Theor Appl Genet (1992) 84: 528–534

Isozyme and cytological markers of some *Psathyrostachys juncea* accessions

M. D. H. M. William and A. Mujeeb-Kazi

CIMMYT, Lisboa 27, Apartado Postal 6-641,Delegación Cuauhtémoc, 06600 México, D. F., México

Received: July 8, 1991; Accepted: January 10, 1992
Communicated by K. Tsunewaki

Summary. *Psathyrostachys juncea* (synonymous to *Elymus junceus;* 2n=2x=14, NN) has unique biotic and abiotic attributes that could contribute towards wheat improvement. The effectiveness of such an intergeneric hybridization program depends greatly on being able to establish diagnostic markers of the alien chromosomes. Isoelectric focusing (IEF) analyses of six enzyme systems have identified five biochemical markers – malate dehydrogenase (MDH), esterase (EST), shikimate dehydrogenase (SKDH), phosphoglucomutase (PGM), and β-amylase (β-AMY) – to be of positive diagnostic value; glucosephosphate isomerase (GPI) banding profiles were of no definite value in the background of *Triticum aestivum* cvs 'Chinese Spring' and 'Seri-82', the potential recipients of *Ps. juncea* chromosomes. The Giemsa C-banding karyotype distinctively separates the *Ps. juncea* chromosomes from each other and from those of *T. aestivum* with little banding site polymorphisms prevalent among its accessions analyzed, indicating the usefulness of C-bands as cytological markers.

Key words: *Psathyrostachys juncea* – *Triticum aestivum* – Isozyme markers – Chromosome banding – Intergeneric hybridization

Introduction

The introgression of genetic material from alien species into hexaploid wheat (2n=6x=42) has the potential of enhancing the variability of the wheat germ-plasm currently being used in breeding as well as the prospect of incorporating desirable genes from the alien species into wheat. The characterization of alien chromosomes in the wheat background can be done by chromosome pairing, differential banding techniques, and the use of morphological, biochemical, and molecular markers. Of the later, isozyme markers have been successfully used for characterizing wheat-rye (Tang and Hart 1975), wheat-barley (Hart et al. 1980), and wheat-*Aegilops* ventricosa (Mena et al. 1989) chromosome addition lines.

Psathyrostachys is a genus of the Eurasian interior, where its species grow on rocky open slopes from the Middle East and European Russia across Central Asia to North China (Tzvelev 1976). This grass was first introduced to the USA in 1927 (Hanson 1972), and since 1950 its potential importance in revegetating depleted rangelands has been exploited. The genus contains about ten species, all of which contain the basic N genome (Jensen et al. 1990). *Ps. juncea* (synonymous to *Elymus junceus*) is known to possess a tolerance to salinity and drought (Dewey 1984), and recently it has also been reported to possess resistance to barley yellow dwarf virus (Plourde et al. 1990). These biotic and abiotic attributes of *Ps. juncea* make the species an invaluable source of germplasm for the incorporation of such traits into wheat (Mujeeb-Kazi and Asiedu 1990) and for breeding programs.

We report here on some isozyme markers and cytologically characterize *Psathyrostachys juncea* accessions in the anticipation that such data could be used in the tracking of *Ps. juncea* chromosomes in a wheat background. The isozyme markers studied are malate dehydrogenase (MDH, E.C.1.1.1.37), esterase (EST, E.C.3.1), shikimate dehydrogenase (SKDH, E.C.1.1.1.25), phosphoglucomutase (PGM, E.C.2.7.5.1), β-Amylase (β-AMY; α-1,4-glucan maltohydrolase, E.C.3.2.1.2), and glucosephosphate isomerase (GPI, D-glucose 6-phosphate ketol-isomerase, E.C. 5.3.1.9).

Correspondence to: A. Mujeeb-Kazi

Table 1. Accessions[a] used in the enzyme and C-band analyses with their geographical location and ploidy level

Accession analyzed	Geographical location of collection	Ploidy
AJC 600	USSR	2n=2x=14
PI 314668	USSR	2n=2x=14
PI 531825	China	2n=2x=14
PI 206684	Turkey	2n=2x=14
K-Asay-27	Nevada/USA	2n=2x=14
PI 531828	Idaho/USA	2n=4x=28[b]
Vinall	N. Dakota/USA	2n=2x=14
Swift	Canada	2n=2x=14

[a] All accessions received from Dr. D. R. Dewey, USDA/ARS, Logan, Utah, USA
[b] Colchicine-induced autotetraploid

Materials and methods

Germ-plasm

Psathyrostachys juncea accessions were obtained from Dr. D. R. Dewey of the USDA/ARS laboratory in Logan, Utah, USA. Eight different accessions from diverse geographical regions were utilized (Table 1). Eight individual seeds plus a composite of 15 seeds from each accession were evaluated for each enzyme system. *Triticum aestivum* cv 'Chinese Spring' and cv 'Seri-82' were used as the hexaploid wheat cultivars (2n=6x=42, AABBDD) whose banding patterns were compared with those of *Ps. juncea* (2n=2x=14, NN).

