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Biochemical versatility of amphiploids derived from crossing *Dasypyrum villosum* Candargy and wheat: genetic control and phenotypical aspects

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Summary. The biochemical complexity and its consequence has been investigated in the amphiploids M × v and CS × v derived from crossing the tetraploid wheat *Triticum turgidum* var *durum* cv 'Modoc' and the hexaploid wheat *T. aestivum* cv 'Chinese Spring', respectively, with *Dasypyrum villosum*. Electrophoretic analysis of variation in six enzyme systems (GOT, ADH, GPI, SOD, EST, and LPX) and in high molecular weight glutenin seed storage proteins indicated that in the amphiploids these proteins were specified by a minimum of seven sets of homologous genes on wheat and *D. villosum* chromosomes and that in each set there were allelic differences. The enzymes detected in each amphiploid were fully accounted for by simple additivity of protomers specified by the homologous genes inherited from their parents. The amphiploids also expressed novel oligomeric enzymes not produced in either one of their parents. The ascertained expression for all the alleles inherited by both parents and the resulting biochemical complexity suggested that some peculiar feature of the amphiploids such as high nitrogen content in the plant and in the kernels and their immunity to the powdery mildew disease caused by both *Erysiphe graminis* f.sp. *tritici* and *E. graminis* f.sp. *haynaldiae* may be the consequence of the indicated complexity but specified by other sets of genes. The biochemical complexity of the M × v amphiploid may be the basis for its versatility as new crop species.

Key words: Electrophoretic analysis – *Dasypyrum villosum* – Seed storage proteins – *Erysiphe graminis* – Amphiploid

Introduction

Gottlieb (1973) indicated that the broad tolerance to environmental fluctuations of polyploid plants may depend to a certain extent on the enzymes coded by replicated loci that are fixed for alternate alleles. Therefore, the wider ranges of environmental tolerance observed for polyploid plants compared to their progenitors (Stebbins 1950) may be due to their biochemical versatility.

The biochemical versatility of polyploid individuals is due to: (1) the production of multimeric enzymes variants formed by the association of polypeptides coded by different alleles at the replicated loci; and (2) the absence of segregation in each generation among individuals of the polyploid population (Barber 1970).

Functional replicated loci for isozymes have been described in tetraploid and hexaploid wheat (Heart 1969, 1970, 1975), in the allotetraploid *Stephanomeria elata* (Gottlieb 1973), and in *Tragopogon* polyploids (Roose and Gottlieb 1976).

The presence of functional duplicated loci for isozymes and their biochemical versatility to a wider tolerance of environmental fluctuations has been explored by Gottlieb (1973) and Roose and Gottlieb (1976) in naturally occurring allotetraploid species. No information on biochemical versatility for new synthesized hexaploid and octoploid species are available, except the data of Tang and Hart (1975) on *Triticale*.

In the Triticeae there are many naturally occurring allotetraploid and allohexaploid species of the *Triticum, Aegilops, Agropyron,* and other genera. Wide crosses among species belonging to different Triticeae genera have been produced (Sakamoto 1973; Sharma and Gill 1983) and fertile amphiploids obtained, e.g., *Secale* × *Triticum* (Müntzing 1979) or *Dasypyrum* ×

Triticum (Jan et al. 1986). In the first case, triticale (× *Triticosecale* Wittmack) is a crop plant growing in habitats overlapping those of the parental species. In the second case, the polyploid × *Triticodasypyrum* (syn × *Haynaldoticum* Meletti et Onnis) obtained, although more productive in green matter than the *Dasypyrum* or *Triticum* parents (Montebove 1985), has not yet been explored for its versatility as a new crop species for marginal areas occupied by *Dasypyrum* and the arable areas in which *Triticum* grows.

A previous study (Montebove et al. 1987) showed that *D. villosum* chromosomes carry structural genes encoding isozymes that are expressed in a wheat genetic background. Therefore, the resulting amphiploid and derived disomic addition lines showed multiplicity of enzymes compared to both the wheat and *D. villosum* parents as well as a high proportion of novel oligomeric enzymes (interallelic heterodimers). These aspects may be related to the occurrence in the synthesized amphiploids of homoeologous sets of wheat and *D. villosum* chromosomes carrying structural gene loci orthologous (genome V of *D. villosum*) to the triplicate sets of paralogous genes located on the corresponding homoeologous wheat chromosomes of the A, B, and D genomes. Thus each set of functionally replicated loci in the amphiploid may control the production of enzymes or proteins with greater multiplicity, activity, and concentration, than the parents, thereby increasing the efficiency of certain biosynthetic pathways. As consequence of this increased biochemical versatility of the amphiploids, an increase in concentration of a certain enzyme product and/or the contemporary presence of gene products specified by the orthologous alleles inherited from their parents at a set of replicated loci may be expected. The resulting multiplicity in gene products may be the basis for an improved response of an amphiploid to varying environments.

Some attractive phenotypic feature of the *Triticum* × *Dasypyrum* amphiploids such as increased resistance to powdery mildew, earliness, kernel protein content, and seed storage subunit multiplicity, were not present in the wheat parent and may be a consequence of their increased biochemical complexities (Montebove 1985).

In this paper we present a biochemical genetic analysis of certain isozymes of the hexaploid amphiploid derived from *Triticum turgidum* var. *durum* × *D. villosum* and of the octoploid amphiploid derived from *T. aestivum* × *D. villosum*. The aim was to ascertain the degree of biochemical complexity of these genotypes to gain insights to the kind of expression observed in the amphiploids for biochemical-related phenotypic features, such as nitrogen content and powdery mildew resistance. The information obtained can be used to infer the versatility of the wheat × *Dasypyrum* amphiploids as potential new crop species.

Materials and methods

Genotypes

Dasypyrum villosum ($2n=2x=14$, VV) (Dv) plants used for establishing the zymogram phenotypes for the isozyme systems described below were obtained from seeds of two populations collected at Valenzano, Bari, Italy and indicated as DV-Va11 and DV-Va12, and within the population DV-84.16 collected at Bomarzo, Viterbo, Italy.

Triticum turgidum var *durum* cv 'Modoc' × *D. villosum* ($2n=6x=42$, AABBVV) (M × v) and *T. aestivum* cv 'Chinese Spring' × *D. villosum* ($2n=8x=56$, AABBDDVV) (CS × v) amphiploids were obtained through embryo culture and colchicine treatment of the F_1 seedlings as described by Jan et al. (1986).

