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

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Summary. The zymogram phenotypes of glucose-phosphate isomerase (GPI), alcohol dehydrogenase-1 (ADH-1), glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), lipoxygenase (LPX), esterase (EST) and the banding patterns of gliadin and glutenin seed storage proteins were determined for *Triticum aestivum* cv. 'Chinese Spring' (CS), *Dasypyrum villosum*, the octoploid amphiploid *T. aestivum* cv. 'Chinese Spring' *D. villosum* (CS × v) (2n = 8x = 56; AABBDDVV), and for five CS-*D. villosum* disomic addition lines. The genes *Gpi-V1*, *Adh-V1*, *Got-V2*, and *Sod-V2* coding for GPI-1, ADH-1, GOT-2, and SOD-2 isozymes were located in *D. villosum* on chromosome 1V, 4V, 6V, and 7V, respectively. Genes coding for gliadin- and glutenin-like subunits are located in *D. villosum* chromosomes 1V. There are no direct evidence for chromosomal location of genes coding for GOT-3, EST-1 and LPX-2 isozymes. The linkage between genes coding for glutenin-like proteins and GPI-1 isozymes in chromosome 1V is evidence of homoeology between chromosome 1V and the chromosomes of homoeologous group 1 in wheat.

Key words: Isozymes – Gliadins – Glutenins – Gene markers – *Dasypyrum*

Introduction

The grass species *Dasypyrum villosum* (L.) Candargy, 2n = 2x = 14, VV, (syn. *Triticum villosum* and *Haynaldia villosa* Schur) is a member of the subtribe *Triticinae* of the tribe *Triticeae* (family *Gramineae*). It is a widespread annual of many disturbed habitats (mainly road-sides) found in the Miditerranean regions and Caucasus.

D. villosum can easily be crossed to both tetraploid and hexaploid wheats (Jan et al. 1986) and is a potential source of useful genes for improving wheat (Qualset et al. 1981). A prerequisite for any conventional gene transfer procedure is the availability of chromosomal gene markers. Such chromosomal markers are useful for mapping other genes of the same species and in identifying alien or varietal chromosomes in intra- and interspecific cross-progenies.

Biochemical markers of chromosomes are more suitable than cytological (C- and N-banding patterns) or biomolecular ('in situ' and 'ex situ' nucleic acid hybridization) markers in breeding programs aimed at interspecific gene transfer through crossing. The use of isozymes and seed storage proteins as chromosome markers in the *Triticinae* species group has been reviewed by Hart (1983) and Law et al. (1983), respectively. Such biochemical markers are useful not only for breeding purposes but also for studying homoeologous relationships among *Triticinae* genome and species.

D. villosum is recognized as a potential donor of useful genes to cultivated wheats; however, no chromosomal gene markers have been reported in the species. In this paper information will be given on the chromosomal location of genes controlling isozymes and seed storage proteins to provide preliminary knowledge on independent marker genes that can be used as reliable biochemical controls in transferring *D. villosum* chromosomes into the wheat genome.

Materials and methods

1 Genotypes

The genotypes used to ascertain the chromosomes carrying the genes controlling isozyme and storage protein formation were the following: (1) *Triticum aestivum* cv. 'Chinese Spring' (CS);

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(2) samples of *Dasypyrum villosum* collected in central Italy; (3) the octoploid amphiploid *T. aestivum* cv. 'Chinese Spring' × *D. villosum* ('CS × v') (2n = 8x = 56; AABBDDVV) obtained by C.C. Jan and C. De Pace at the University of California, Davis, USA; and (4) five disomic CS-*D. villosum* addition lines (designated as CS-1V, CS-2V, CS-4V, CS-6V and CS-7V on the basis of the implied homoeologies of *D. villosum* chromosome markers and the same markers in wheat) containing *D. villosum* chromosomes in a 'Chinese Spring' wheat background, kindly provided by E.R. Sears.

2 Isozyme analysis

Crude extracts from leaves of 4 to 5-week-old plants were extracted with Carlson's buffer as modified by Hart (1982) (tissue/buffer ratio: 1 mg/4 µl) in order to analyze GOT (glutamate oxaloacetate transaminase; E.C.2.6.1.1), EST (esterase; E.C.3.2.1.), and SOD (superoxide dismutase; E.C.1.15.1.1.). Extracts were centrifuged at 6,000 rpm, 4 °C for 7 min. The supernatant was either used directly for electrophoresis or stored at −80 °C. To analyze ADH-1 (alcohol dehydrogenase-1, E.C.1.1.1.1.) and GPI (glucosephosphateisomerase; E.C.5.3.1.9.), half seeds were soaked for 18 h in distilled and deionized water in Petri dishes and extracted with 50 µl (for ADH-1) or 100 µl (for GPI) of an aqueous solution of 12.5% sucrose. Extracts were centrifuged at 6,000 rpm, 4 °C for 5 min. The supernatant was used immediately for electrophoresis. Extracts of coleoptile from 4-day-old green seedlings grown in Petri dishes were used for LPX (lipoxygenase; E.C. 1.13.11.12). A tissue/Carlson's buffer ratio 1 mg/2 µl was used for LPX extraction.