Enzyme extraction

For all of the enzyme systems, single, mature seeds of *Ps. juncea*, 'Chinese Spring', and 'Seri-82' were used. Single seeds were dehulled and ground, and the flour was extracted with 60 µl of extraction buffer for *Ps. juncea* accessions. For MDH, GPI, SKDH, and PGM the extraction buffer used was 0.2 *M* TRIS-HCl (pH 7.5). Beta-amylase was extracted with the same buffer, supplemented with 0.2 *M* 2-mercaptoethanol; for EST, 0.05 *M* Na_2HPO_4 (pH 7.5) was used. Mature grains of 'Chinese Spring' and 'Seri-82' were extracted with 300 µl of the buffer solutions used for the respective enzyme sytems. Composites of individual *Ps. juncea* accessions were extracted using 15 seeds of a particular accession with 300 µl of the respective buffer solution. The extractions were made at 4 °C for 1 h for all enzyme systems except for β-AMY, which was extracted overnight. Samples were vortex mixed, centrifuged at 20,000 rpm for 5 min, and the supernatant was utilized for enzyme analysis.

Isoelectric focusing

Isozymes of all six enzyme systems were separated by isoelectric focusing (IEF) on Ampholine PAG plates (Pharmacia). For MDH, GPI, EST, and SKDH, pH 3.5–9.5 gels were used, whereas PGM and β-AMY isozymes were separated on pH 4.0–6.5 gels. The cathode and anode buffers for the pH 3.5–9.5 gels were 1 *M* NaOH and 1 *M* H_3PO_4, respectively. For pH 4.0–6.5 gels, the cathode buffer was 0.1 *M* β-alanine and the anode buffer was 0.5 *M* H_3PO_4. Isoelectric focusing was performed on a flat-bed LKB Multiphor 11 Electrophoresis unit. Prefocusing was done for 45 min at a constant power of 12 W per gel. Paper wicks (Whatman 3 mm) were laid on the gel cm from the cathode, 20 µl of sample extracts was then applied onto the wicks, and the paper wicks were removed after 1 h. The total running time was 6 h. The isoelectric points of the isozymes of different enzyme systems were computed by running IEF standard mixtures supplied by Sigma Chemical Co.

Native polyacrylamide gel electrophoresis

Isozymes of β-AMY were separated by native polyacrylamide gel electrophoresis in addition to IEF. A running gel of 8.5% acrylamide and a stacking gel of 5.0% acrylamide were used. The seed extracts for IEF were mixed with 0.005% bromophenol-blue dye marker before being loaded onto the gel. The gel dimensions were (180 × 160 × 1.5 mm), and the runs were conducted at 60 mA until the dye front reached the bottom of the gel. The gel composition and the running buffers used were according to Davis (1964).

Staining procedure

The staining procedure for enzyme activity was adopted from Vallejos (1983) with modifications in the cases of MDH, GPI, EST, PGM, and SKDH. For EST, Fast blue RR (75 mg) and β-naphthyl acetate (30 mg) were dissolved in 5 ml acetone and filtered; 95 ml of extraction buffer was then added to the filtered solution. The staining solution for PGM contained 100 ml TRIS-HCl (pH 7.5), 1 ml $MMgCl_2$, 50 mg glucose-1-phosphate, 7.5 mg NADP, 10 mg 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2 mg phenazine-methosulfate (PMS), and 10 µl of 700 units/ml glucose-6-phosphate-dehydrogenase. For SKDH, the staining solution consisted of 100 ml TRIS-HCl (pH 7.5), 50 mg shikimic acid, 7.5 mg NADP, 10 mg MTT, and 2 mg PMS. Gels for MDH were stained with a solution of 100 ml TRIS-HCl (pH 7.5), 3 ml 1 *M* D.L. malate (pH 7.5), 12 mg NAD, 15 mg MTT, and 2 mg PMS. GPI staining was according to Chojecki and Gale (1982). Gels for all enzyme systems except β-AMY were stained at 37° in the dark until the bands developed, rinsed with water, and then left in a 10% acetic acid solution. Beta-amylase was stained by first immersing the gel in a 3% starch solution (soluble potato starch) for 20 min, rinsing with water, and then staining with a 3% acetic acid solution containing 5.4×10^{-3} *M* KI and 2.4×10^{-3} *M* iodine at room temperature.