Isozyme analysis

The progeny of a random sample of plants of the indicated genotypes were assayed individually by polyacrylamide gel electrophoresis to identify zymogram phenotypes for six enzyme systems: glutamate-oxaloacetate-transaminase (GOT; E.C. 2.6.1.1), alcohol dehydrogenase (ADH; E.C. 1.1.1.1), glucosephosphate isomerase (GPI; E.C. 5.3.1.9), superoxide dismutase (SOD; E.C. 1.15.1.1), esterase (EST; E.C. 3.2.1.–) and lipoxygenase (LPX; E.C. 1.13.11.12). Coleoptiles, leaves and half kernels were mainly used as materials to prepare crude extracts for the analysis.

Leaf blades of 4 to 5-week-old plants were ground in a mortar in Carlson's buffer as modified by Hart (1982) using a tissue/buffer ratio: 1 mg/4 μl. The slurry was centrifuged at 12,000 rpm at 4 °C for 7 min and the supernatant was electrophoresed to visualize GOT, SOD, and EST isozymes.

Extracts of 4-day-old coleoptile (tissue/Carlson's buffer ratio: 1 mg/2 μl) from kernels germinated in Petri dishes were centrifuged at 12,000 rpm at 4 °C for 7 min and the supernatant was submitted to electrophoresis to visualize LPX isozymes.

Half kernels (endosperm end) were soaked for 18 h in distilled water in Petri dishes and extracted with an aqueous solution of 12.5% sucrose. Each half seed was crushed in a 1.5 ml microcentrifuge polypropylene tube using a glass rod and 50 μl of sucrose solution to obtain a liquid extract for analysis of ADH isozymes (10 μl of sucrose solution were used to prepare extract for analyzing GPI isozymes). The liquid extracts were centrifuged at 6,000 rpm at 4 °C for 7 min and the supernatant was used immediately for electrophoresis. Forty μl of supernatant of each sample extract were used for gel analysis.

Electrophoresis was carried out in polyacrilamide gels in a vertical apparatus using a discontinuous buffer system. The composition of gels and buffers used for the electrophoretic separation of ADH, GOT, GPI and LPX isozymes (adapted from Hart 1982) and of the gels and buffers utilized to separate SOD and EST isozymes (adapted from Shumaker et al. 1984) are reported in Table 1. Additional details on the preparation of samples and electrophoresis running conditions were reported in Montebove et al. (1987).

The staining solutions used to visualize the isozymes (Table 2) have been adapted from Hart (1982) for GOT, ADH, SOD, EST and LPX and from Arus and Orton (1984) for GPI.

Seed storage protein analysis

High molecular weight (HMW) glutenins were electrophoretically analyzed using a SDS-PAGE procedure adapted from Fullington et al. (1983) to separate the glutenin subunits. Gliadins were extracted and electrophoresed using the acid-PAGE procedure and buffers described in Montebove et al. (1987).

Table 1. Composition of the stacking gels, resolving gels and tray buffers used for isozyme electrophoresis (amount for two 18 × 16 × 0.15 cm gels)

Isozyme system	Composition of resolving gel			Composition of stacking gel			Tray buffer
	Stock solution		Amount	Stock solution		Amount	
GOT	A	1 M Tris-HCl (pH 8.9)	8.75 ml	B	0.416 M Tris-HCl (pH 6.9)	2.50 ml	0.04 M Tris-glycine
ADH	C	28.0% acrylamide + 0.735% Bis-acrylamide	17.50 ml	D	10% acrylamide + 2.5% Bis-acrylamide	5.00 ml	(pH 8.3)
GPI							
LPX	E_2	0.114% ammonium persulfate	43.75 ml	F	40% sucrose	10.00 ml	
	E_2	(only for LPX) 0.560% ammonium persulfate	5.00 ml	E_1	0.5% ammonium persulfate	2.50 ml	
		1% starch	38.75 ml				
		TEMED	20 μl		TEMED	20 μl	
EST	A	0.8 M Tris-citrate	22.00 ml	D	0.8 M Tris-borate (pH 8.5)	1.30 ml	0.2 M Tris-borate
SOD		(pH 9.0)		F	0.15 M Na_2-EDTA	0.20 ml	(pH 8.5)
	B	0.15 M Na_2-EDTA	0.70 ml	G	6.66% acrylamide + 0.37% Bis-acrylamide	15.00 ml	
	C	20.41% acrylamide + 0.54% Bis-acrylamide	34.30 ml	E	0.56% ammonium persulfate	3.50 ml	
	E	0.56% ammonium persulfate	13.00 ml				
		TEMED	20 μl		TEMED	15 μl	

Table 2. Staining solution for GOT, ADH, GPI, SOD, EST and LPX isozymes (amount for 1 gel)

Isozyme system		Isozyme system	
ADH	4 mg NAD 2 mg PMS 9 mg NBT 5 ml 0.1 M Tris-HCl (pH 7.5) 2 ml EtOH bring to 50 ml with distilled water	GOT	300 mg aspartic acid 150 mg α-ketoglutaric acid 100 mg fast-blue-BB salt 0.75 mg piridoxal-5′-phosphate dissolve in 50 ml of 0.2 M Tris-HCl (pH 8)
GPI	4 mg NADP 16 mg MTT 4 mg PMS 11 mg D-fructose-6-phosphate 5 ml 10% $MgCl_2$ 40 units glucose-6-phosphate dehydrogenase dissolve in 50 ml of 0.2 M Tris-HCl (pH 8)	SOD	3 mg riboflavine 5 mg NBT 12.5 ml 0.2 M Tris-HCl (pH 8) bring to 50 ml with distilled water
LPX	0.5 g linoleic acid with a drop of Tween-80 in 25 ml DDD H_2O and bring to 50 ml. Use 25 ml of this solution and bring to 50 ml with 0.05 M Tris-HCl (pH 8.3)	EST	30 mg α-naphtyl-acetate 10 mg β-naphtyl-acetate 1 ml acetone 200 mg fast-blue-RR salt bring to 50 ml with distilled water

Analysis of morphological features

The weight of mature plant biomass (culms, leaves, spikes and kernels) and weight of kernels at 12% water content were scored from plants grown in greenhouse kept at 20±1 °C and 16 hours light and 8 hours dark photoperiod. These characters were used to estimate the harvest index (HI) as the ratio kernel weight/biomass weight. Hundred seed weight and nitrogen content (using a Tecator Kjeltek System I for micro-Kjeldahl determinations of N) were also evaluated.