Electrophoresis was carried out in polyacrylamide gels. Details of the composition of the gels used for ADH-1, GOT, GPI, SOD and LPX (adapted from Hart 1982) and the gels utilized to analyze EST (adapted from Shumaker et al. 1984) are reported in De Pace et al. (1986, in prep.). Forty µl of supernatant of each crude sample extract were used for gel analysis. Electrophoresis was performed with a Protean I (Biorad, Richmond, CA) vertical apparatus containing two gels. The running buffer was kept at 4 °C using a circulating refrigerant. For ESTs electrophoresis was carried out at 40 mA for 5 h or until the borate front migrated 13 cm from the upper edge of the resolving gel. All the other isozymes were electrophoresed at: 25 mA for 1 h then at 40 mA for 1 h, and finally at 50 mA until the bromophenol blue front migrated out the resolving gel.

The staining solutions used to visualize the isozymes have been adapted from Hart (1982) for ADH-1, GOT, and SOD; from Arus and Orton (1984) for GPI; and from Shumaker et al. (1984) for EST (De Pace et al. 1986, in prep.).

3 Seed storage proteins analysis

Analyses of high molecular weight (H.M.W.) glutenins were performed using a procedure adapted from Fullington et al. (1983). Half-grains were extracted with a solution (20 µl/mg) containing 0.75 ml 2-mercaptoethanol, 4.25 ml stock solution (Table 1) and 2 ml glycerol dissolved in 15 ml distilled and deionized water. Extracts were centrifuged at 6,000 rpm for 5 min.

Electrophoresis was carried out in polyacrylamide gels in a Protean I apparatus containing two gels. For details on the gel composition, see Table 1. 20 µl of supernatant of each sample was analyzed. The proteins were electrophoresed at 50 mA until the pyronin front migrated to the lower edge of the gel. Gels were stained for 12 h, then destained for 24 h, with a change of destaining solution after 12 h (see Table 1).

Table 1. Solutions used for the electrophoresis of total seed storage proteins (doses for two 18 × 16 × 0.15 cm gels)

Stock solution for sample buffer	6.25 ml 1M Tris-HCl (pH 6.8) 2 g SDS 10 mg pyronin Y bring to 50 ml with distilled water
Resolving gel	26.25 ml 1M Tris-HCl (pH 8.8) 40.25 ml 30% acrylamide + 0.135% Bis-acrylamide 0.7 ml 1% ammonium persulfate 0.7 ml 10% SDS 30 µl TEMED
Stacking gel	2.5 ml 1M Tris-HCl (pH 6.8) 16.8 ml 3.6% acrylamide + 0.05% Bis-acrylamide 1.0 ml 1% ammonium persulfate 0.2 ml 10% SDS 20 µl TEMED
Staining solution	0.3 g Coomassie brillant blue R 250 200 ml methanol 48 g TCA (Tricloro acetic acid) 70 ml glacial acetic acid dissolve TCA and acetic acid in distilled water and bring to 800 ml. In this solution filter the Coomassie blue dissolved in methanol
Tray buffer	70.55 g glycine 15.00 g Tris 2.00 g SDS dissolve with distilled water titrate to pH 8.3 and bring to 5 l
Destaining solution	4.8% TCA

Table 2. Solution used for the electrophoresis of the gliadins (doses for two 18 × 16 × 0.15 cm gels)

Resolving gel	1.6 ml K-lactate stock solution made by: – 3.5 g KOH – 23 ml lactic acid (85%) – 50 ml distilled water check pH and if necessary titrate to pH 3.1 with lactic acid 20 ml 28% acrylamide + 1.2% Bis-acrylamide 40 ml 0.004 N ammonium persulfate 17 ml distilled water 0.8 ml $AgNO_3$
Stacking gel	10 ml of the following solution: 6.0 g acrylamide 0.3 g Bis-acrylamide 20.0 mg ascorbic acid 2.5 mg $FeSO_4$ 2 ml of the K-lactate stock solution bring to 100 ml with distilled water
Prerun buffer	The same K-lactate stock solution of resolving gel diluted 1:50 with distilled water
Tray buffer	0.625 g Al-lactate 0.7 ml lactic acid (85%) 500 ml distilled water check if pH is 3.1, otherwise titrate with lactic acid
Staining solution	60 g TCA 0.1 g Coomassie brillant blue R-250 25 ml ethanol bring to 500 ml with distilled water

For gliadins, a procedure modified from that for the first dimension of the two-dimensional (two pH) electrophoresis method described by Lafiandra et al. (1984) was used.

Half-grains were extracted in a solution (20 µl/mg) composed of 1.2 ml NN'-dimethylformamide, 1.5 g sucrose, 10 mg methylgreen and 10 mg methylviolet brought to 10 ml with distilled and de-ionized water. Extracts were centrifuged at 6,000 rpm and 20 µl of supernatant for each sample was electrophoresed in polyacrylamide gels prepared with the solutions indicated in Table 2.

Gels were prerun at 90 mA (2 gel apparatus) with inverted electrodes (i.e., cathode and anode electrodes reversed) for 1h with the prerun-buffer. After the prerun, the prerun buffer in the top tank of the apparatus was replaced with tray buffer, and electrophoresis was carried out at 90 mA for about 2 h. Gels were stained for 2 to 4 h with the staining solution described in Table 2.

Results

Isoenzymes

ADH-1. Figure 1 presents the ADH-1 isozyme phenotypes for 'Chinese Spring' (CS), the disomic CS-*D. villosum* addition lines 1, 2, 4, 6, and 7, the amphiploid CS×v, and *D. villosum*. 'Chinese Spring' and the disomic addition lines 1, 2, 6, and 7 exhibited three ADH-1 isozymes (and *D. villosum* exhibits only one ADH-1 isozyme that had an electrophoretic mobility slower than the CS isozymes). The bands were very faint in disomic addition line 1 due to the small amount of sample extract loaded on the gel. Other faint bands were visible for *D. villosum* and the disomic addition line 6 which were artifacts due to unnoticed migration of sample during gel loading. Among the disomic addition lines, only CS-4V differs from 'Chinese Spring'. It produced a zymogram with five isozymes, three corresponding to those of 'Chinese Spring', one corresponding to that of *D. villosum*, and a new isozyme specific for this addition line. The same electrophoretic pattern with five forms of ADH-1 was shown by CS×v.