Cytology

Somatic analysis. Seeds for *Ps. juncea* (PI 314668) were germinated and individually transplanted into peat pots. Root tips were periodically sampled from each of the ten plants and processed according to the procedure of Mujeeb-Kazi and Miranda (1985).

C-banding. After the pre-treatment some root tips were fixed in 0.1% aceto-carmine for 48 h, then squashed in 45% acetic acid, following which the cover glass was removed by the dry ice method. The dehydration, barium hydroxide [$Ba(OH)_2$] treatment, 2 × SSC incubation, and staining procedures were essentially similar to those of Bennett et al. (1977) and Jahan et al. (1990).

Photography. Representative cells were photographed on a Kodak Technical Pan Film 2415 (Estar-AH base) at 18 din, 12 V with a Collins special green filter cum yellow 54 barrier filter combination.

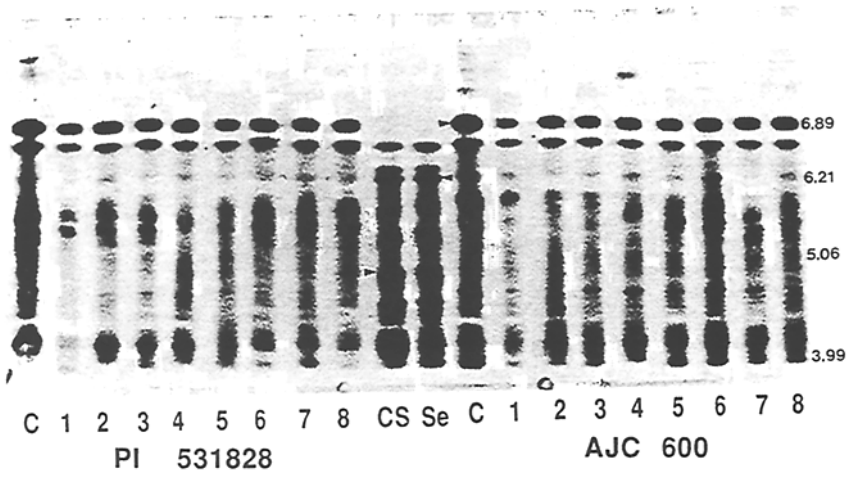

Fig. 1. Grain malate dehydrogenase banding profiles of *Ps. juncea* accessions PI 531828 and AJC 600, and *T. aestivum* cvs 'Chinese Spring' (*CS*) and 'Seri-82' (*Se*) on IEF (pH 3.5–9.5) polyacrylamide gels. *Lanes 1–8* of *Ps. juncea* accessions show the banding profiles of the individual seeds, with *C* indicating the composite within each accession. Diagnostic marker bands are indicated by an *arrowhead*

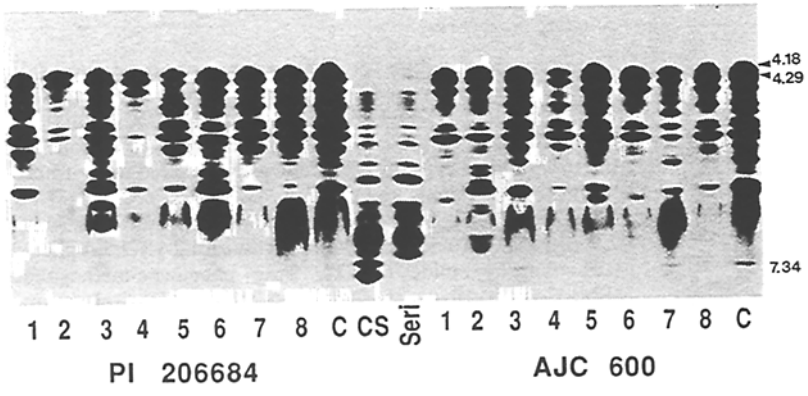

Fig. 2. Grain esterase banding profile of *Ps. juncea* accessions PI 206684 and AJC 600 and *T. aestivum* cvs 'Chinese Spring' (*CS*) and 'Seri-82' (*Seri*) on IEF (pH 3.5–9.5) polyacrylamide gels. *Lanes 1–8* of *Ps. juncea* accessions show the banding profiles of the individual seeds, with *C* indicating the composite within each accession. Diagnostic marker bands are indicated by an *arrowhead*

