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The genetic control of hexokinase isozymes in wheat

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SUMMARY

In extracts of mature wheat grains, 13 hexokinase isozymes were distinguished by IEF. The genes controlling the production of five isozymes were located on chromosome arms 1BS, 1DS and 3BS by nullisomic analysis. The three loci, part of two homoeoallelic series (*Hk-1* and *Hk-2*) are designated *Hk-B1*, *Hk-D1* and *Hk-B2* respectively. Analysis of chromosome 1D short-arm terminal deletions indicated the *Hk-D1* locus to be located proximally to the glucose phosphate isomerase locus, *Gpi-D1* on the short arm. Three variant HK phenotypes were distinguished amongst 55 hexaploid wheats examined. Analysis of seven Chinese Spring/*Agropyron elongatum* chromosome addition lines showed that *Ag. elongatum* isozymes were expressed in the wheat background in additions IV and V.

1. INTRODUCTION

There is currently much interest in the development of isozyme markers for the identification of the chromosomes of hexaploid wheat (*Triticum aestivum*, $2n = 6x = 42$) and its related species. The study of isozyme marker genes which allows individual chromosomes or segments of chromosomes to be followed through complex genetic manipulations is proving to be a powerful cytogenetic tool. Apart from their application in genetic analyses, enzyme markers are presently being successfully used during the development and verification of wheat inter-varietal chromosome substitution lines and alien-wheat chromosome addition and substitution lines. The ultimate aim, however, is to enable small segments of wheat or alien chromosomes to be rapidly identified in breeding lines where such segments include the marker gene linked with a potentially useful gene. For example, chromosome segments carrying genes conferring disease resistance could be readily screened in situations where present tests are time-consuming and environmentally sensitive.

Only a relatively small number of enzyme systems have been characterized in wheat. Although approximately 70 loci have been identified on specific chromosome arms (Hart, 1982), no single chromosome has more than seven suitable marker loci (e.g. 6B, 6D) and there are significant gaps in the map, particularly with respect to the group 2 chromosomes, where no enzyme structural genes have been identified. A more complete system of isozyme marker genes is therefore required in wheat before these systems can be exploited regularly, especially with a view to transferring genes of agronomic importance from related species.

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Hexokinase (ATP: D-hexose-6-phosphotransferase, E.C. 2.7.7.1) catalyses the phosphorylation of D-hexose sugars by ATP to yield D-hexose-6-phosphate, and is an enzyme for which no genetic study has been reported in wheat.

Hexokinase activity, which is present in soluble and particulate fractions from a variety of plant species and tissues (Feingold & Avigad, 1980), has been demonstrated in wheat, both in primary leaves (Lunderstadt, 1966) and in wheat germ (Saltman, 1953). Four isozymes from wheat germ have been isolated (Meunier, Buc & Ricard, 1971; Higgins & Easterby, 1974).

The purpose of the present study was to investigate the isozymes of hexokinase in wheat endosperm by isoelectric focusing and to identify the chromosomal control and if possible the location(s) within the chromosome(s) of the structural genes.

2. MATERIALS AND METHODS

(i) Genotypes

(a) *Aneuploids and alien chromosome addition lines*

The nullisomic-tetrasomic (NT) and ditelosomic series in the variety Chinese Spring (CS) developed by Professor E. R. Sears of the University of Missouri, Columbia were used in the nullisomic analysis. The following NT lines were not available: CSN2A-T2B, CSN4A-T4B, CSN4D-T4B, CSN6B-T6D.

The seven addition lines of *Agropyron elongatum* chromosomes to wheat produced by Dr J. Dvorak of the University of California, Davis (Dvorak & Knott, 1974) were also investigated.