GOT. The GOT isozymes contained in the youngest leaf of 4 to 5-week-old plants of CS, CS×v, and the five CS-*D. villosum* disomic addition lines were resolved into three major zones on the basis of their migrational distance. Each zone was composed of either one or three or more isozymes, depending on the genotype (Fig. 2a). The isozymes of the three zones were designated in sequential order as GOT-1 (the most anodal), GOT-2, and GOT-3 (see Fig. 2 and Hart 1975).

The GOT-3 zymogram phenotypes produced by CS and CS-*D. villosum* addition lines were indistinguishable and characterized by three bands. *D. villosum* shows one or three bands in this zone (Fig 2b). In the triple-banded *D. villosum* phenotype, two isozymes were slower and one isozyme was as slow as the slower isozyme of CS zymogram in this zone (Fig. 2c). The CS×v amphiploid showed five bands (only four bands are visible in Fig. 2a) that overlap both the CS and the *D. villosum* GOT-3 bands.

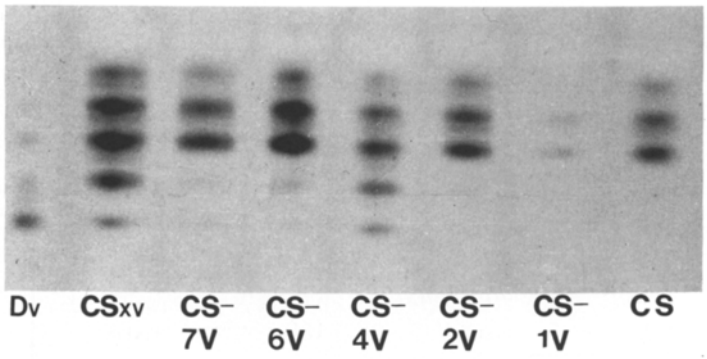

Fig. 1. ADH-1 zymogram phenotypes obtained by PAGE electrophoresis. Dv = *Dasypyrum villosum;* CSxv = *Triticum aestivum* cv. 'Chinese Spring' × *D. villosum* (2n = 8x = 56, AABBDDVV); CS-1V, CS-2V, CS-4V, CS-6V, and CS-7V are the disomic addition lines of *D. villosum* chromosome 1V, 2V, 4V, 6V, and 7V, respectively, in the *T. aestivum* cv. 'Chinese Spring' genome background according to Sears designation (Sears, pers. commun.). ⊕: anode; ⊖ cathode; → direction of isozyme migration

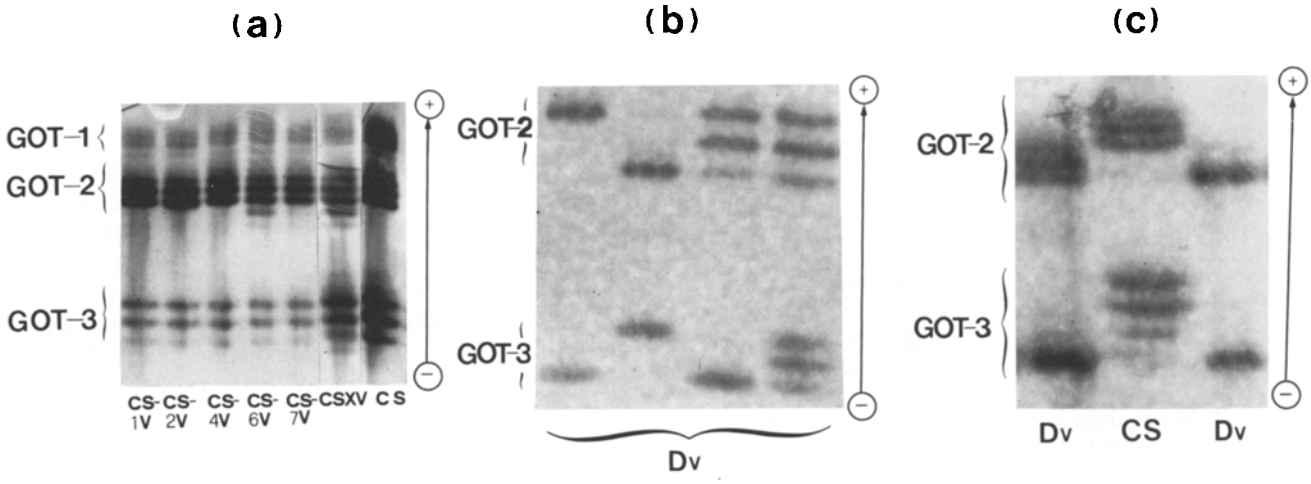

Fig. 2. GOT-1, GOT-2, and GOT-3 zymogram phenotypes obtained by PAGE electrophoresis. Explanation for abbreviations and symbols as in Fig. 1

CS showed three GOT-2 bands and *D. villosum* showed one or three bands (Fig. 2c). The one-banded *D. villosum* phenotype showed a band that corresponds either to the faster or to the slower isozymes of the triple-banded phenotype (Fig. 2b). The fastest *D. villosum* isozyme comigrated with the slowest CS isozyme. CS-*D. villosum* addition lines 1, 2, 4, and 7 showed three bands and addition line CS-6V showed five bands. Five isozyme bands were also present on the GOT-2 zymogram of CS×v and these isozymes comigrated with those of the CS-6V addition line.