Analysis of powdery mildew reaction

Erysiphe graminis DC ex Mérat f. sp. *tritici* conidia were isolated from a population of mildew pustules occurring on leaves of adult plants of 'Modoc' durum wheat, and *Erysiphe graminis* f. sp. *haynaldiae* conidia were isolated from a population of mildew pustules occurring on leaves of greenhouse grown *D. villosum* plants. Three groups (replicates) of 10 seedlings of the amphiploids and of the parents were each isolated under a clear plastic tube 30 cm tall (10 cm ∅). Conidia were brushed

directly from mildew pustules of leaves kept above the isolating tube and were allowed to deposit on the seedlings (settling tower technique). Uniform levels of conidia were deposited on inoculated leaves. Adult plants of the same genotypes at anthesis were also infected with the two *E. graminis* strains. Number of pustules were recorded 10 days after inoculation.

Results and discussion

Genetical aspects

Polymorphism and genetics of isozymes Glutamate oxaloacetate transaminase, GOT. Three zones of isozyme activity were observed for GOT. The isozymes of the three zones were designated in sequential order as GOT-1 (the anodal zone), GOT-2 and GOT-3 (the cathodal zone) (Fig. 1). The resolution of the isozymes (visualized as bands of enzyme activity during the staining procedure) for GOT-1 was not always good, although three bands seemed to characterize the electrophoretic phenotype in this zone for the analyzed genotypes (Fig. 1 a).

Five bands of isozyme activity were observed in the GOT-2 zone among the analyzed genotypes. The isozymes of the GOT-2 zone of M comigrated to the same position on the gel and were visualized as one band (band 3, Fig. 1 a). The adjacent anodal bands may be due to either the activity of isozymes of the GOT-1 zone or to intergenic isozymes, i.e., hybrid (oligomeric) isozymes formed by dimerization of protomers coded by alleles at nonhomoeologous loci. The GOT-2 zymogram phenotype of M × v was composed by the three most cathodal bands (bands 3–5 in Fig. 1 a) and Dv showed only one band (band 5) in Fig. 1 a, although Dv phenotypes with only band 3 or with three bands as in M × v were found previously (Montebove et al. 1987). Dv showed an intense band between the GOT-2 and GOT-3 zones, probably due to intergenic heterodimers.

The zymogram phenotype exhibited by CS was different from that of M and Dv and was composed of the three most anodal bands (bands 1–3; Fig. 1 b and c). CS × v exibited a five-banded phenotype (Fig. 1 b and c).

The observed positional variation of the bands within the GOT-2 zymograms provided evidence that these bands were the sites of a group of isozymes encoded by a set of four homologous genes, three paralogous genes on wheat chromosomes and one orthologous gene on a Dv chromosome.

The three genes located on wheat chromosomes of homoeologous group 6 governing the isozymes of the GOT-2 zone have been designed *Got-A2, Got-B2* and *Got-D2* (Hart 1975) and the subunits (protomers) they encoded as α_2, β_2 and δ_2 respectively. CS contained all three genes while M contained only the *Got-A2* and *Got-B2* genes.

The gene(s) located on Dv chromosome 6V (Montebove et al. 1987) was designated *Got-V2* and showed two allelic forms: *Got-V2a,* the most frequent (De Pace 1987) and *Got-V2b.* The subunits encoded by the two alleles were indicated as V_{2a} (the heavier protomer coded by the

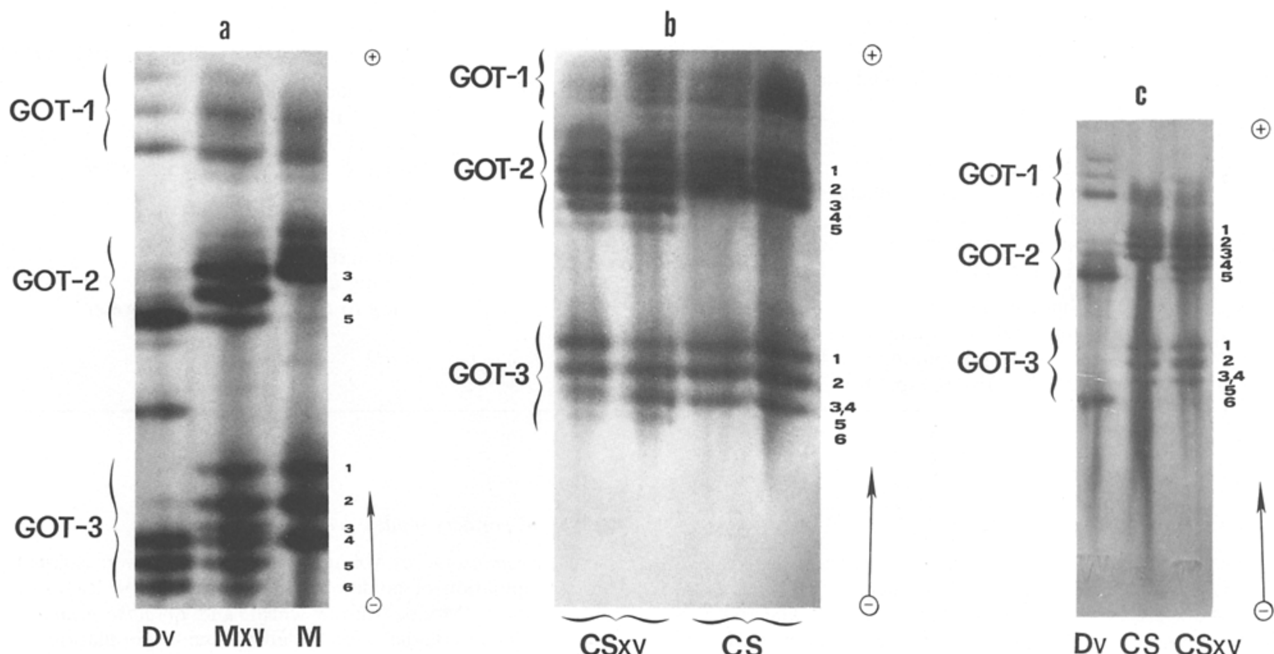

Fig. 1 a–c. GOT zymogram phenotypes. Dv = *Dasypyrum villosum*; M × v = *Triticum turgidum* var *durum* cv 'Modoc' × *Dasypyrum villosum*; CS × v = *Triticum aestivum* cv 'Chinese Spring' × *Dasypyrum villosum*; M = *T. turgidum* var *durum* cv 'Modoc'; CS = *T. aestivum* cv 'Chinese Spring'. + = anode; – = cathode; – – > = direction of migration