(b) *Varieties*

Fifty-five hexaploid genotypes were examined: Alpe 3, Atlas 66, Azteca 67, Bersée, Besostaya 1, Bounty, Brigand, C306, Capitole, Cappelle-Desprez, Champlein, Cheyenne, Chinese Spring, Ciano 67, Darius, Desprez 80, Dillikot, Glennson, Hahn 'S', Highbury, Hobbit, Hobbit 'S', Holdfast, Hope, Jitpur, Koga II, Little Joss, Lomsom, Lutescens 62, Manella, Mara, Maris Dove, Maris Huntsman, Maris Ranger, Maris Sportsman, Minister Dwarf, Poros, Sappo, Sava, Sicco, Spica, Sprint 3, Sprint 4, Synthetic (amphidiploid of *Triticum dicoccum* × *Aegilops squarrosa* produced by Professor E. R. Sears, of University of Missouri, Columbia (McFadden and Sears, 1946)), SD1, SD2, Timmo, Timstein, Tom Thumb, *T. macha*, *T. spelta*, Veery 'S', Vilmorin 27, VPM and Wembley.

(ii) *Enzyme extraction*

For each extract, a single mature dry grain was milled in a microhammer mill (Paulis & Wall, 1979). Extraction solution (1:1 w/v of 20% sucrose and 0·01 M dithiothreitol at 4 °C) was added to the flour and the mixture sonicated briefly and allowed to stand overnight. The extracts were centrifuged at 12000 ***g*** for 15 min. The supernatant was used immediately for isoelectric focusing. All operations were carried out at 4 °C.

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(iii) *Isoelectric focusing*

Flat-bed IEF was carried out using Multiphor apparatus (LKB) with a self-regulating power supply (LKB) on Ampholine PAG plates pH 4·0–6·5 (LKB). A constant power of 1 W/cm width of gel was applied with cooling at 4 °C. Small filter-paper pieces (Whatman 3MM, 5 × 10 mm) were soaked with 40 μl of extract and applied to the gel 20 mm from the cathode, after 30 min prefocusing. The sample application pieces were removed after 30 min and electrofocusing was terminated after a further 2 h. pH gradients were measured by taking readings at 10 mm intervals across the gel with a surface electrode.

(iv) *Enzyme visualization*

Gels were stained for hexokinase activity with: 40 ml 0·2 M Tris buffer pH 7·5, 9 mg glucose, 100 mg ATP (NA_2 salt), 7·5 mg NADP, 5 mg MTT, 5 mg PMS, 100 μl glucose-6-phosphate dehydrogenase (140 units/ml); 10 ml 0·02 M-$MgCl_2$. Staining was carried out in the dark at 37 °C.

3. RESULTS

(i) *Nullisomic analysis*

In extracts from mature whole grains of Chinese Spring (CS), 13 isozymes can be consistently distinguished, with pIs ranging from 5·15 to 5·9 (numbered 1–13 from the anodal end, Fig. 1).

Analysis of all the available nullisomic–tetrasomic combinations in CS showed two chromosome groups, groups 1 and 3, to be implicated in the production of hexokinase (Fig. 2). Nullisomy for chromosome 1B (CSN1B–T1A, CSN1B-T1D) results in the removal of isozyme band 12. The structural gene(s) encoding band 12 can be further located to the short arm of 1B, as band 12 is absent from the zymogram of CSDT1BL but present in CSDT1BS (Fig. 2). Removal of chromosome 1D (CSN1D-T1A, CSN1D-T1B) causes removal of band 13. Since the isozyme is also absent in the CSDT1DL zymogram, this structural gene must be located on the short arm of 1D. These two loci are assumed to form part of a series of homoeoloci, *Hk-1*, and have been designated *Hk-B1* and *Hk-D1*.

Removal of chromosome 3B (CSN3B-T3A, CSN3B-T3D) causes the removal of isozymes 3, 4 and 5. The short arm of 3B is implicated as these bands are absent from the zymogram of CSDT3BL. The locus encoding isozymes 3, 4 and 5 has been designated *Hk-B2*.

No conclusive difference in hexokinase isozymes was apparent when chromosomes 1A, 3A or 3D were nullisomic, and the remaining eight isozymes were not able to be assigned to a chromosome by nullisomic analysis.

(ii) *Varietal variation*

The 55 genotypes surveyed for hexokinase showed very conserved zymograms and only three variants were observed (Fig. 3) although differences in band

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intensities were relatively common. Cheyenne had a hexokinase zymogram with bands 1 and 2 considerably reduced in intensity. Ciano 67 also had bands 1 and 2 much reduced in intensity. In addition, band 3 (3BS) was absent in Ciano 67 and band 12 (1BS) was increased in intensity. In Mara, band 13 (1DS) was absent and bands 3 and 4 appeared very intense.