Results and discussion

Malate dehydrogenase (MDH)

The *Mdh* genes were first located on the long arms of homoeologous group 1 chromosomes using starch and polyacrylamide gel electrophoresis (Benito and Salinas 1983). Another set of *Mdh* genes was later located on the short arms of homoeologous group 5 chromosomes using IEF (Liu and Gale 1989). The banding profiles of 8 individual seeds of *Ps. juncea* accessions AJC 600 and PI 531828, of composites of these accessions, and of *T. aestivum* cvs 'Chinese Spring' and 'Seri-82' are shown in Fig. 1. The MDH banding profiles were in the range of isoelectric points (pI) between pH 3.99 and 6.89. 'Chinese Spring' and 'Seri-82' gave identical banding patterns with 13 bands. The banding resolution was better at the basic side of the gel. Individual *Ps. juncea* accessions gave slightly polymorphic banding profiles, but the composite seed samples contained all of the bands that each of the individual seeds analyzed possessed. 'Chinese Spring' and 'Seri-82' had 2 bands with pIs of 6.21 and 5.06, respectively which were not present in any of the *Ps. juncea* accessions, while the latter had a unique band with a pI of 6.89 that was not present in the two wheat cultivars. With this distinctive MDH band as a biochemical marker it may be possible to identify the *Ps. juncea* chromosome homoeologous to group 1 or 5 or wheat in a wheat background.

The polymorphism in the banding pattern of individual seeds within the *Ps. juncea* accessions is presumably an attribute of its self-incompatibility nature. This polymorphism, however, will not interfere in the use of the distinct MDH band (pI 6.89) in intergeneric hybridization programs since there was no polymorphism for this locus.

Esterase (EST)

'Chinese Spring' and 'Seri-82' had banding profiles with an array of EST bands of pI values between 4.18 and 7.34 (Fig. 2). The smear of bands at the acidic end have been previously excluded in the analysis of EST isozymes (Ainsworth et al. 1984). There were differences in the banding profiles of 'Chinese Spring' and 'Seri-82': the 2 most basic bands of 'Chinese Spring', encoded by chromosome 3A (Ainsworth et al. 1984), were absent in 'Seri-82'.

All eight *Ps. juncea* accessions showed a certain degree of polymorphism in the banding profiles for esterases; a presumable consequence of its self-incompatibility nature. The polymorphism may also be due to allelic variation present in *Ps. juncea* as the material evaluated did not sufficiently conform to the allelic nature reported earlier for hexaploid wheat (Ainsworth et al. 1984). The induced autotetraploid accession (PI 531828) with improved self-fertility also showed some polymorphism. The polymorphism exhibited by 8 individual seeds of each of accessions AJC 600 and PI 206684 (Fig. 2) is specifically presented, with individual seed variation readily identifiable in the composite track of each accession; this latter observation suggests that the composite can be effectively used in diagnostic analysis associated with an alien gene transfer program. Cooke et al. (1986) reported that the majority of *T. aestivum* cultivars possess the same alleles at the seed EST loci, although Ainsworth et al. (1984) observed allelic variation among bread wheat cultivars. All accessions of *Ps. juncea* analyzed had 2 bands in the acidic range with pI values of 4.18 and 4.29, bands which were not present in 'Chinese Spring' and 'Seri-82'. Consequently, these bands can be effectively used as biochemical markers to track the presence of *Ps. juncea* chromosomes of homoelogous group 3.

Shikimate dehydrogenase (SKDH)

Koebner and Shepherd (1983) investigated SKDH-1 of *T. aestivum* using polyacrylamide gel electrophoresis, and the genes for SKDH were reportedly located on the short arms of homoeologous group 5 chromosomes in wheat. Benedettelli and Hart (1988) expanded on the study of SKDH using IEF and identified 8 bands in leaf tissue. Seeds of the two wheat cultivars used in this study ('Chinese Spring' and 'Seri-82') had identical banding profiles with 6 bands, of which 3 were very distinct (Fig. 3). All bands were in the pI range of 4.71 to 6.14. The banding profiles of the composites of the *Ps. juncea* accessions, which had a pI range of 5.56 to 7.09, also contained 5–6 bands of which 2 (6.47 and 7.09) were distinct and always present in the individual seed analyses. The individual seeds had some vague bands that overlapped with the banding profiles of 'Chinese Spring' and 'Seri-82', but the 2 distinct bands of pI 6.47 and 7.09 may serve as diagnostic markers for detecting and tracking an alien *Ps. juncea* chromosome of homoeologous group 5.

