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Chromosomal location of seed storage protein genes in the genome of *Dasypyrum villosum* (L.) Candargy

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Summary. Genes coding for glutenin-like subunits and for several prolamin subunits with electrophoretic mobilities (lactate-PAGE) corresponding to those of omega- and gamma-gliadins of wheat were located in *Dasypyrum villosum* chromosome *1V*. Genes controlling four gliadin-like subunits with electrophoretic mobilities corresponding to those of alpha- and gamma-gliadins were located on the short arm of chromosome *6V* and on the long arm of chromosome *4V*. N-terminal amino acid sequences of these four components were also determined and homology with alpha-type gliadins was demonstrated. The presence of genes coding for glutenin- and gliadin-like subunits on chromosomes *1V* and *6V* demonstrates homoeology between the *D. villosum* chromosomes *1V* and *6V* and the chromosomes of homoeologous groups 1 and 6 in wheat. It is likely that the additional locus *Gli-V3* on chromosome *4V* originated by translocation from the *Gli-V2* locus.

Key words: Gliadins – Glutenins – Gene location – *Dasypyrum villosum* – Wheat

Introduction

The storage proteins of *durum* and bread wheat grains, which include gliadins and glutenins, form part of the nutritional reservoir for the seed and young germinating plant, and determine to a large extent the nutritional and technological properties of flours (Shewry and Miflin 1985). The genetic and biochemical aspects of these proteins have been extensively investigated in recent years (Payne 1987). In 1984, Shewry et al. designated the gliadins and glutenins of wheat and related proteins from other cereals as prolamins, based on their solubility in alcohol mixtures either in the native state or as reduced subunits. Genetic analysis of wheat endosperm proteins was made possible by the availability of aneuploid lines of the wheat variety "Chinese Spring", developed by Sears (1954), and by the improved procedures of one- and two-dimensional electrophoresis in polyacrylamide gels (PAGE). The development of alien chromosome addition lines has allowed us to question whether the genes controlling seed proteins in other genomes in the tribe *Triticeae* have a similar chromosomal distribution.

Dasypyrum villosum (L.) Candargy, a wild species synonymous with *Haynaldia villosa* (L.) Schur., is an allogamous annual grass of the subtribe *Triticinae*, native to the Mediterranean region and south-west Asia. It is a diploid ($2n=14$) species (genome designated by the letters VV) with one or more genes for disease resistance (powdery mildew, stem and leaf rust, *Gaeumannomyces graminis*), tolerance to drought, high seed protein content, and other desirable morpho-physiological characteristics (Blanco and Simeone 1989). Montebove et al. (1987) and Shewry et al. (1987) reported the characterization of the prolamins of *D. villosum* and determined the chromosomal location of the structural genes for some components, using an incomplete set of "Chinese Spring" – *D. villosum* disomic addition lines.

This paper provides further information on the chromosomal location of genes controlling prolamin proteins in the genome of *D. villosum*, obtained by one- and two-dimensional gel electrophoresis of the complete series of the "Creso" durum wheat-*D. villosum* monosomic addition lines.

Materials and methods

Seeds

The genotypes analyzed included cv "Creso" of *Triticum turgidum* L. var. *durum* (2n=4x=28, AABB), accession HV 17 of *Dasypyrum villosum* (L.) Candargy (2n=2x=14, VV), the respective amphiploid (2n=6x=42, AABBVV), and seven mono-

somic addition lines (2n = 29) of the Institute of Plant Breeding, University of Bari (Blanco et al. 1984, 1987). Some monotelosomic or ditelosomic addition lines for the long and short arms of chromosomes *4V* and *6V* were also investigated.

Electrophoretical techniques

Total seed protein extraction and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis were carried out with 10% polyacrylamide (Payne et al. 1981). Monomeric prolamins were extracted from crushed single grain halves with 1.5 *M* dimethylformamide at a 1 : 5 w/v ratio. After centrifugation (15 min at 10,000 × *g*), the clear supernatant was used for electrophoretic separation (Lafiandra and Kasarda 1985).