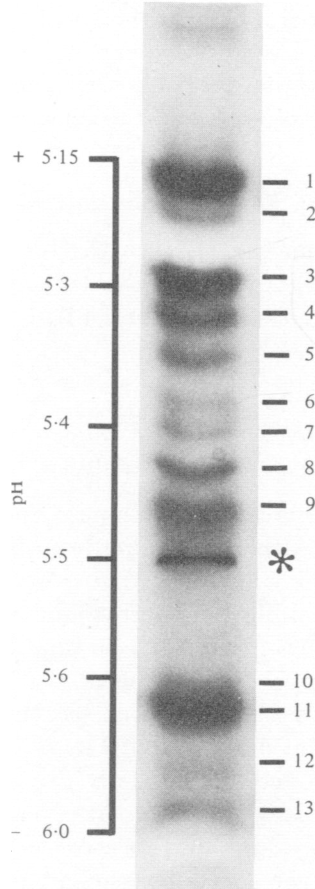

Fig. 1. Chinese Spring euploid hexokinase zymogram. * indicates the position of the anodal end of the sample application wick which is seen in all zymograms and does not represent an isozyme band.

(iii) *Alien addition lines*

Although the accession of *Agropyron elongatum* ($2n = 2x = 14$) was not the same as that used by Dvorak to make the CS/*Ag. elongatum* chromosome addition series, two hexokinase isozymes which are unique to *Ag. elongatum* are expressed in additions IV and V (Fig. 4). This suggests that the *Agropyron* chromosomes in additions IV and V are homologous, at least for the hexokinase locus, and that they are in part homoeologous to the chromosomes of wheat group 1 or 3. Because the original *Ag. elongatum* parent was unavailable, interpretation of the addition

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chromosome zymograms is difficult. Bands are evident in the addition I and III zymograms which are not seen in either parent. In additions II and VI, some CS bands are absent. However, resolution of this region of the zymogram is poor, and similar inconsistencies can be seen in the NT and DT lines (Fig. 2).

In addition, a number of other alien addition series involving rye, barley and other *Aegilops* species in which differences between the alien and wheat hexokinase

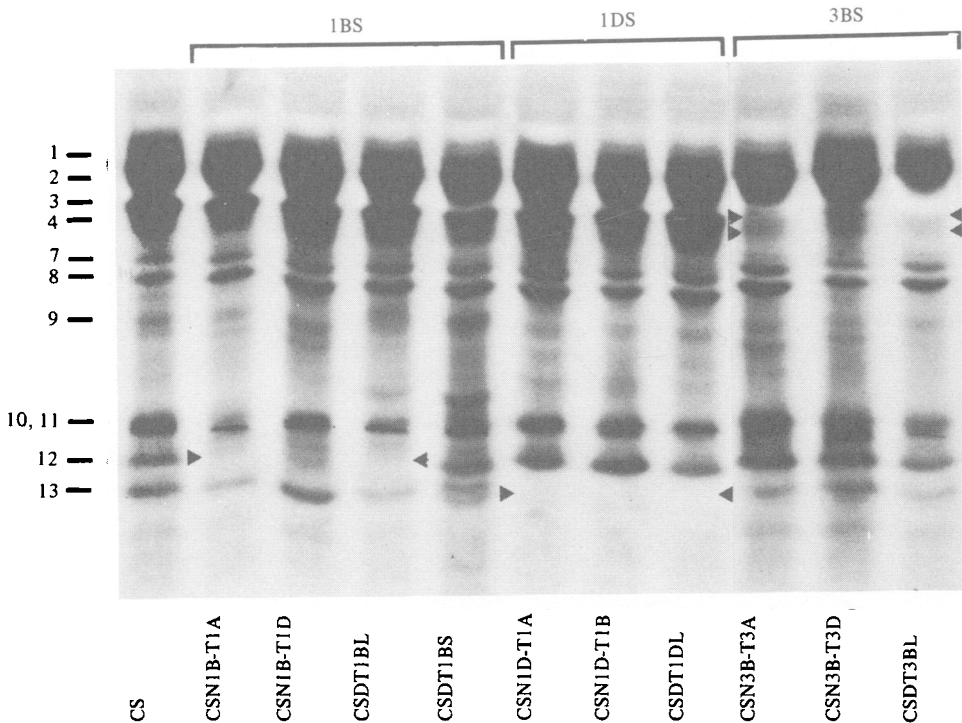

Fig. 2. Hexokinase zymogram of chromosome 1B, 1D and 3B nullisomic–tetrasomic and ditelosomic aneuploid genotypes in Chinese Spring. Arrows indicate the absence of CS isozyme bands.