Table 3. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the GOT-2 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Got-2a							1/16	—	$\delta_2\delta_2$				1/9	—	$\delta_2\delta_2$
2	Got-2b							4/16	▬	$\alpha_2\delta_2$, $\beta_2\delta_2$				4/9	▬	$\alpha_2\delta_2$, $\beta_2\delta_2$
3	Got-2c	1/4	—	$V_{2b}V_{2b}$	4/9	▬	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$	6/16	█	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$, $V_{2a}\delta_2$	1	▬	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$	4/9	▬	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$
4	Got-2d	2/4	▬	$V_{2a}V_{2b}$	4/9	▬	$V_{2a}\alpha_2$, $V_{2a}\beta_2$	4/16	▬	$V_{2a}\alpha_2$, $V_{2a}\beta_2$						
5	Got-2e	1/4	—	$V_{2a}V_{2a}$	1/9	—	$V_{2a}V_{2a}$	1/16	—	$V_{2a}V_{2a}$						

Table 4. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the GOT-3 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Got-3a				1/9	—	$\beta_3\beta_3$	4/16	█	$\beta_3\beta_3$, $\delta_3\delta_3$, $\beta_3\delta_3$	1/4	—	$\beta_3\beta_3$	4/9	█	$\beta_3\beta_3$, $\delta_3\delta_3$, $\beta_3\delta_3$
2	Got-3b				2/9	▬	$\alpha_3\beta_3$	4/16	█	$\alpha_3\beta_3$, $\alpha_3\delta_3$	2/4	▬	$\alpha_3\beta_3$	4/9	█	$\alpha_3\beta_3$, $\alpha_3\delta_3$
3	Got-3c				2/9	▬	$V_{3a}\beta_3$	4/16	█	$V_{3a}\beta_3$, $V_{3a}\delta_3$						
4	Got-3d	1/4	—	$V_{3b}V_{3b}$	1/9	—	$\alpha_3\alpha_3$	1/16	—	$\alpha_3\alpha_3$	1/4	—	$\alpha_3\alpha_3$	1/9	—	$\alpha_3\alpha_3$
5	Got-3e	2/4	▬	$V_{3a}V_{3b}$	2/9	▬	$V_{3a}\alpha_3$	2/16	▬	$V_{3a}\alpha_3$						
6	Got-3f	1/4	—	$V_{3a}V_{3a}$	1/9	—	$V_{3a}V_{3a}$	1/16	—	$V_{3a}V_{3a}$						

Got-V2a allele) and V_{2b} (the lighter protomer encoded by the *Got-V2b* allele). When the Dv genotypes were homozygous for *Got-V2a* or *Got-V2b* they exhibited a one banded phenotype (only band 3 or band 5) and the *Got-V2a/Got-V2b* heterozygous genotype exhibited a triple-banded phenotype (Montebove et al. 1987), indicating that GOT-2 isozymes were dimeric.

The genes coding for GOT-2 isozymes in the amphiploids were *Got-A2, Got-B2*, and *Got-V2a* in M × v and *Got-A2, Got-B2, Got-D2*, and *Got-V2a* in CS × v. The presence of the *Got-V2a* allele instead of the *Got-V2b* allele in the amphiploids was implied by: (1) the zymogram phenotypes showed by M × v and Cs × v, and (2) the fact that the *Got-V2a* allele was the most frequent in different Dv populations (De Pace 1987) and therefore *Got-V2a* had the greatest probability to be present in the haploid genotype of the Dv pollen grain that fertilized the wheat genotype.

A schematic model for the subunit composition of the GOT-2 isozymes of the genotypes analyzed is shown in Table 3. The model, following Hart (1975), assumed: (1) the four types of protomers were produced in equal quantities; (2) the protomers associated randomly in all possible combinations; and (3) α_2 and β_2 protomers gave homodimers and heterodimers of the same mobility. Although this model was consistent with the assumption of random subunit association, it did not account for the observed staining intensities (Fig. 1 b) of the bands in CS × v. As a matter of fact, if the staining intensities given by the isozymes at each position (band) were proportional to the amount of those isozymes, then bands 1 and 5 should have had (according to the assumption of equal production of each subunit) one-fourth of the staining intensity of bands 2 and 4, and one-sixth of the staining intensity of band 3. This expectation seemed to be verified for band 5, but not for band 1. Therefore, in CS × v there was either an over-production of GOT-2 protomers coded by wheat genes or an under-production of GOT-2 protomers coded by Dv genes. The last hypothesis seemed more reasonable.

Up to six bands of activity were expressed for the GOT-3 isozymes. The GOT-3 zymogram phenotype of M included bands 1, 2 and 4 (Fig. 1 a). Dv showed three phenotypes: only band 4, only band 6 (Fig. 1 c), or a triple-banded phenotype with bands 4–6 (Fig. 1 a and Montebove et al. 1987). Band 4 was common to both M and Dv. M × v exhibited a six-banded phenotype with bands 1, 2 and 4–6 at the same position as in the M and Dv zymogram phenotypes and band 3 that was not present in either parent.

CS exhibited a zymogram phenotype composed of bands 1, 2 and 4 as in M, and CS × v exhibited a six-banded phenotype as in M × v (only five of these bands are visible in Fig. 1 b). Bands 3, 5, and 6 were very faint in freshly prepared gels and were not always seen in CS × v zymograms of extracts from leaf blades of both young (6 to 14-day-old seedlings) (Fig. 1 b) and adult plants (Fig. 1 c).