Phosphoglucomutase (PGM)

'Chinese Spring' gave a banding profile of 3 bands with pIs between 4.67 and 5.20. All single seeds plus the respective composites of *Ps. juncea* accessions gave a single diagnostic band with a pI of 4.47 (Fig. 4) that was anodal to the banding profile of 'Chinese Spring' and could serve as a marker for the *Ps. juncea* chromosome of homoeologous group 4, on which group the genes are located (Benito et al. 1984). Some *Ps. juncea* accessions expressed few faint bands that overlap with 'Chinese Spring' bands (Fig. 4) in the more basic range and hence cannot be positively utilized as markers.

Beta-amylase (β-AMY)

Genetic loci controlling β-AMY are located on two sets of homoeologous chromosomes, i.e., 4A, 5A (possible translocation from 4B), and 4D; 2A, 2B, and 2D (Sharp et al. 1988). Using IEF Ainsworth et al. (1983) identified 33 bands in wheat. Similarly, a range of bands with pIs between 4.41 and 5.93 were identified in this study for cvs 'Chinese Spring' and 'Seri-82', with the banding profiles of both cultivars being quite similar. The β-amylase IEF zymogram and the zymogram of native PAGE is presented in Figs. 5 and 6, respectively.

Individual seeds of different *Ps. juncea* accessions had banding patterns with 2–4 distinct bands in a pI range between 4.33 and 4.5 and an array of faint bands with higher pI values of a resolution inadequate for differentiation from the wheat cultivars (Fig. 5). Some individual seed samples among all eight accessions of *Ps. juncea* had only two sharp bands that overlapped the low pI bands of 'Chinese Spring' and 'Seri-82'. Each accession exhibit-

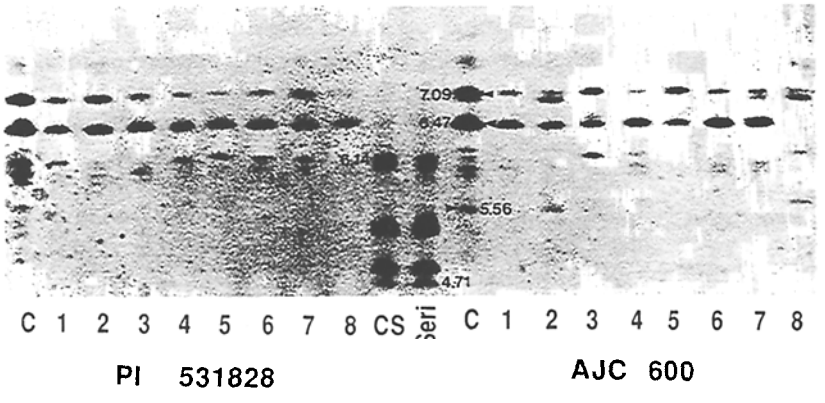

Fig. 3. Grain shikimate dehydrogenase banding profiles of *Ps. juncea* accessions PI 531828 and AJC 600 and *T. aestivum* cvs 'Chinese Spring' (*CS*) and 'Seri-82' (*Seri*) on IEF (pH 3.5–9.5) polyacrylamide gels. *Lanes 1–8* of *Ps. juncea* accessions show the banding profiles of the individual seeds, with *C* indicating the composite within each accession. Diagnostic marker bands are indicated by an *arrowhead*