The GOT-1 phenotype was composed of three bands in all of the genotypes analyzed.

GPI-1. All the samples analyzed except CS-1V exhibited three major cathodal bands and additional anodal bands. Our analysis is confined to the three main cathodal bands, whose genetic control was determined by Hart (1979a). These three electrophoretically cathodal GPI isozymes composed the GPI-1 system. *D. villosum* exhibited an isozyme coincident in mobility to the faster of the three anodal isozymes of CS and two slower isozymes with slightly greater electrophoretic mobilities than the other two cathodal CS isozymes (Fig. 3). Except for the CS-1V disomic addition line, which showed an enlargement of the two slower bands in the anodal direction, the CS-*D. villosum* disomic addition lines all showed a zymogram identical to CS. The observed variation in the GPI-1 bands indicate that some isozymes coded by genes on *D. villosum* chromosome 1V and some isozymes coded by genes on CS chromosomes of homoeologous group 1 in the CS-1V genotype migrate as closely adjacent isozymes in the electrophoretic system used. The CS×v amphiploid expresses a zymogram that is identical to that of 'Chinese Spring'.

These results are consistent with a schematic model for the subunit composition of GPI-1 isozymes of 'Chinese Spring', *D. villosum,* and CS-1V shown in Table 3. The model assumes that a set of four GPI-1 genes located in chromosomes 1A, 1B, 1D, and 1V and designated *Gpi-A1, Gpi-B1, Gpi-D1* (Hart 1979a), and *Gpi-V1*, respectively, control the expression of GPI-1 isozymes. According to Hart (1979a) it is assumed that *Gpi-A1* and *Gpi-D1* as well as *Gpi-V1* structural genes produce equal

Table 3. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II), and schematic model for the subunit (III) composition of the GPI-1 isozymes produced by *D. villosum* (Dv), *T. aestivum* cv. 'Chinese Spring' (CS) and the CS-*D. villosum* disomic addition line 1 (CS-1V)

Band no.	Isoenzyme	CS			CS-1V			Dv			
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	
1	Gpi-1a	1/4		$\alpha_1\alpha_1$, $\delta_1\delta_1$, $\alpha_1\delta_1$	4/25		$\alpha_1\alpha_1$, $\delta_1\delta_1$, $\alpha_1\delta_1$	1/4		$\nu_{1b}\,\nu_{1b}$	⊕
2	Gpi-1b′				4/25		$\nu_{1a}\alpha_1$, $\nu_{1a}\delta_1$	2/4		$\nu_{1a}\nu_{1b}$	
	Gpi-1b	2/4		$\alpha_1\beta_1$, $\delta_1\beta_1$	8/25		$\alpha_1\beta_1$, $\delta_1\beta_1$				
3	Gpi-1c′				1/25		$\nu_{1a}\nu_{1a}$	1/4		$\nu_{1a}\nu_{1a}$	
	Gpi-1c″				4/25		$\nu_{1a}\beta_1$				
	Gpi-1c	1/4		$\beta_1\beta_1$	4/25		$\beta_1\beta_1$				⊖

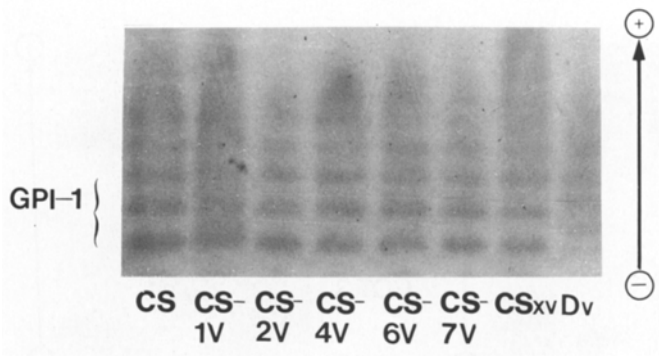

Fig. 3. GPI-1 zymogram phenotypes obtained by PAGE electrophoresis. Explanation for abbreviations and symbols as in Fig. 1

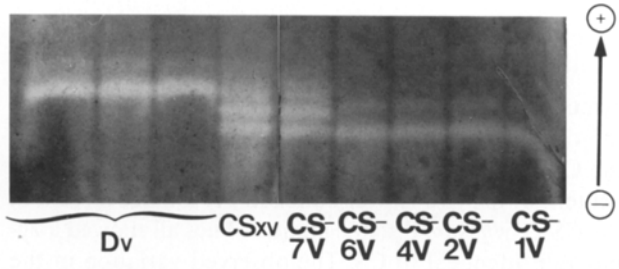

Fig. 4. SOD-2 zymogram phenotypes obtained by PAGE electrophoresis. Explanation for abbreviations and symbols as in Fig. 1