Protein electrophoretical analyses of the addition lines were carried out on the grain halves proven to be monosomic additions at mitosis (2n = 29). Root tips were pretreated for 4 h with 0.05 colchicine and then fixed in Carnoy's 3 : 1 fixative. Standard Feulgen staining and squash procedures were used for cytological examination.

N-terminal amino acid sequencing

Monomeric prolamins were transferred from two-dimensional gels to Immobilon PVDF membrane, according to the manufacturer's instructions (Immobilon Tech Protocol TP008), and briefly stained with Comassie BBR 250 (0.1% w/v) in 50% (v/v) methanol with 10% (v/v) TCA. Stained spots from several separations were bulked and N-terminal sequences were determined using an Applied Biosystem 477A Protein Sequencer at the University of Bristol Molecular Recognition Center.

Results

Electrophoretical separation of prolamins

One-dimensional polyacrylamide gel electrophoretic patterns (lactate, pH 3.1) of monomeric prolamins extracted from single seeds of the seven durum wheat-*Dasypyrum villosum* monosomic addition lines and the respective parental lines (durum wheat cultivar "Creso," amphiploid, and *D. villosum*) are shown in Fig. 1. The electrophoretic pattern of the *durum* wheat cv "Creso" represents a typical gliadin pattern of tetraploid wheats: components are spread over the entire range of mobility from the alpha to the omega region. The banding pattern of the "Creso" – *D. villosum* amphiploid (2n = 42, AABBVV) displays three groups of bands (indicated as V1–V2 and V3 in Fig. 1) in addition to those normally present in the pattern of "Creso." Bands V1 and V2 with mobilities equivalent to omega- and gamma-gliadins, respectively, are controlled by genes on *D. villosum* chromosome *1V*, since they are present in the pattern of addition line *1V*. The strong unresolved band V3 with mobility intermediate between alpha- and beta-gliadins is controlled by genes on *D. villosum* chromosomes *4V* and *6V*, since this band is present in the patterns of addition lines *4V* and *6V*. Tests of the monotelosomic or ditelosomic additions for chromosomes *4V* and *6V* have shown that band V3

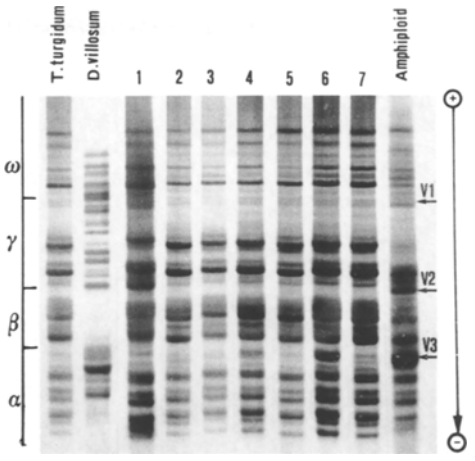

Fig. 1. One-dimensional electrophoretic separation at pH 3.1 of gliadins from *T. turgidum* cv "Creso", *D. villosum* monosomic addition lines *1V–7V*, and amphiploid *T. turgidum – D. villosum*. The *arrows* V1, V2, V3 indicate the *Dasypirum* bands present in the amphiploid, in addition to the *durum* wheat ones

is controlled by genes on the long arm of chromosome *4V* and on the short arm of chromosome *6V*. The banding patterns of addition lines *2V*, *3V*, *5V*, and *7V* are identical to each other and to the profile of the parental line "Creso."

Because of the complexity of one-dimensional profiles, it is difficult to distinguish the profiles of the *4V* and *6V* addition lines and to provide unequivocal evidence of the chromosomal location of the *Dasypyrum* genes coding for prolamin proteins. Monomeric prolamins of "Creso," the "Creso" – *D. villosum* amphiploid, the monosomic addition line *1V*, and ditelosomic additions *4V*L and *6V*S were therefore fractionated by two-dimensional (2-pH) polyacrylamide gel electrophoresis (Fig. 2). This allowed every major component in the amphiploid profile (Fig. 2b) that could not be attributed to "Creso" (Fig. 2a) to be attributed to *D. villosum* (these are numbered 1–11 in Fig. 2). All these components were present in the profiles of the addition lines *1V*, *4V*, or *6V* (Fig. 2c, d, e), supporting the conclusion from one-dimensional gels that the *Dasypyrum* prolamin genes, expressed in the wheat background, are located on three chromosomes of the *D. villosum* genome. The *1V* addition line (Fig. 2c) contained seven (1–7) of the 11 components labelled in Fig. 2b, most of which belonged to the omega and gamma regions. The strong prolamin component, with mobility intermediate between alpha- and beta-gliadins in the one-dimensional gel (indicated as V3 in Fig. 1), was separated into four components in the addition lines *4V* and *6V* by two-dimensional electrophoresis. Subunits 10 and 11 are coded by genes on the long arm of chromosome *4V*, and subunits 8 and 9 by genes on the short arm of chromosome *6V*.