The observed positional variation of the bands within the GOT-3 zymograms analyzed provided evidence that these bands were the sites of a group of isozymes encoded by a set of four genes, three paralogous genes on wheat chromosomes and one orthologous gene on a DV chromosome. The three GOT-3 genes located on wheat chromosomes (those of homoeologous group 3; Hart 1975) have been designated *Got-A3, Got-B3* and *Got-D3* and the protomers they encoded as α_3, β_3 and δ_3, respectively (Hart 1975). A gene located on one Dv chromosome (probably 3V) was designated *Got-V3*, which showed two allelic forms: *Got-V3a*, which was the most frequent in natural population (De Pace 1987) and *Got-V3b*. The subunits these alleles encoded were designated as V_{3a} (the heavier protomer coded by the *Got-V3a* allele) and V_{3b} (the lighter protomer coded by the *Got-V3b* allele). When the Dv genotypes were homozygous for the *Got-V3a* or *Got-V3b* allele they exhibited a one-banded phenotype with only band 4 or band 6 present, respectively, (Montebove et al. 1987). The heterozygous *Got-V3a/Got-V3b* Dv genotypes exhibited a triple-banded phenotype indicating that the GOT-3 isozymes were dimeric.

The genes coding for GOT-3 isozymes in the amphiploids were: *Got-A3, Got-B3*, and *Got-V3a* in M × v, and *Got-A3, Got-B3, Got-D3*, and *Got-V3a* in CS × v. The presence of the *Got-V3a* allele instead of the *Got-V3b* allele in the amphiploid was implied: (1) by the zymogram phenotypes showed by M × v and CS × v; and (2) by the fact that the *Got-V3a* allele was the most frequent in Dv populations and therefore had the highest chance to be present in the haploid genotype of the DV pollen grain that fertilized the wheat genotype during the hybridization process.

A schematic model (Table 4) for the subunit (protomer) composition of the GOT-3 isozymes of the analyzed genotpyes follows the same assumptions as given earlier for GOT-2. The staining intensities of the bands (Fig. 1) seemed to be consistent with these assumptions and fit the expected quantitative distribution, although bands 3 and 4 usually appeared as one thick band in CS × v and band 6 was barely visible in the zymogram phenotype of CS × v. The proportion of the total GOT-3 isozymes present in band 6 was expected to be 6.25% in CS × v and 11.1% in M × v. This latter proportion was sufficient to make the isozymes in band 6 visible, the former proportion was insufficient.

Alcohol dehydrogenase, ADH. The ADH isozymes were observed as five bands in a single activity zone designated as ADH-1 (Fig. 2). M exhibited the three anodal bands 1–3 (the slightly stained bands 4 and 5 were due to sample migration from adjacent wells) and Dv exhibited

Table 5. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the ADH-1 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Adh-1a				1/9	—	$\alpha_1\alpha_1$	1/16	—	$\alpha_1\alpha_1$	1/4	—	$\alpha_1\alpha_1$	1/9	—	$\alpha_1\alpha_1$
2	Adh-1b				2/9	▮	$\alpha_1\beta_1$	4/16	▮	$\alpha_1\beta_1$, $\alpha_1\delta_1$	2/4	▮	$\alpha_1\beta_1$	4/9	▮	$\alpha_1\beta_1$, $\alpha_1\delta_1$
3	Adh-1c				3/9	▮	$\beta_1\beta_1$, $V_1\alpha_1$	6/16	▮	$\beta_1\beta_1$, $\delta_1\delta_1$, $\beta_1\delta_1$, $V_1\alpha_1$	1/4	—	$\beta_1\beta_1$	4/9	▮	$\beta_1\beta_1$, $\delta_1\delta_1$ $\beta_1\delta_1$
4	Adh-1d				2/9	▮	$V_1\beta_1$	4/16	▮	$V_1\delta_1$, $V_1\beta_1$						
5	Adh-1e	1	—	V_1V_1	1/9	—	V_1V_1	1/16	—	V_1V_1						

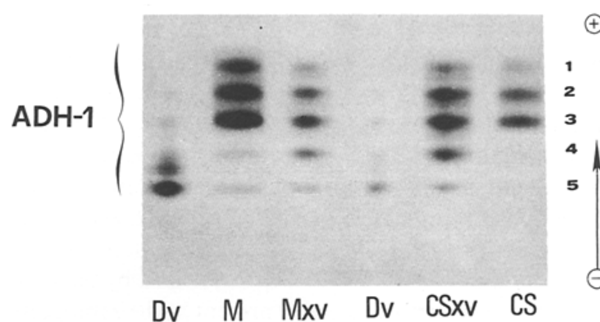

Fig. 2. ADH-1 zymogram phenotypes. Explanation for abbreviations and symbols as in Fig. 1

only the cathodal band 5 (the additional anodal bands in the zymogram of Fig. 2 may be due to conformational variants of ADH-1). M × v exhibited five bands: band 1, 2, and 3 as in M, band 5 as in Dv and band 4, which was not present in either parental zymogram phenotypes. The zymogram phenotype of CS resembled that of M; CS × v showed the same five-banded zymogram phenotype as M × v.

The observed variation in the ADH-1 zymogram phenotypes provided evidence that these bands were the sites of a group of isozymes that were encoded by three paralogous genes on wheat chromosomes and one orthologous gene on a Dv chromosome. The three *Adh-1* genes located on wheat homoeologous group 4 chromosomes have been designated *Adh-A1*, *Adh-B1*, and *Adh-D1* and the protomers they encoded as α_1, β_1 and δ_1, respectively (Hart 1970). The gene(s) located on Dv chromosome 4V (Montebove et al. 1987) was designated *Adh-V1* and the protomer it encoded as V_1. Therefore the genes coding for ADH-1 isozymes in the amphiploids were *Adh-A1*, *Adh-B1* and *Adh-V1* in M × v, and *Adh-A1*, *Adh-B1*, *Adh-D1*, and *Adh-V1* in CS × v.

A schematic model (Table 5) for the subunit (protomer) composition of the ADH-1 isozymes of the analyzed genotypes assumes (as in Hart 1970) that: (1) four types of subunits were produced in equal quantities; (2) they associated randomly in all possible combinations; and (3) β_1, and δ_1 protomers gave homodimers and heterodimers with the same mobility. The number and staining intensities observed in Fig. 2 seemed to be consistent with this model.

Glucosephosphate isomerase, GPI. Two zones of isozyme activity were observed in the GPI zymograms of all the genotypes analyzed. No clear bands were observed in zone 2 (the most cathodal zone), but for zone 1, the six isozymes produced by the activity of the GPI-1 isozyme system as indicated by Hart (1979) were visualized in

bands 1–6 of Fig. 3 (see also Table 3 in Montebove et al. 1987). In zone 1 there were two additional anodal bands common to all genotypes, which will not be considered here.