quantities of subunits α_1, δ_1, and ν_1, respectively and that *Gpi-B1* produces double quantity of subunits β_1 compared to the other GPI-1 subunits. The random association of those subunits give the dimeric GPI-1 isozymes depicted in Table 3 and shown in Fig. 3. Furthermore, it is assumed that the three isozymes produced by *D. villosum* are due to the presence of two alleles at one locus (indicated as *Gpi-V1a* coding for the slow ν_{1a} subunit and *Gpi-V1b* coding for the fast ν_{1b} subunit). In the hybridization process, only one allele would have been transferred from *D. villosum* into the CS genetic background. So the only hypothesis in full agreement with the results obtained (Fig. 3) and with the considerations given is one which assumes: (1) that in Cs×v the *Gpi-V1b* allele for GPI-1 transferred from *D. villosum* produces the subunit ν_{1b} of the same electrophoretic mobility as the subunits α_1 and δ_1 coded by the *Gpi-A1* and *Gpi-D1* alleles of 'Chinese Spring', respectively, and (2) that in the formation of the disomic addition line CS-1V a chromosome 1V of *D. villosum* that carried the allele *Gpi-V1a* was involved, which produces a subunit that differs from those of CS in electrophoretic mobility (for further details see Montebove 1985; De Pace et al., in prep.).

SOD. Three major zones of activity are observed for SOD. The most cathodal SOD zone in wheat has been indicated as SOD-C by Jaaska (1982) and this corresponds to the mitochondrial form of *Triticeae* SOD. However, Neuman and Hart (1986) gave the designation SOD-1 to this enzyme in order to be consistent with the practice of assigning consecutive Arabic numerals to the members of different sets of homoeologous wheat gene loci. Therefore, the most anodal zone of SOD activity, indicated as SOD-A by Jaaska (1982), can be indicated (following the criteria of Neuman and Hart 1986) as SOD-2. Only SOD-2 showed variability (Fig. 4), while SOD-1 was visualized as one-banded phenotypes in all strains analyzed. In the SOD-2 zone, CS and *D. villosum* showed a double-banded phenotype with one major and one minor band. The most anodal band for both CS and *D. villosum* may be an artifact or due to conformational isozymes. The two isozymes of the CS zymogram migrated slower than those of *D. villosum*. The CS-*D. villosum* disomic addition lines 1, 2, 4, and 6 showed the same SOD phenotype as that of CS, while the disomic addition line CS-7V and the CS×v amphiploid exhibited a phenotype composed of three isozymes, one with the same electrophoretic mobility as the *D. villosum* isozyme, another in the same position as the slower of the two CS isozymes, and a third band intermediate between the first two.

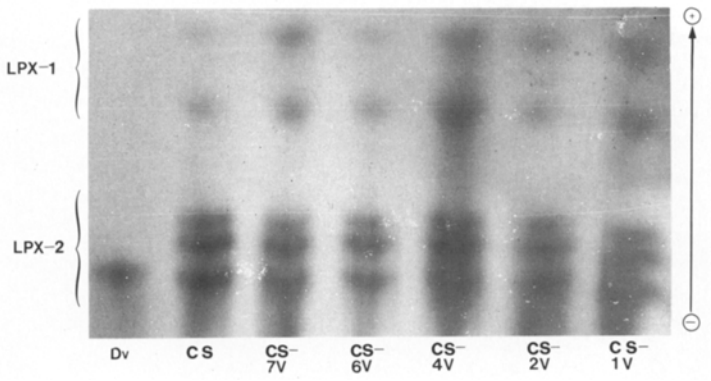

Fig. 5. LPX-1 and LPX-2 zymogram phenotypes obtained by starch-PAGE electrophoresis. Explanation for abbreviations and symbols as in Fig. 1

LPX. Five bands of LPX activity were resolved on starch-acrylamide slab gels for CS and CS-Dv disomic addition lines 1, 2, 4, 6, and 7 and one band for *D. villosum* (Fig. 5). The five bands of CS and CS-Dv disomic addition lines occurred in two major zones of LPX activity designated LPX-1 and LPX-2. LPX-1 consists of the two most anodal bands and LPX-2 of the three more cathodal bands. According to Hart and Langston (1977), the two most anodal bands of CS occurring in the LPX-1 zone contain isozymes encoded by genes on chromosomes 4A, 4B, and 4D. The product of the two structural genes on 4B and 4D have coincident electrophoretic mobilities and both contribute to the production of the slowest band of the LPX-1 zone. *D. villosum* does not show clear LPX-1 activity.

The three bands of the LPX-2 zone contain isozymes encoded by genes located in the long arms of the 5A, 5B, and 5D chromosomes. The *D. villosum* phenotype produced in this zone consists of one band of LPX-2 activity. According to the model for LPX composition in wheat and wheat-*D. villosum* derivatives (De Pace et al. in prep.), a double enzyme activity is to be expected for the most cathodal of the three LPX-2 bands in comparison to the other two. This situation is not visualized in any of the five CS-*D. villosum* addition lines available. This occurrence is compatible with the hypothesis that chromosomes 1V, 2V, 4V, 6V, and 7V of *D. villosum* do not carry genes for LPX-2 and that due to gene synteny it is probably chromosome 5V and not 3V that carries genes encoding for LPX-2 in *D. villosum*.

EST. We have arbitrarily divided the EST isozymes into three zones. In zone 1 of the faster moving esterase isozymes, first studied by Barber et al. (1968) and designated EST-1 by Hart (1979 b), neither CS×v nor the disomic addition lines studied exhibited differences when compared to CS; however, *D. villosum* does differ from CS in this zone (Fig. 6). For the other two esterase zones, the zymogram was not clear, although gross differences between the disomic addition lines were not evident. However, CS×v showed some bands that are not present in CS (in zone 2) (Fig. 6).