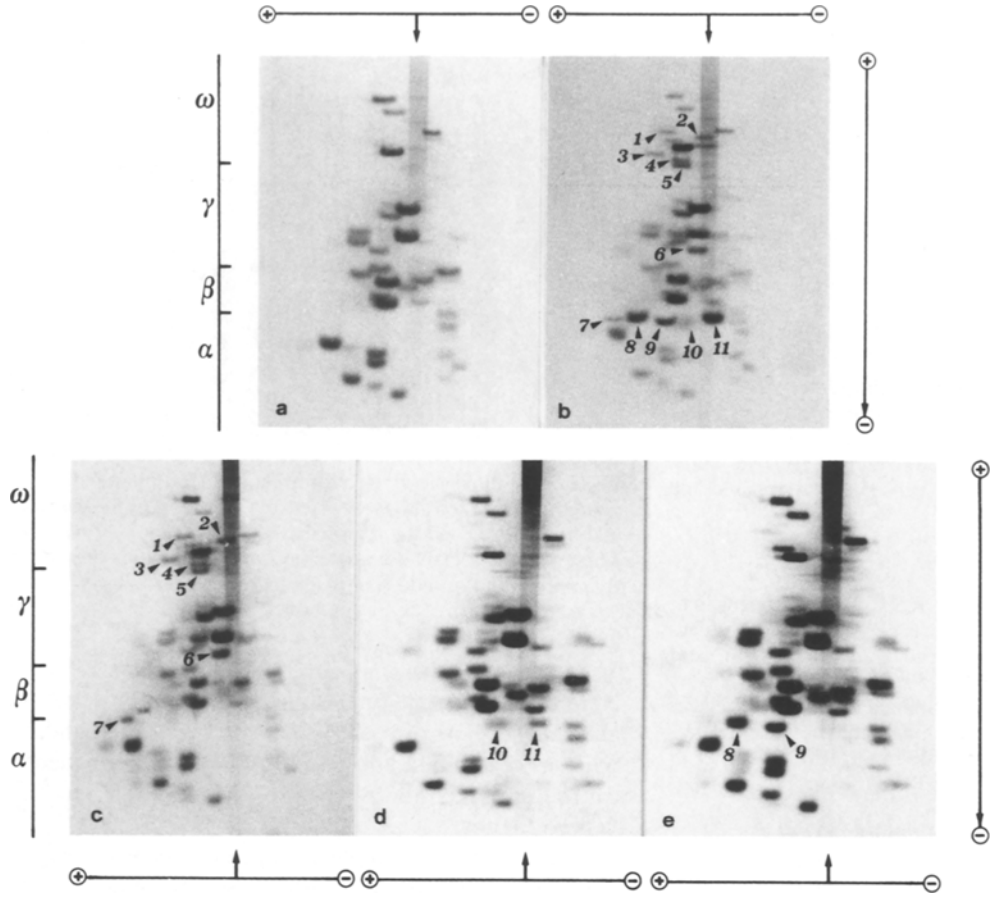

Fig. 2. Fractionation by two-dimensional polyacrylamide gel electrophoresis of gliadin proteins of *durum* wheat cv. "Creso" (*a*), *durum* wheat cv "Creso" – *D. villosum* amphiploid (*b*), and *durum* wheat – *D. villosum* monosomic addition lines for chromosomes *1V* (*c*), *4V* (*d*), and *6V* (*e*). The numbers indicate the subunits additional to the "Creso" ones and encoded by the *D. villosum* chromosome *1V* (bands 1–7), *4V* (bands 10–11), and *6V* (bands 8–9)