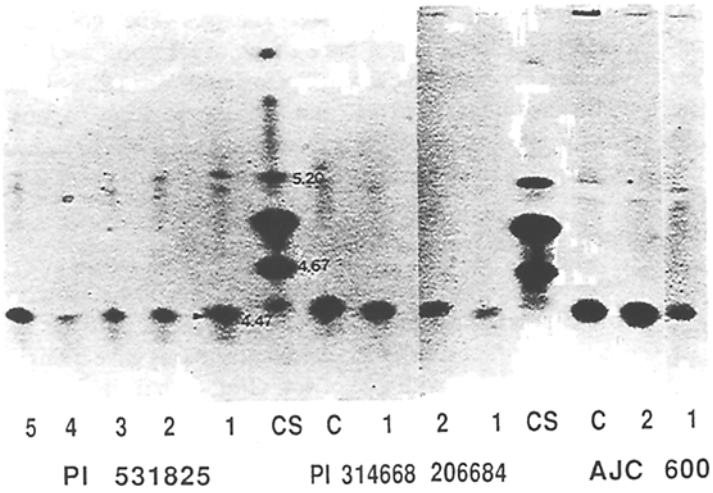

Fig. 4. Grain phosphoglucomutase banding profiles of four *Psathyrostachys juncea* accessions (PI 531825, PI 314668, PI 206684, AJC 600) and *T. aestivum* cv 'Chinese Spring' (*CS*) on IEF (pH 4–6.5) polyacrylamide gels. *Lane numbers* of *Ps. juncea* accessions show the banding profiles of the individual seeds, with *C* indicating the composite within each accession. Diagnostic marker bands are indicated by an *arrowhead*

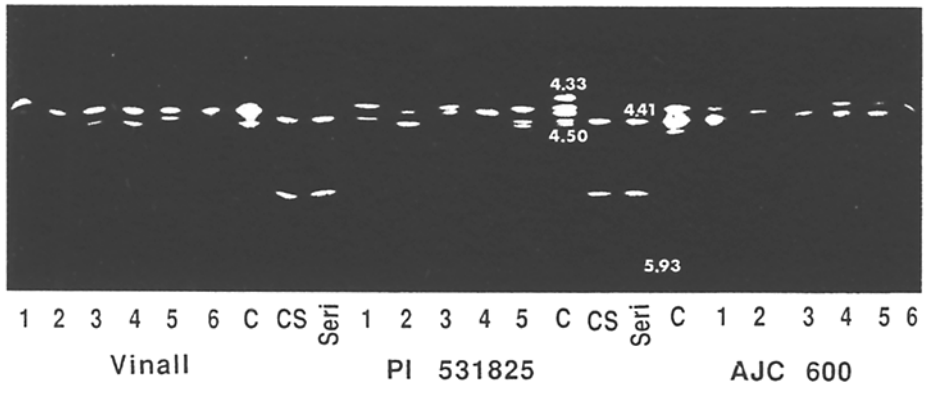

Fig. 5. Beta-amylase isozyme banding profiles of *Ps. juncea* accessions Vinall, PI 531825 and AJC 600 and *T. aestivum* cvs 'Chinese Spring' (*CS*) and 'Seri-82' (*Seri*) on IEF (pH 4.0–6.5) polyacrylamide gels. *Lane numbers* of *Ps. juncea* accessions show the banding profiles of the individual seeds, with *C* indicating the composite within each accession

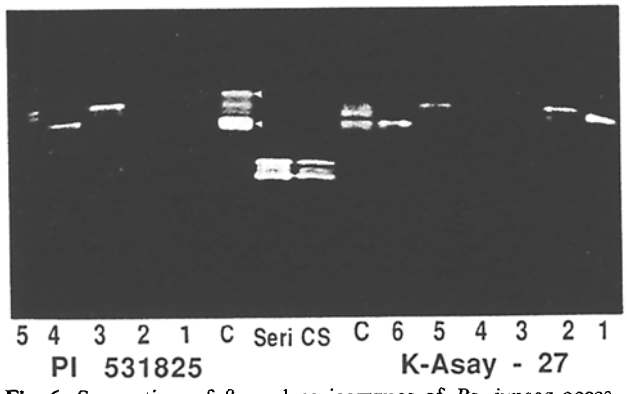

Fig. 6. Separation of β-amylase isozymes of *Ps. juncea* accessions PI 531825 and K-Asay27 and *T. aestivum* cvs 'Chinese Spring' (CS) and 'Seri-82' (*Ser*) on 8.5% native polyacrylamide gels. *Lane numbers* of *Ps. juncea* accessions show the banding profiles of individual seeds, with C indicating the composite within each accession. Marker bands in the composite are identified by an *arrowhead*

ed considerable polymorphism, and this would consequently complicate the possibility of identifying *Ps. juncea* chromosomes in a wheat background using this marker with IEF.