zymogram were detected, and which are known to contain group 1 or 3 additions, were investigated. Expression of alien hexokinase isozymes in a wheat background genotype could not be demonstrated for any series.

(iv) *Gene localization*

Five wheat genotypes, which have been shown to have partial deletions of the short arm of chromosome 1D: Sprint 3, Darius, Alpe 3, Lomsom and Dillikot (Payne *et al.*, unpublished) were examined. All five zymograms were identical to that of CS and band 13, the isozyme controlled by chromosome 1D, was present in each case.

These genotypes have been assayed for the presence of the glucose phosphate isomerase locus (*Gpi-D1*) (Chojecki & Gale, 1982) and ω-gliadin locus (*Gli-D1*)

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(Payne *et al.* 1982), which are both located on the short arm of chromosome 1D. *Gli-D1* is 34 map units outside *Gpi-D1* which is 36 map units from *Glu-D1*, a high molecular weight glutenin subunit locus carried near the centromere on the long arm (Chojecki *et al.* 1983). It is suggested that there have been small terminal deletions of parts of the short arms of 1 D in Alpe 3, Dillikot, Darius and Lomsom,

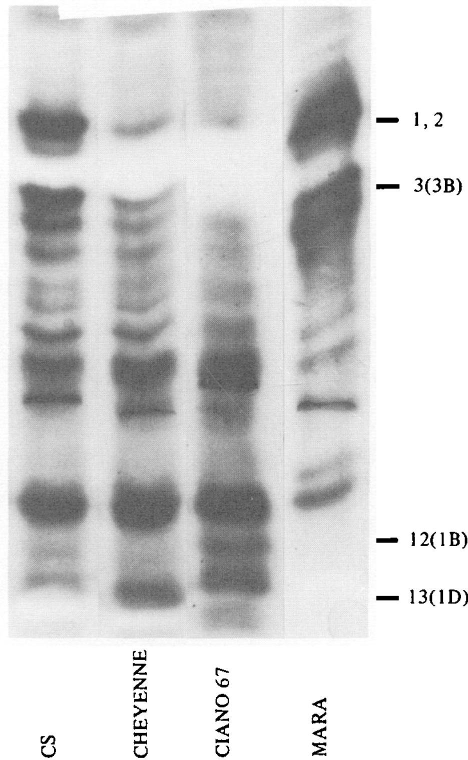

Fig. 3. Hexokinase zymograms of Chinese Spring and three varieties exhibiting variation in isozyme pattern.

where there is no expression of the *Gli-D1* locus, and a larger deletion in Sprint 3 where neither *Gli-D1* nor *Gpi-D1* is expressed (Payne *et al.*, unpublished). *Hk-D1* is active in all five genotypes. Therefore the locus is located proximally to *Gpi-D1* on the short arm of chromosome 1D.

4. DISCUSSION

The chromosomal locations of the structural genes controlling the production of hexokinase isozymes in wheat endosperm have not previously been reported. The three hexokinase loci reported here, *Hk-B1*, *Hk-D1* and *Hk-B2*, on chromosomes 1B, 1D and 3B, are probably, by analogy with other enzyme structural genes reported in wheat, e.g. ADH (Hart, 1970), part of two homoeoallelic series of loci (*Hk-1* and *Hk-2*) located on the short arms of the group 1 and 3 chromosomes

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respectively. Although the remaining chromosomes making up the two homoeoallelic series, 1A, 3A and 3D, were not shown to control the production of hexokinase isozymes, eight isozymes were not removed by nullisomy for any group 1 or 3 chromosome. The hexokinase system is therefore similar to β-amylase, where several isozymes were not removed by nullisomy for any group 4 or 5 chromosome