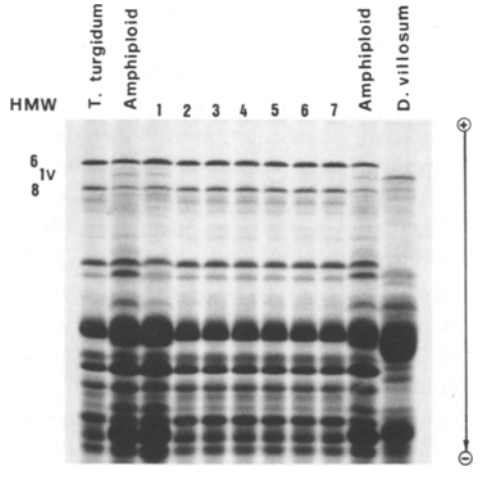

Fig. 3. SDS-PAGE patterns of total seed proteins of *T. turgidum* cv "Creso," amphiploid *T. turgidum* cv "Creso" – *D. villosum*, monosomic addition lines *1V–7V*, and *D. villosum* (HMW = high-molecular-weight prolamins)

High-molecular-weight prolamins

The electrophoretic pattern obtained with crude extracts of total seed proteins of the wheat cultivar "Creso" in the presence of sodium dodecyl sulphate is shown in Fig. 3. The two slowest moving bands in this pattern represent high-molecular-weight prolamins commonly referred to in wheat as high-molecular-weight (HMW) subunits of glutenin. The two subunits are controlled by the locus *Gli-B1* on the long arm of chromosome *1B* and correspond to the pair of subunits designated 6+8 by Payne and Lawrence (1983). The banding pattern of the "Creso" – *D. villosum* amphiploid displays a single band in addition to those normally present in the pattern of "Creso." This band is controlled by gene(s) on *D. villosum* chromosome *1V*, since it is present only in the pattern of addition line *1V*. The arm location has not been determined.

Table 1. N-terminal amino acid sequences of components controlled by genes on chromosome *4V* (components *10V* and *11V*) and *6V* (*8V* and *9V*) of *D. villosum*

	1				5					10
D. villosum[a]	(Q)	L	R	V	P	V	P	Q	L	Q S
Chinese Spring[b]	V		R	V	P	V	P	Q	L	Q P
Creso *6V* add. line (*8V*)	V		R	V	P	V	P	Q	L	Q L
Creso *6V* add. line (*9V*)	V		R	V	P	V	P	Q	L	Q P
Creso *4V* add. line (*10V*)	V	L	X	V	P	V	P	Q	L	Q
Creso *4V* add. line (*11V*)	V	L	R	V	P	V	P	Q	L	X

Standard single letter abbreviations for amino acids are used: Q, glutamine; V, valine; L, leucine; R, arginine; P, proline; S, serine; X, unidentified

[a] Shewry et al. (1987)

[b] Kasarda et al. (1984)

N-terminal amino acid sequences of prolamins of D. villosum controlled by genes on chromosomes 4V and 6V

N-terminal amino acid sequences of components of *D. villosum* monomeric prolamin components numbered as V8, V9 (controlled by genes present on chromosome *6V*), V10, and V11 (controlled by genes on chromosome *4V*) were determined (Table 1). The N-terminal amino acid sequences of a low M_r monomeric prolamin from *D. villosum*, previously determined by Shewry et al. (1987), and of alpha-type gliadin components controlled by genes on the short arm of chromosome *6A* in bread wheat (Kasarda et al. 1984) are included for comparison.

The two components controlled by chromosome *6V* of *D. villosum* are identical for the first nine and ten residues, respectively, to the N-terminal sequences of typical alpha-type gliadins of bread wheat. In contrast, the two components controlled by chromosome *4V* of *D. villosum* show an additional leucine residue inserted at position 2. These two sequences are identical to that reported by Shewry et al. (1987) for a purified prolamin of low molecular weight from *D. villosum*, except for the tentative identification of glutamine instead of valine at position 1. This difference may, in fact, be an artifact of the solid-phase sequencing methods used in the previous study.