Native polyacrylamide gel electrophoresis showed the differences between *Ps. juncea* and *T. aestivum* more distinctively than what was observed with IEF (Fig. 6). Isoelectric focusing separates proteins according to their net charge. The sensitivity of the resolving power of native PAGE, however, is not as high as that in IEF, but this does not preclude the detection of differences in the banding profiles by native PAGE. Despite the within and among species polymorphism, the various accessions of *Ps. juncea* had 2–4 sharp bands at different migratory positions than the *T. aestivum* cultivars, supporting the diagnostic interpretation that β-AMY could serve as a distinct marker in a *T. aestivum* × *Ps. juncea* hybridization program.

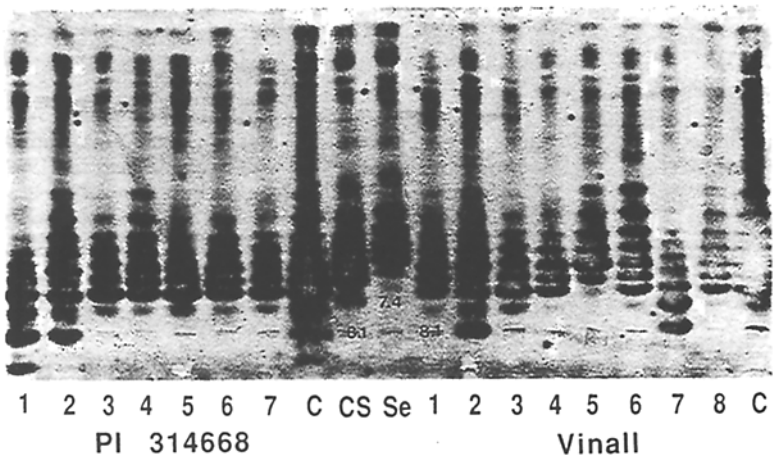

Fig. 7. Grain glucosephosphate isomerase banding profiles of *Ps. juncea* accessions PI 314668 and Vinall with *T. aestivum* cvs 'Chinese Spring' (*CS*) and 'Seri-82' (*Se*) on IEF (pH 3.5–9.5) polyacrylamide gels. *Lane numbers 1–8* under *Ps. juncea* accessions show the banding profiles of the individual seeds, with C indicating the composite within each accession

Fig. 8. A C-banded karyotype of *Ps. juncea* PI 314668 (2n=2x=14, NN) with diagnostic band positive sites for each of the seven chromosomes

Glucose phosphate isomerase (GPI)

'Chinese Spring' exhibited a banding profile of 10 bands with the majority of bands having basic pI values, the most basic being of pI 7.4. The bands on the anodal side of the gel were poorly resolved and consequently excluded in the analysis. These bands may be isozymes of 6PGD, as suggested by Chojecki and Gale (1982), since the GPI stain can also stain 6PGD (Hart 1979). The range of pI values is somewhat different from the range of pIs reported by Chojecki and Gale (1982), probably due to the differences in gel types used to separate GPI isozymes in the two studies.

Individual seeds of the different *Ps. juncea* accessions showed considerable banding polymorphism (Fig. 7). The banding profile of individual seeds and the composite of a *Ps. juncea* accessions (PI 314668 and Vinall) had a band of pI value 8.1 (Fig. 7). This was more basic than the most basic band of 'Chinese Spring'. However, due to the polymorphic nature of all individual seeds of a particular accession no diagnostic marker bands could be identified, rendering GPI unsuitable for tracking homoeologous group 1 chromosomes in a *T. aestivum* × *Ps. juncea* hybridization program.

Cytology

The C-banded karyotype of *Ps. juncea* (Fig. 8) shows unique differential banding sites for each of its chromosomes, and the karyotype is distinct from the C- and N-banded chromosomes of *T. aestivum* (Endo 1986; Gill 1987). C-banding of *Ps. juncea* was first reported by Gill (1981), subsequently by Endo and Gill (1984), and now by us (Fig. 8), with retention of the earlier reported alphabetical designations.

Polymorphisms were minimal for the banding sites (Fig. 8) In comparison to the previous reports chromosome E had an additional interstitial site on the long arm, chromosome F a faint terminal site on the long arm, and chromosome G an interstitial banding site on the long arm. The banding distinctiveness of each of the *Ps. juncea* chromosomes should facilitate their adequate identification in a *T. aestivum* background.