In zone 1, three bands (indicated as bands 1, 3 and 6, corresponding to isozymes Gpi-1a, Gpi-1b and Gpi-1c, respectively) were visible in CS. The GPI-1 zymogram phenotypes of M, M × v and CS × v were indistinguishable from that of CS and were characterized by bands 1, 3, and 6. Three bands were also present in the zymogram of Dv, but with only band 1 common to the other genotypes; bands 2 and 4 were more anodal and narrower than bands 3 and 6 in M, M × v, CS, and CS × v. Bands 2 and 4 contained the Gpi-1b′ and Gpi-1c′ isozymes, respectively. The presence of band 5 containing isozyme Gpi-1c″, was assumed on the basis of the zymogram phenotype of CS + 1V disomic addition line (Montebove et al. 1987).

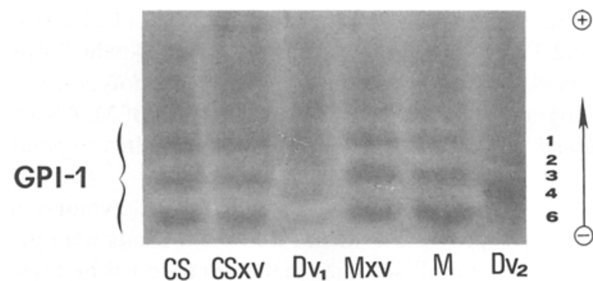

Fig. 3. GPI-1 zymogram phenotypes. Explanation for abbreviations and symbols as in Fig. 1

Previously, Hart (1979) identified a set of three paralogous genes *Gpi-A1*, *Gpi-B1* and *Gpi-D1*, located on wheat chromosomes of homoeologous group 1 that in CS coded for the protomers α_1, β_1 and δ_1, respectively. According to Hart (1979), the *Gpi-A1* and *Gpi-D1* structural genes produced equal quantities of subunits α_1 and δ_1, respectively, and *Gpi-B1* produced a double quantity of subunit β_1 compared to the other GPI-1 subunits. The gene located on Dv chromosome 1V (Montebove et al. 1987) was designated *Gpi-V1* with two alleles, *Gpi-V1a* and *Gpi-V1b*. The subunits encoded by the two alleles were indicated as V_{1a} (the heavier protomer coded by the *Gpi-V1a* allele) and V_{1b} (the lighter protomer encoded by the *Gpi-V1b* allele). It was assumed that when either one of these alleles was present in CS × v, it produced the same amount of protomer V_1 as was produced by the *Gpi-A1* or *Gpi-D1* alleles. On Dv the homozygotes *Gpi-V1a*/*Gpi-V1a* and *Gpi-V1b*/*Gpi-V1b* produced bands 4 (Dv-2, Fig. 3) or 1, respectively. Heterozygous *Gpi-V1a*/*Gpi-V1b* Dv genotypes produced bands 1, 2 and 4 (Dv-1, Fig. 3). The triple-banded phenotype produced by heterozygous Dv individuals was an indication that GPI-1 isozymes were dimeric. The triple-banded phenotype observed in M, M × v, CS and CS × v were produced by the isozymes whose protomeric composition and proportions are reported in Table 6. The

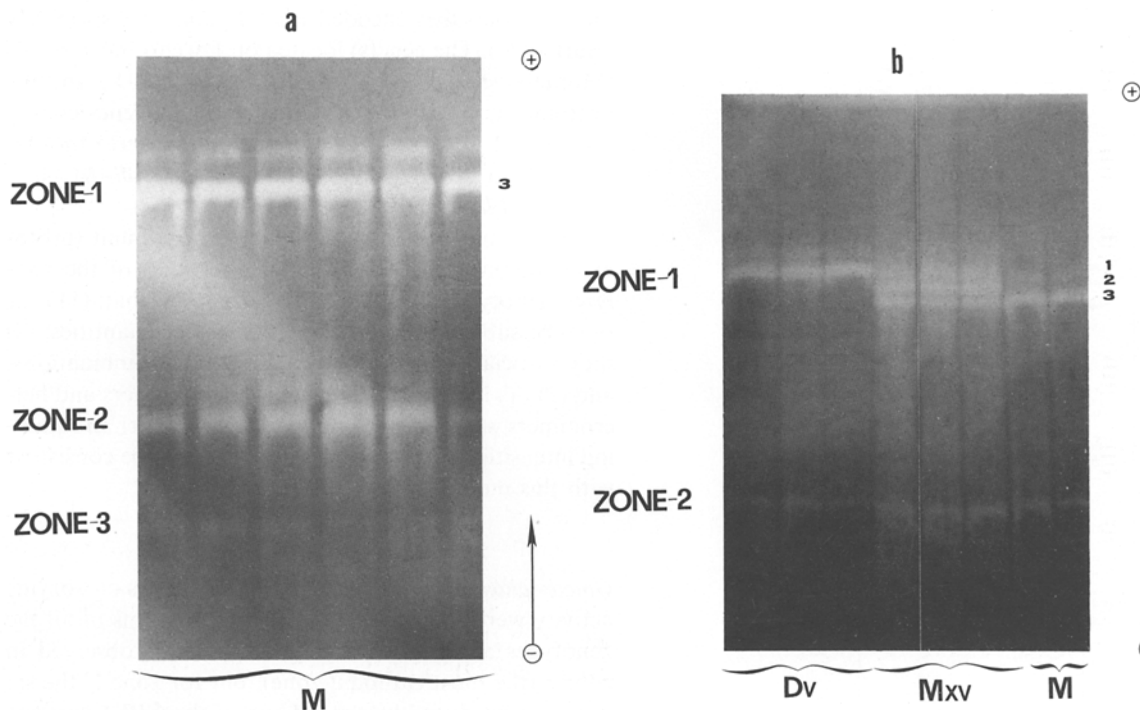

Fig. 4a and b. SOD zymogram phenotypes. Explanation for abbreviations and symbols as in Fig. 1