Discussion

The results reported here show that *D. villosum* endosperm proteins analogous to the wheat HMW glutenin subunits and to the wheat omega- and gamma-gliadin components are all encoded by genes on chromosome *1V*. Genes coding for these protein groups occur on the homoeologous chromosomes in other species of the Triticeae, namely, chromosomes *1A*, *1B*, and *1D* in wheat. Thus, it is likely that chromosome *1V* of *D. villosum* is also a homoeologue, and the loci should be designated *Glu-V1* and *Gli-V1*, respectively.

The *D. villosum* prolamins analogous to the wheat alpha- and gamma-gliadin subunits are encoded by genes, designated *Gli-V2* and *Gli-V3*, located on chromosome arms *6V*S and *4V*L.

Montebove et al. (1987) and Shewry et al. (1987) used an incomplete set of addition lines of *D. villosum* in the bread wheat "Chinese Spring" to locate prolamin genes in the *D. villosum* genome. Montebove et al. (1987) were able to locate genes coding for HMW glutenin-like subunits on chromosome *1V* and for alpha-gliadin-like subunits on chromosomes *1V* and *4V*. Shewry et al. (1987) located genes for HMW glutenins and gamma-type gliadins on chromosome *1V* and genes for alpha-type gliadins on chromosome *6V*. The failure to detect all three *Gli* loci of the *D. villosum* genome could possibly be due to allelic diversity in the *D. villosum* stock used in synthesizing the "Chinese Spring" – *D. villosum* amphiploid and disomic addition lines, and/or to the overlapping of wheat and *Dasypyrum* proteins in one-dimensional electrophoresis. Indeed, the alpha- and gamma-gliadin-like subunits coded by genes on chromosomes *4V* and *6V*, present in the addition lines analyzed in the current study, overlapped in one-dimensional electrophoresis and were only resolved in the two-dimensional separation.

In *Triticum* and *Aegilops*, the genes controlling the HMW prolamins are located on the long arms of the group 1 chromosomes, and the genes controlling the monomeric alpha-, beta-, gamma-, and omega-type prolamins are located on the short arms of the homoeologous group 1 and 6 chromosome (Payne 1987). The same chromosomal locations of genes for endosperm proteins have been found in *Agropyron elongatum* (Dvorak et al. 1986) and *Agropyron intermedium* (Foster et al. 1987). The spatial separation of the prolamin genes has been attributed to an ancient interchromosomal translocation originated in the diploid progenitor of the *Triticum*, *Aegilops*, and *Elytrigia* diploid species (Shepherd and Jennings 1971; Dvorak et al. 1986). According to Payne et al. (1982) and Shewry et al. (1984), it is likely that the locus *Gli-2* originated through translocation of a gamma-type gene from chromosome 1 to chromosome 6, followed by divergence of the coding sequence to give rise to the alpha-type sequence.

The chromosomal distribution is different in rye, with two loci encoding gamma-type prolamins present on chromosomes *1R* and *6R* in the wild rye *Secale montanum* and on chromosomes *1R* and *2R* in the cultivated rye *Secale cereale* (Shewry and Miflin 1985; Shewry et al. 1986). In the *Hordeum* species thus far analyzed, the prolamin genes are located on chromosomes $1H^{ch}$ and $7H^{ch}$ in the wild species *H. chilense*, but only on chromosome *1H* in the cultivated barley *H. vulgare* (Lawrence and Shepherd 1981; Shewry et al. 1983; Payne et al. 1987). It is possible that the prolamin genes on *6R* and *2R* in rye species and on $7H^{ch}$ in wild barley had independent origins due to independent translocations of ancestral gene(s) from chromosome 1 (Shewry et al. 1984; Payne et al. 1987).

The *D. villosum* genome contains two prolamin loci on chromosomes *1V* and *6V*, and a third locus on chromosome *4V*. It is possible that the additional locus *Gli-V3* originated by translocation of genes from the *Gli-V2* locus, as they both encode prolamin subunits which have electrophoretic mobilities within the range of the alpha-type gliadins, and have similar but not identical N-terminal amino acid sequences.