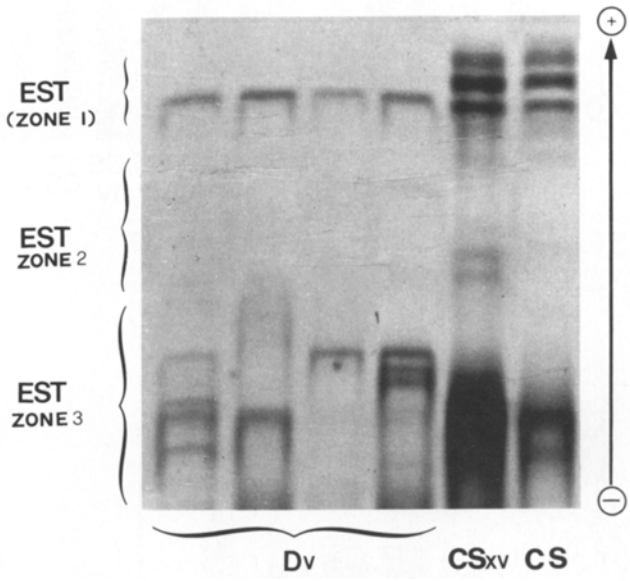

Fig. 6. EST zymogram phenotypes obtained by PAGE electrophoresis. Explanation for abbreviations and symbols as in Fig. 1

HMW glutenins

For the HMW storage proteins or glutenins (Fig. 7), CS showed four bands, named 2, 7, 8 and 12 according to Payne et al. (1984). In *D. villosum*, we can observe one or more HMW "glutenin-like" bands only in the zone between bands 7 and 8 of CS (Fig. 7 and De Pace, unpublished data). In disomic CS-*D. villosum* addition lines 2, 4, 6, and 7 we do not note differences in the profile of HMW glutenins in comparison to that of CS. In the addition line CS-1V and in the CS×v amphiploid, the electrophoretic pattern of HMW glutenins is characterized by an additional band which is located between bands 7 and 8 of CS. This additional band on the SDS-

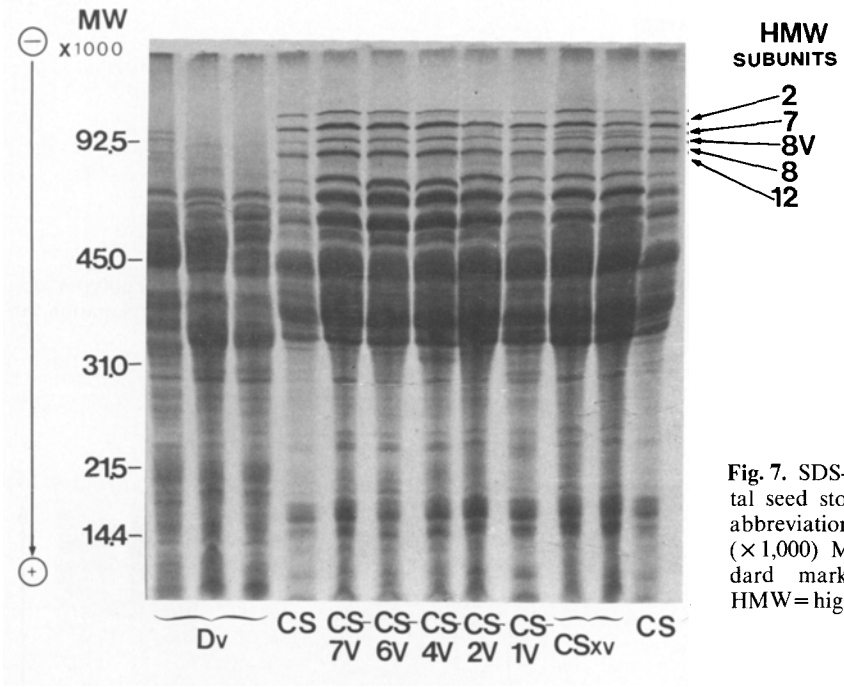

Fig. 7. SDS-PAGE electrophoresis of total seed storage proteins. Explanation for abbreviations and symbols as in Fig. 1. MW (× 1,000) Molecular weight for size standard marker expressed in K-daltons. HMW = high molecular weight subunits

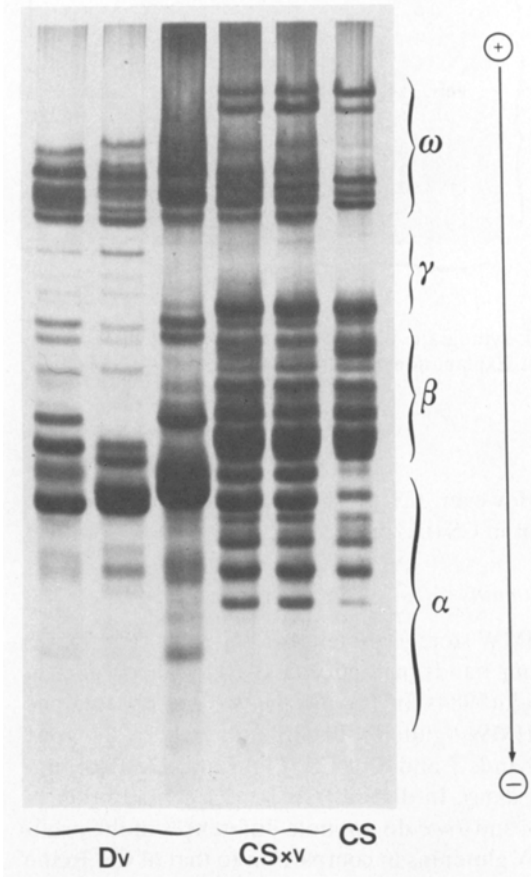

Fig. 8. Al-lactate PAGE electrophoresis of dimethylformamide extract of seed storage proteins. α, β, γ and ω are groups of polypeptides that share some amino acid sequence homology. Explanation for abbreviations and symbols as in Fig. 1