Table 6. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the GPI-1 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Gpi-1a	1/4	—	$V_{1b}V_{1b}$	1/4	—	$\alpha_1\alpha_1$, $V_{1b}V_{1b}$, $V_{1b}\alpha_1$	9/25	▬	$\alpha_1\alpha_1$, $\delta_1\delta_1$, $V_{1b}V_{1b}$, $\alpha_1\delta_1$, $V_{1b}\alpha_1$, $V_{1b}\delta_1$	1/9	—	$\alpha_1\alpha_1$	1/4	—	$\alpha_1\alpha_1$, $\delta_1\delta_1$, $\alpha_1\delta_1$
2	Gpi-1b′	2/4	▬	$V_{1a}V_{1b}$												
3	Gpi-1b				2/4	▬	$\alpha_1\beta_1$, $V_{1b}\beta_1$	12/25	▬	$\alpha_1\beta_1$, $\beta_1\delta_1$, $V_{1b}\beta_1$	4/9	▬	$\alpha_1\beta_1$	2/4	▬	$\alpha_1\beta_1$, $\beta_1\delta_1$
4	Gpi-1c′	1/4	—	$V_{1a}V_{1a}$												
5	Gpi-1c″															
6	Gpi-1c				1/4	—	$\beta_1\beta_1$	4/25	—	$\beta_1\beta_1$	4/9	▬	$\beta_1\beta_1$	1/4	—	$\beta_1\beta_1$

type and number of bands composing the M × v and CS × v zymogram phenotypes indicated that, in addition to the *Gpi* genes from the wheat parent, they carried also the *Gpi-V1b* allele from Dv but not the *Gpi-V1a* allele. If the allele *Gpi-V1a* from Dv was present in chromosomes of the two amphiploids, a six-banded phenotype should have appeared. On the other hand, if the resolution was poor, a triple-banded phenotype should have appeared, with the intermediate band composed of the isozymes Gpi-1b′ and Gpi-1b (Table 6) and the most cathodal band composed of isozymes Gpi-1c″, Gpi-1c′ and Gpi-1c (Table 6) thicker than in M and CS. Such a phenotype was observed in the CS-1V disomic addition line (Montebove et al. 1987) with an enlargement of the intermediate and cathodal bands indicating that the allele *Gpi-V1a* was present in this disomic addition line and that resolution with the electrophoretic system used did not allow the separation of the six expected isozymes.

Superoxide dismutase, SOD. Three zones of activities were observed in the SOD zymograms (Fig. 4a). In zone 1, the anodal zone, CS (Fig. 4 in Montebove et al. 1987), M, and Dv (Fig. 4b) showed a double-banded phenotype with one major and one minor band. The minor band (the most anodal) may have been due to conformational isozyme variants of SOD, or oxidoreductase enzymes that were coordinately regulated with SOD. The SOD isozymes of the CS and M zymograms of zone 1 migrated slower than those of DV. In zone 1, the amphiploids M × v (Fig. 4b) and CS × v (Fig. 4 in Montebove et al. 1987) showed a three-banded phenotype, with the two extreme bands, band 1 and 3, common to Dv and wheat, respectively, plus a third intermediate band (band 2). In zone 2 (the intermediate position zone), all genotypes showed a one-banded phenotype. In zone 3 (the cathodal zone), the SOD isozymes were visible as one diffuse band for all genotypes examined.

When gels were stained in the presence of 2 m*M* KCN (Fig. 5a) the SOD activity in zone 1 was inhibited, the SOD activity of zone 2 appeared to be almost nil, and the SOD activity of zone 3 was not inhibited.

Similar results were obtained by Jaaska and Jaaska (1982). Fridovich (1975) indicated that only copper and zinc containing SODs in eukaryotic cytosol were reversibly inhibited by a millimolar concentration of cyanide, while manganese-containing SODs, found in mitochondria and prokaryotes, were not inhibited by cyanide. Therefore, on the basis of this evidence, Jaaska and Jaaska (1982) suggested that: (1) SOD-A (in zone 1) and SOD-B (in zone 2) found among the species of the subtribe Triticinae were copper and zinc containing SODs, and SOD-C (in zone 3) was a manganese-containing enzyme; and (2) SOD-A, SOD-B, and SOD-C were the chloroplastic, the cytosol and the mitochondrial SOD isozymes, respectively. They found also that SOD-A,

SOD-B and SOD-C had a pI of 4.5, 5.4–5.7 and 6.1, respectively.

Neuman and Hart (1986) designated as SOD-1 the mitochondrial SOD-C isozymes, with pIs of approximately 6.0–6.35. The SOD-1 isozymes were not visualized in all the gels (Fig. 4b and 5b); however, they were clearly visible in cyanide-treated gel after negative staining (Fig. 5a).

Montebove et al. (1987) designated the partially cyanide-sensitive chloroplastic SOD isozymes of zone 1 as SOD-2, which corresponded to SOD-A with a pI 4.5 designated by Jaaska and Jaaska (1982).

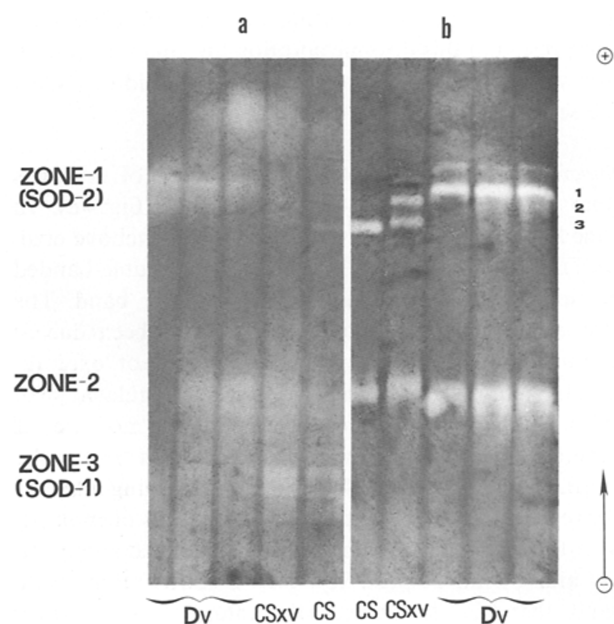

Fig. 5a and b. SOD zymogram phenotypes obtained **a** in the presence and **b** absence of 2 m*M* KCN in the staining solution. Explanation for abbreviations and symbols as in Fig. 1

The sets of paralogous wheat genes coding for SOD-2 isozyme have not been identified yet, but the orthologous gene on Dv has been located on chromosome 7V. Because this was the second SOD gene identified in Triticeae and because of gene synteny, the existence of paralogous wheat genes coding for homologous isozymes to that coded by chromosome 7V is implicated. The designation of SOD-2 for these isozymes and of *SOD-2* for the genes that encode them seems reasonable.