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References

Blanco A, Simeone R (1989) Use of *Dasypyrum villosum* (L.) Candargy in durum wheat improvement. In: Wittmer G (ed) Proc 3rd Int Symp Durum Wheat, Foggia, Italy, pp 215–228

Blanco A, Simeone R, Orecchia C (1984) Cytology, morphology and fertility of the amphiploid *Triticum durum* Desf. × *Haynaldia villosa* (L.) Schur. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 171–177

Blanco A, Simeone R, Resta P (1987) The addition of *Dasypyrum villosum* (L.) Candargy chromosomes to *durum* wheat *Triticum durum* (Desf.). Theor Appl Genet 74: 328–333

Dvorak J, Kasarda DD, Dietler MD, Lew EJL, Anderson OD, Litts JC, Shewry PR (1986) Chromosomal location of seed storage protein genes in the genome of *Elytrigia elongata*. Can J Genet Cytol 28: 818–830

Foster BP, Reader SM, Forsyth SA, Koebner RMD, Miller TE, Gale MD, Cauderon Y (1987) An assessment of the homoeology of six *Agropyron intermedium* chromosomes added to wheat. Genet Res 50: 91–97

Kasarda DD, Okita TW, Bernardin JE, Baecker PA, Nimmo CC, Lew EJL, Dietler MD, Greene FC (1984) Nucleic acid (cDNA) and amino acid sequences of α-type gliadins from wheat (*Triticum aestivum*). Proc Natl Acad Sci USA 81: 4712–4716

Lafiandra D, Kasarda DD (1985) One and two-dimensional (2-pH) polyacrylamide gel electrophoresis in a single gel: separation of wheat proteins. Cereal Chem 62: 314–319

Lawrence GJ, Shepherd KW (1981) Inheritance of glutenin protein subunits of wheat. Theor Appl Genet 60: 333–337

Montebove L, De Pace C, Jan CC, Scarascia Mugnozza GT, Qualset CO (1987) Chromosomal location of isozyme and seed storage protein genes in *Dasypyrum villosum* (L.) Candargy. Theor Appl Genet 73: 836–845

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38: 141–153

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for the high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11: 29–35

Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. 1. Allelic variation in subunits amongst varieties of wheat. Theor Appl Genet 60: 229–236

Payne PI, Holt LM, Reader SM, Miller TE (1987) Chromosomal location of genes coding for endosperm proteins of *Hordeum chilense*, determined by two-dimensional electrophoresis of wheat-*H. chilense* chromosome addition lines. Biochem Genet 25: 53–65

Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 3. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. Theor Appl Genet 63: 129–138

Sears ER (1954) The aneuploids of common wheat. Univ Mo Agric Exp Stn Res Bull 572: 59

Shepherd KW, Jennings AC (1971) Genetic control of rye endosperm proteins. Experentia 27: 98–99

Shewry PR, Miflin BJ (1985) Seed storage proteins of economically important cereals. In: Pomeranz Y (ed) Advances in cereal science and technology, Vol 7. American Association of Cereal Chemists St Paul/MN, pp 1–83

Shewry PR, Finch RA, Parmar S, Franklin J, Miflin BJ (1983) Chromosomal location of *Hor3*, a new locus governing storage proteins in barley. Heredity 50: 179–189

Shewry PR, Miflin BJ, Kasarda DD (1984) The structural and evolutionary relationships of the prolamin storage proteins of barley, rye and wheat. Philos Trans R Soc Lond Ser B 304: 333–339

Shewry PR, Parmar S, Fulrath N, Kasarda DD, Miller TE (1986) Chromosomal locations of the structural gene for secalins in wild perennial rye (*Secale montanum* Guss.) and cultivated rye (*S. cereale* L.) determined by two-dimensional electrophoresis. Can J Genet Cytol 28: 76–83

Shewry PR, Parmar S, Pappin DJC (1987) Characterization and genetic control of the prolamins of *Haynaldia villosa*: relationship to cultivated species of the *Triticeae* (rye, wheat, and barley). Biochem Genet 25: 309–325