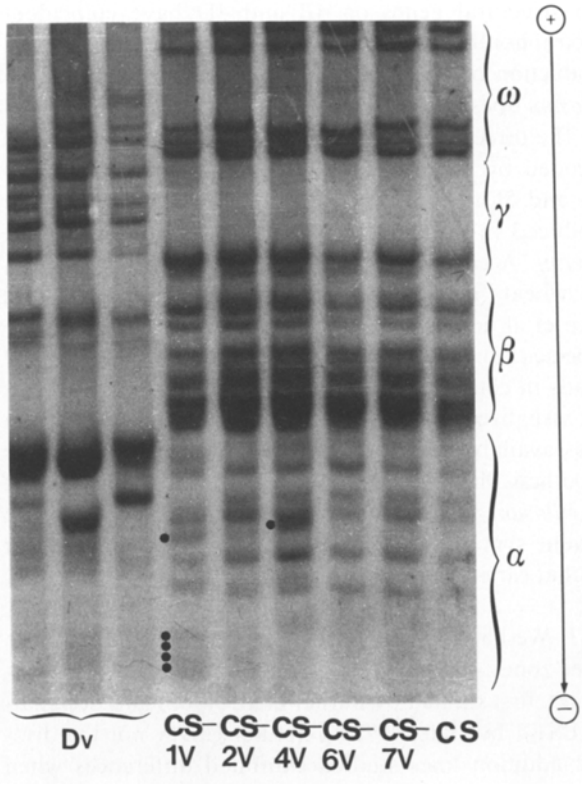

Fig. 9. Al-lactate PAGE electrophoresis of dimethylformamide extract of seed storage proteins. Explanation for abbreviations and symbols as in Figs. 1 and 8

PAGE pattern of HMW glutenins of CS×v and CS-1V has been designated band 8V (Fig. 7).

Gliadins

CS×v, in comparison with CS, showed two additional gliadin polypeptides in the region of the ω-gliadins (Fig. 8). These gliadin polypeptides had an electrophoretic mobility similar to some subunits that have been found in *D. villosum*. In the region of α-gliadins, the bands of Cs×v are more intensely stained in comparison to CS. In the α-gliadin region the electrophoretic pattern of *D. villosum* is particularly rich. The gliadins of the five disomic CS-*D. villosum* disomic addition lines were similar in their electrophoretic patterns to CS, with small differences observed for the α-gliadins of CS-1V and CS-4V disomic addition lines (Fig. 9). The pattern of the α-gliadins of CS-1V and CS-4V showed additional bands (indicated by dots in Fig. 9) in comparison to the CS gliadin pattern.

Discussion

The designations of the *D. villosum* chromosomes included in the genomic background of each of the five CS-*D. villosum* disomic addition lines used in this study follow Sears (Sears, pers. commun.). Sears' designations were based on (1) isozymes and morphological markers and (2) on the supposed highly conservative synteny of genes within the *Triticinae* subtribe. Therefore, there is not necessarily a correspondence between the chromosome designations used here and those of von Berg (1934) based on chromosomal arm ratios.

The results of the present study clearly demonstrate that chromosome 4V possesses a gene (or genes) involved in the production of the ADH-1 isozymes and that it can be designated *Adh-V1*. The evidence for the presence of the *Adh-V1* gene on chromosome 4V is that the disomic CS-4V addition line that carries chromosome 4V of *D. villosum* is the only one showing both the isozymes of the wheat parent and those of the *D. villosum* parent, and that its phenotype is identical to that of the CS×v amphiploid which carries all of the chromosomes of CS and *D. villosum*. It is unlikely that ADH-1 genes are present on chromosomes 3V and 5V because only one *Adh-1* locus has been detected in hexaploid wheat; specifically, in the chromosomes of homoeologous group 4 in wheat (Hart 1970).

Similar findings indicate that chromosome 6V of *D. villosum* carries a GOT-2 gene locus, chromosome 1V a GPI-1 gene locus and chromosome 7V a SOD-2 gene locus. The genes controlling the production of ADH-1, GOT-2, GPI-1, and SOD-1 isozymes in *D. villosum* are designated as *Adh-V1*, *Got-V2*, *Gpi-V1*, and *Sod-V2*, respectively.

The banding patterns observed for ADH-1, GOT-2, GPI-1, and SOD-2 isozymes in the disomic addition lines CS-4V, CS-6V, CS-1V, and CS-7V, respectively, and in the CS×v amphiploid, are explained on the basis of the dimeric nature of the enzyme formed by the random association of the subunits coded by the alleles carried by the CS and *D. villosum* chromosomes in those lines (Montebove 1985; De Pace et al., in prep.).

Hart (1970) demonstrated that each of the chromosomes of homoeologous group 4 of common wheat possesses a gene (or genes) involved in the production of the ADH-1 isozymes; since in *D. villosum* the chromosome carrying the *Adh-V1* alleles is 4V, it follows that chromosome 4V has some homoeology with the chromosomes of homoeologous group 4 of wheat.

