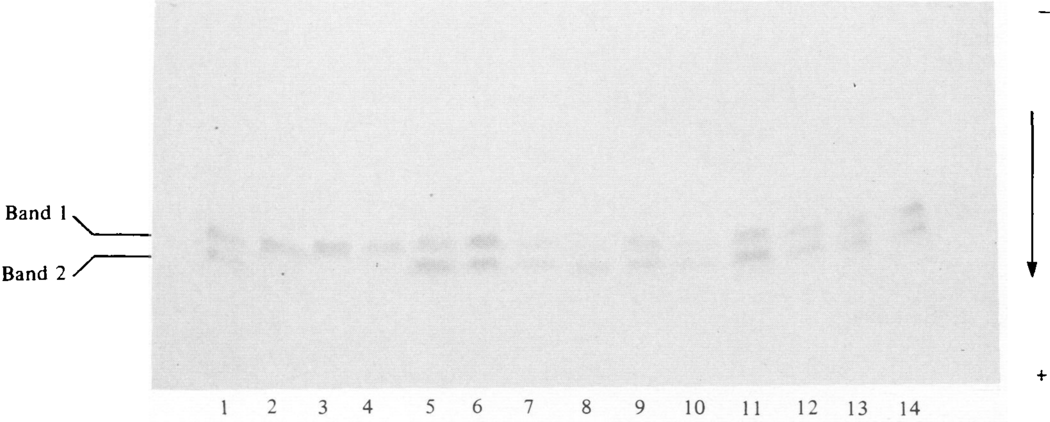

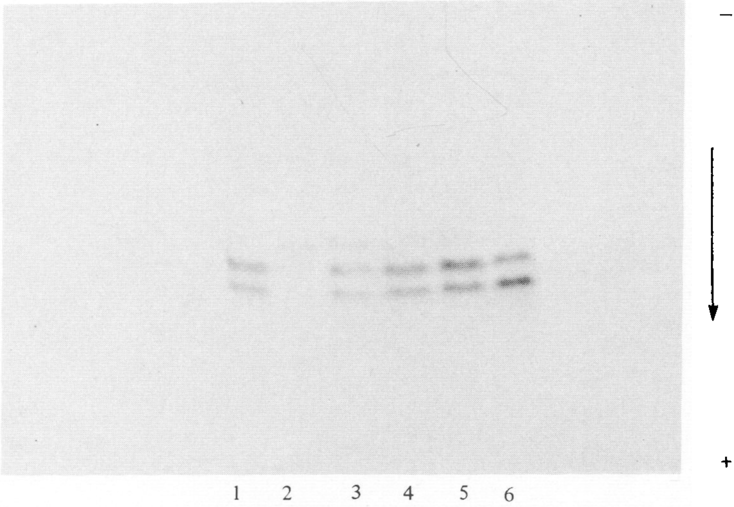

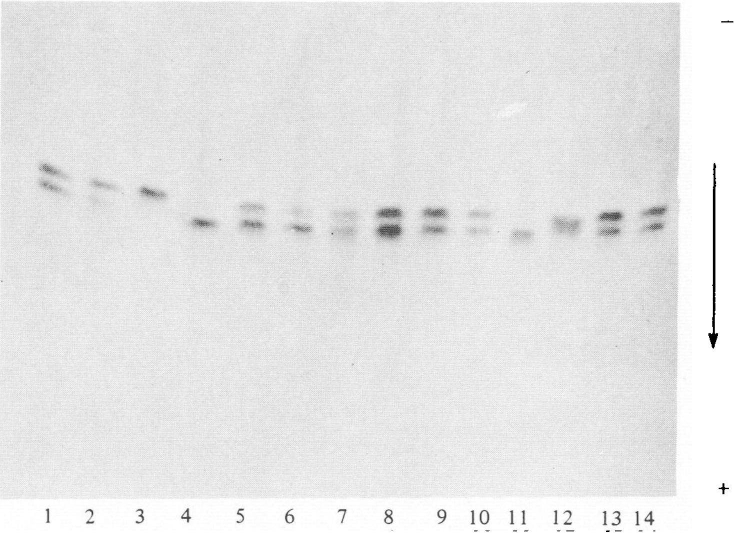

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EXPLANATION OF PLATE

Plate 1

Fig. 1. Shikimate dehydrogenase (SKDH) zymogram phenotypes of (1) Chinese Spring wheat (CS); (2) nyllisomic 5A-tetrasomic 5B (N5AT5B); (3) N5AT5D; (4) ditelocentric 5AL (dit 5AL): (5) N5BT5A; (6) N5BT5D; (7) dit 5BL; (8) N5DT5A; (9) N5DT5B; (10) dit 5DL; (11) tetrasomic 5A (T5A); (12) T5B; (13) T5D; (14) CS. Samples derived from 40 h embryos.

Fig. 2. SKDH zymogram phenotypes of (1) CS; (2) Imperial rye; (3) amphiploid of CS and Imperial; (4) wheat–rye addition line 5R; (5) translocation line 4Aα-5RL; (6) translocation line 5BL-5RL.
Samples derived from 40 h embryos.

Fig. 3. SKDH zymogram phenotypes of (1) CS; (2) substitution line of $5C^u$ for 5A ($5C^u$ (5A)); (3) dit 5AL; (4) $5C^u$ (5B); (5) dit 5BL; (6) $5C^u$ (5D); (7) amphiploid of CS and *Ae. umbellulata*; (8) wheat – *Ae. umbellulata* addition line $5C^u$ ($CS + 5C^u$); (9) $CS + 1C^u$; (10) CS; (11) *Ae. umbellulata*; (12) Betzes barley; (13) wheat–barley addition line 7; (14) CS.
Samples derived from 40 h embryos.

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