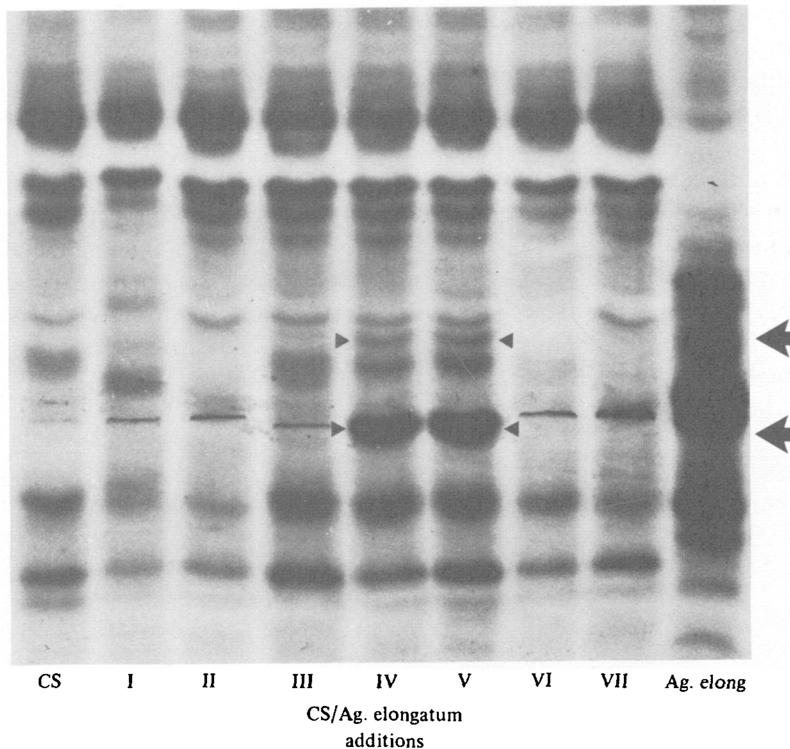

Fig. 4. Hexokinase zymograms of the Chinese Spring/*Agropyron elongatum* addition lines. Two isozyme bands (arrowed) from *Ag. elongatum* are present in additions IV and V.

(Ainsworth, Gale & Baird, 1983) rather than to α-amylase (Gale *et al.* 1983), GPI (Chojecki & Gale, 1982) or ADH (Hart, 1970), where most or all bands are accounted for as the unique products of several loci. It may well be that chromosomes 1A, 3A and 3D do control the production of hexokinase isozymes but that these co-focus with isozymes controlled by another chromosome, using the IEF technique employed here.

The hexokinase system appears fairly conserved in that only three variant phenotypes were detected which were consistently different from CS. Variation in band intensity occurred in a number of varieties but it was difficult to assess. Only in Cheyenne, Ciano and Mara were bands absent. No novel isozyme was found. Null variants appear to be the most common type of allelic variant as has been shown for β-amylase (Ainsworth *et al.* 1983) and esterase (Ainsworth *et al.*, 1984). This implies that mutations which result in the loss of enzyme activity, as

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a result either of production of a non-functional protein or there being no protein transcribed at all, are more common than mutations which alter protein structure and pI but do not affect activity.

Hexokinase may not be an ideal enzyme system for the elucidation of alien-wheat chromosome homoeologies because of the existence of two series of loci producing HK products which may not be distinct with respect to pI. This is the case for most enzyme systems with more than one homoeoallelic series of loci. A notable exception is α-amylase, where the group 6 and 7 products (α-AMY-1 and α-AMY-2) have distinctly different pIs (Nishikawa & Nobuhara, 1971).

Analysis of the CS/*Agropyron elongatum* addition series shows that the added *Agropyron* chromosomes in additions IV and V both control the production of HK isozymes and are therefore partially homologous with each other. This result is consistent with those of Hart & Tuleen (1983), who studied eleven enzyme systems and found esterase structural genes on chromosomes IV and V, indicating homoeology with wheat group 3. The homoeology of these two chromosomes is not, however, straightforward. It appears that both IV and V have the same short arm, which is homoeologous to wheat group 3, but that the long arms are homoeologous to wheat groups 7 and 2 respectively (Dvorak, 1980). The hexokinase locus in *Agropyron elongatum* is designated *Hk-E2*.