Hart (1975, 1979a) dimonstrated that the chromosomes involved in the production of GOT-2 and GPI-1 isozymes in wheat are those of homoeologous groups 6 and 1, respectively. Thus, it follows that, since the 6V and 1V chromosomes of *D. villosum* carry genes for these two isozymes, they exhibit some homoeology with the chromosomes of wheat of homoeologous groups 6 and 1, respectively.

GOT-3 isozymes are produced in CS by genes on the chromosomes of homoeologous group 3 (Hart 1975). Since (1) we did not notice any differences between the GOT-3 isozyme patterns of the five disomic addition lines in comparison to the pattern of CS, (2) the disomic addition line CS-3V is not available, and (3) CS×v shows isozymes characteristic of both CS and *D. villosum*, it can be supposed that, by analogy with the other isozymes discussed, a *Got-V3* gene (or genes) might be located on chromosome 3V that produces GOT-3 protomers. However, it can also be supposed that (a) the gene coding for the GOT-3 isozymes might be located on chromosome 5V or (b) the *Got-V3* gene might be present in the addition lines studied but has not been detected because the disomic addition line involved carries a *Got-V3* allele that produces GOT-3 subunits of the same mobility as those of CS. Because of the high conservation of gene synteny in the *Triticinae* subtribe, it is highly probable that the *Got-V3* locus of *D. villosum* is in chromosome 3V.

In common wheat genes controlling EST-1 isozymes are present in the chromosomes of homoeologous group 3 (Barber et al. 1968, 1969) and genes controlling LPX-2 isozymes are present on chromosomes of homoeologous group 5 (Hart and Langston 1977).

In our study, no differences in EST-1 and LPX-2 isozymes were observed between the disomic CS-*D. villosum* addition lines studied. For the EST-1 and LPX-2 isozymes, the conclusions indicated for GOT-3 can be repeated. We will be able to give a definitive answer on the chromosomal location of the genes controlling GOT-3, EST-1, and LPX-2 isozymes in *D. villosum* only when the complete set of seven possible disomic CS-*D. villosum* lines are available.

For SOD-2, Jaaska (1982) detected the presence of a monomorphic phenotype consisting of one isozyme on the zymograms of all wheats, and for *D. villosum* he detected a single isozyme having a higher electrophoretic mobility than that found in wheat. On the basis of the expression of three isozymes by *Ae. mutica*, an outcrossing diploid, Jaaska (1982) supposed a dimeric nature for this isozyme. Our results are in accordance with this view. Furthermore, one of the two CS SOD-2 bands (the most anodal) does not appear on all gels, therefore, it cannot be a genetically determined isozyme. It may be (1) a product of the conformational alteration of the slower form of the SOD-2 isozyme or (2) an artifact due to the extracting method or electrophoresis system.

As matter of fact, the expression of only one group of comigrating isozymes in CS and in *D. villosum* and of three groups of comigrating isozymes in CS×v and the disomic addition line CS-7V is further evidence of the dimeric composition of SOD-2. The subunit compositions of CS, *D. villosum*, CS×v and CS-1V SOD-2 isozymes have been worked out by Montebove (1985) and De Pace et al. (in prep.).

In hexaploid wheat, glutenin subunits are coded by genes on the long arm of chromosomes of homoeologous group 1 (Payne et al. 1984). Since CS-1V is the only disomic addition line which produces a glutenin subunit similar to those most frequently found in *D. villosum*, we can suppose that there is a gene(s) coding for seed storage proteins similar to the one coding for wheat glutenins, on chromosome 1V of *D. villosum*.

The additional HMW protein subunit that in CS×v and CS-1V is coded by *D. villosum* gene(s) and the glutenin-like HMW subunits more frequently found in *D. villosum* both migrate to a position between bands 7 and 8 of CS. Since HMW glutenin genes of wheat that produce subunits migrating to this region are in chromosome 1B (Payne et al. 1984) it may be suggested that 1V is homoeologous to 1B. Furthermore, the contemporary presence of genes for glutenin-like proteins and GPI-1 isozymes, on chromosome 1V, is evidence of homoeology between chromosome 1V and the chromosomes of homoeologous group 1 in wheat, which also carry genes for glutenins and GPI-1 proteins (Chojecki et al. 1983).

The presence of genes coding for gliadin-like-subunits in chromosome 1V and 4V of *D. villosum* is deduced from the presence of additional α-gliadin subunits in the pattern of the disomic addition lines CS-1V and CS-4V. Genes coding for α, β, γ, and ω gliadin subunits of wheat have been located in chromosomes of homoeologous group 1 and 6 (Mecham et al. 1978). The CS×v amphiploid shows additional ω-gliadin-like subunits that are produced by genes in *D. villosum* chromosomes. These type of ω-gliadin subunits are absent in the CS-1V and CS-4V addition lines. Allelic diversity in the *D. villosum* strain used in synthesizing CS×v and the disomic addition lines may explain the difference. Therefore, any conclusive evidence on the chromosomal location for gliadin genes will come when disomic addition lines from CS×v are produced.

The information produced through this study can be useful for breeding purposes and for searching evolutionary affinities in *Triticinae*. As matter of fact, at least four independent gene markers (*Gpi-V1, Adh-V1, Got-V2,* and *Sod-V2*) have been identified which label four distinct *D. villosum* chromosomes. These *D. villosum* chromosomal gene markers are suitable for monitoring gene transfer from *D. villosum* to other *Triticinae* species and also in mapping other *D. villosum* genes. Furthermore, the ascertained homoeology between *D. villosum* and wheat genes for isozymes and seed storage proteins can have some bearing on the studies of affinities between *D. villosum* and *Triticum* genomes.

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