References

Ainsworth CC, Gale MD, Baird S (1983) The genetics of β-amylase isozymes in wheat. Theor Appl Genet 66:39–49

Ainsworth CC, Gale MD, Baird S (1984) The genetic control of grain esterases in hexaploid wheat. Theor Appl Genet 68:219–226

Benedettelli S, Hart GE (1988) Genetic analysis of Triticeae shikimate dehydrogenase. Biochem Genet 26:287–301

Benito C, Salinas J (1983) The chromosomal location of malate dehydrogenase isozymes in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 64:255–258

Benito C, Figueiras AM, Gonzalez-Jean MT (1984) Phosphoglucomutase – a biochemical marker for group 4 chromosomes in the *Triticinae*. Theor Appl Genet 68:555–557

Bennett MD, Gustafson JP, Smith JP (1977) Variation in nuclear DNA in the genus *Secale*. Chromosoma 61:149–176

Chojecki AJS, Gale MD (1982) Genetic control of glucose phosphate isomerase in wheat and related species. Heredity 49:337–347

Cooke RJ, Smith TM, Ainsworth CC (1986) Discrimination between bread wheat, durum wheat, rye and triticale by electrophoresis of seed proteins and enzymes. Seed Sci Technol 14:693–704

Davis BJ (1964) Disc electrophoresis II. Methods and application to human serum proteins. Ann N Y Acad Sci 121:404–427

Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant improvement. Plenum Press, New York, pp 209–279

Endo TR (1986) Complete identification of common wheat chromosomes by means of the C-banding technique. Jpn. J Genet 61:89–93

Endo TR, Gill BS (1984) The heterochromatic distribution and genome evolution in diploid species of *Elymus* and *Agropyron*. Can J Genet Cytol 26:669–678

Gill BS (1981) Evolutionary relationships based on heterochromatin bands in six grass species of the *Triticinae*. J Hered 72:391–394

Gill BS (1987) Chromosome banding methods, standard chromosome band nomenclature, and applications in cytogenetic analysis. In: Heyne EG (ed) Wheat and wheat improvement 2nd edn. Agric Ser No 13, pp 243–254

Hanson AA (1972) Grass varieties in the United States. Agriculture Handbook 170. US Government Printing Office, Washington, D.C., USA

Hart GE (1979) Evidence for a triplicate set of glucose phosphate isomerase structural genes in hexaploid wheat. Biochem Genet 17:585–598

Hart GE, Islam AKMR, Shepherd KW (1980) Use of isozymes as chromosome markers in the isolation and characterization of wheat-barley chromosome addition lines. Genet Res 36:311–325

Jahan Q, Ter-Kuile N, Hashmi N, Islam M, Vahidy AA, Mujeeb-Kazi A (1990) The status of the 1B/1R translocation chromosome in some released wheat varieties and the 1989 candidate varieties of Pakistan. Pak J Bot 22:1–10

Jensen KB, Zhang YF, Dewey DR (1990) Mode of pollination of perennial species of the *Triticeae* in relation to genomically defined genera. Can J Plant Sci 70:215–225

Koebner RMD, Shepherd KW (1983) Shikimate dehydrogenase- a biochemical marker for group 5 chromosomes in the *Triticinae*. Genet Res 41:209–213

Liu CJ, Gale MD (1989) The chromosomal location of a third set of malate dehydrogenase loci, *Mdh-3* in wheat, barley and related species. Theor Appl Genet 78:349–352

Mena M, Orellana J, Lopez-Braña I, Garcia-Olmedo F, Delibes A (1989) Biochemical and cytological characterization of wheat/*Aegilops ventricosa* addition and transfer lines carrying chromosome $4M^v$. Theor Appl Genet 77:184–188

Mujeeb-Kazi A, Miranda JL (1985) Enhanced resolution of somatic chromosome constrictions as an aid to identifying intergeneric hybrids among some Triticeae. Cytologia 50:701–709

Mujeeb-Kazi A, Asiedu R (1990) Wide hybridization- potential of alien genetic transfers for *Triticum aestivum* improvement. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry vol 13. Springer, Berlin, Heidelberg, New York, pp 111–127

Plourde A, Fedak G, St-Pierre CA, Comeau A (1990) A novel intergeneric hybrid in the *Triticeae: Triticum aestivum* × *Psathyrostachys juncea*. Theor Appl Genet 79:45–48

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of β-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Tang KS, Hart GE (1979) Use of isozymes as chromosome markers in wheat-rye addition lines and in triticale. Genet Res 26:187–201

Tzvelev NN (1976) Poaceae URSS. Tribe 111. Triticeae DUM. USSR Academy of Science Press, Leningrad, pp 105–206

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozyme in plant genetics and breeding, part A. Elsevier Science Publ. Amsterdam, pp 469–515