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REFERENCES

Ainsworth, C. C., Gale, M. D. & Baird, S. (1983). The genetics of β-amylase isozymes in wheat. I. Allelic variation among hexaploid varieties and intrachromosomal gene locations. *Theoretical and Applied Genetics* **66**, 39–49.

Ainsworth, C. C., Gale, M. D. & Baird, S. (1984). The genetic control of grain esterases in hexaploid wheat. 1. Allelic variation. *Theoretical and Applied Genetics*. (In the Press.)

Chojecki, A. J. S. & Gale, M. D. (1982). Genetic control of glucose phosphate isomerase in wheat and related species. *Heredity* **49**, 337–347.

Chojecki, A. J. S., Gale, M. D., Holt, L. M. & Payne, P. I. (1983). The intrachromosomal mapping of a glucose phosphate isomerase structural gene, using allelic variation among stocks of Chinese Spring wheat. *Genetical Research* **41**, 221–226.

Dvorak, J. (1980). Homoeology between *Agropyron elongatum* chromosome arms with *Triticum* chromosomes. *Canadian Journal of Genetics and Cytology* **21**, 243–254.

Dvorak, J. & Knott, D. R. (1974). Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. *Canadian Journal of Genetics and Cytology* **16**, 399–417.

Feingold, D. S. & Avigad, G. (1980). Sugar nucleotide transformations in plants. In *The Biochemistry of Plants*, vol. 3, *Carbohydrates, Structure and Function* (ed. J. Preiss), pp. 101–170. New York: Academic Press.

Gale, M. D., Law, C. N., Chojecki, A. J. S. & Kempton, R. A. (1983). Genetic control of α-amylase production in wheat. *Theoretical and Applied Genetics* **64**, 309–316.

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. (1982). Biochemical loci of hexaploid wheat (*Triticum aestivum*, $2n = 42$, Genomes AABBDD). *Genetics Maps* **2**, 373–376.

Hart, G. E. & Tuleen, N. A. (1983). Chromosomal locations of eleven *Elytrigia elongata* (= *Agropyron elongatum*) isozyme structural genes. *Genetical Research* **41**, 181–202.

https://doi.org/10.1017/S0016672300021674 Published online by Cambridge University Press

HIGGINS, T. J. C. & EASTERBY, J. S. (1974). Wheatgerm hexokinase: physical and active-site properties. *European Journal of Biochemistry* **45**, 147–160.

LUNDERSTADT, J. (1966). The effect of rust infection on hexokinase activity and carbohydrate dissimilation in primary leaves of wheat. *Canadian Journal of Botany* **44**, 1345–1364.

MEUNIER, J. C., BUC, J. & RICARD, J. (1971). Isolation, purification and characterization of wheat germ hexokinase. *FEBS Letters* **14**, 25–28.

NISHIKAWA, K. & NOBUHARA, M. (1971). Genetic studies of α-amylase isozymes in wheat. I. Location of genes and variation in tetra- and hexaploid wheat. *Japanese Journal of Genetics* **46**, 345–348.

PAULIS, J. W. & WALL, J. A. (1979). Note on mill for pulverising single kernels of cereals for isoelectric focusing. *Cereal Chemistry* **56**, 497–498.

PAYNE, P. I., HOLT, L. M., WORLAND, A. J. & LAW, C. N. (1982). Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part III. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. *Theoretical and Applied Genetics* **63**, 129–138.

PAYNE, P. I., HOLT, L. M., JACKSON, E. A., CHOJECKI, A. J. S., GALE, M. D. & BENNETT, M. D. (in preparation). Lack of expression of genes at the *Gli-D1* locus, which code for endosperm storage proteins in one variety of wheat, two varietal biotypes and numerous landraces from Nepal.

SALTMAN, P. (1953). Hexokinase in higher plants. *Journal of Biological Chemistry* **200**, 145–154.

https://doi.org/10.1017/S0016672300021674 Published online by Cambridge University Press