The genes coding for the highly cyanide-sensitive cytosol SOD of zone 2 (corresponding to SOD-B with a pI 5.4–5.7 of Jaaska and Jaaska 1982) have not been designated yet in either wheat or alien species. Jaaska (1982) postulated a dimeric nature for the SOD-2 (=SOD-A) isozyme in Triticeae and our results are in accordance with this view.

The observed variation in the number of SOD-2 bands provided evidence that these bands were the sites of a group of isozymes encoded by a set of four SOD-2 genes, three paralogous genes on wheat chromosomes and one orthologous gene on a Dv chromosome. The three SOD-2 genes of wheat, probably located on chromosomes of only one homoeologous group, could be designated *Sod-A2, Sod-B2* and *Sod-D2*, and the protomers they encode as α_2, β_2, and δ_2, respectively. The gene(s) located on Dv chromosome 7V (Montebove et al. 1987) was designated as *Sod-V2* and the protomer it encoded as V_2.

A schematic model for the subunit (protomer) composition of the SOD-2 isozymes for the analyzed genotypes is shown in Table 7. The model assumed that (1) the four types of protomers were produced in equal quantities, (2) they associated randomly in all possible combinations, and (3) α_2, β_2 and δ_2 subunits had the same net charge and size. The number of bands observed in Figs. 4 and 5 are consistent with this model, even though the intensities of band 1 in M × v observed in the zymogram

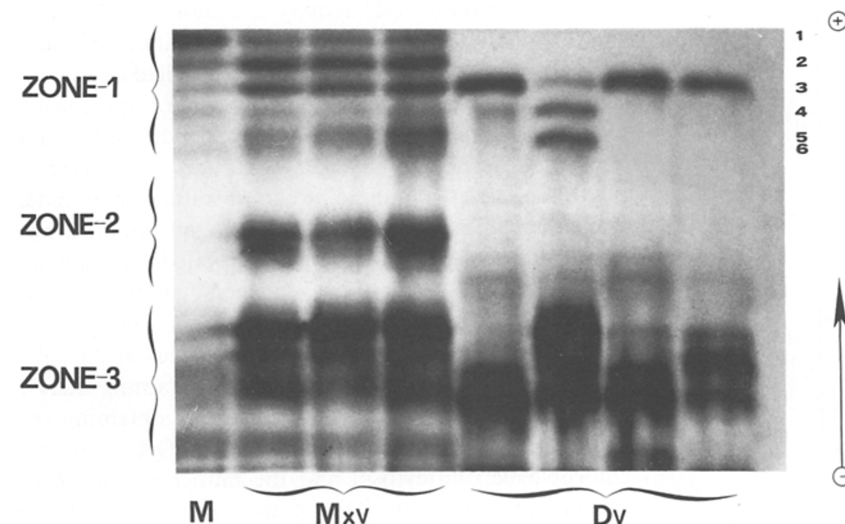

Fig. 6. EST zymogram phenotypes. Explanation for abbreviations and symbols as in Fig. 1

Table 7. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the SOD-2 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Sod-2a	1	——	V_2V_2	1/9	——	V_2V_2	1/16	——	V_2V_2						
2	Sod-2b				4/9	▬	$V_2\alpha_2$, $V_2\beta_2$	6/16	▬	$V_2\alpha_2$, $V_2\beta_2$, $V_2\delta_2$						
3	Sod-2c				4/9	▬	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$	9/16	▬	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\delta_2\delta_2$, $\alpha_2\beta_2$, $\alpha_2\delta_2$, $\beta_2\delta_2$	1	——	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\alpha_2\beta_2$	1	——	$\alpha_2\alpha_2$, $\beta_2\beta_2$, $\delta_2\delta_2$, $\alpha_2\beta_2$, $\alpha_2\delta_2$, $\beta_2\delta_2$

phenotypes of Fig. 4b was higher than expected. This could be due to an unequal production of the subunits or to the higher activity of the V_2V_2 isozyme.

Esterase, EST. The zymogram phenotypes of the studied genotypes (Fig. 6) (Montebove et al. 1987) showed at least three zones of activity.

In zone 1 six bands were observed. In CS and CS × v bands 1–3 of equal intensity were observed (Montebove et al. 1987). In M, the bands 1–4 and 6, with decreasing staining intensity from band 1 to band 6, were observed. In M × v all six bands were observed, with bands 5 and 6 confluent as one thick band. Dv showed at least five phenotypes in zone 1 (De Pace 1987), and in Fig. 6 two phenotypes were present: one with only band 3 and the other with bands 3–5. Evidence from progenies of multi-banded Dv phenotypes indicated that the variability observed for the phenotypes of zone 1 could be explained, assuming that the esterase isozymes occurring in this zone are dimeric and that their genetic control is due to multiple alleles (at least four) at one locus. Barber et al. (1968) showed the dimeric composition of the fast-migrating (anodal) esterase in wheat.

The most frequent esterase phenotype in the Dv populations was expressed as band 3. Therefore the allele coding for the band 3 isozyme was expected to be the most frequent since it was contributed by the haploid genotype of the Dv male gamete that fertilized the CS and M ovules from which CS × v and M × v were derived.

Bands 1–3 of M showed decreasing staining intensity compared to M × v which showed an increasing intensity for the same bands in the following order: bands 1, 3 and 2. This observation indicated that the paralogous wheat genes and the orthologous Dv genes in M × v were all active and coded for subunits that associated randomly to produce esterase homodimers and heterodimers. If this was the case, it was expected that an increased proportion of isozymes would be visible in bands 2 and 3. As a matter of fact, bands 2 and 3 were more intense in M × v than in M (Fig. 6). M × v showed additional esterase isozymes, compared to those of the parents, in both zone 2 and zone 3.

Lipoxygenase, LPX. Two zones of LPX activity were designated in sequential order as LPX-1 (the most anodal) and LPX-2 (the most cathodal) (Fig. 7). Three bands of LPX-2 activity were resolved on acrylamide slab gels for CS, CS × v (Fig. 7b) and M × v (Fig. 7a). M showed only two bands with the same electrophoretic mobility as the two more anodal bands of CS. In Dv three different LPX-2 isozymes were found with the same or slower electrophoretic mobility than the most cathodal isozyme of CS.

The observed variation provided evidence that these bands were the sites of a group of monomeric isozymes

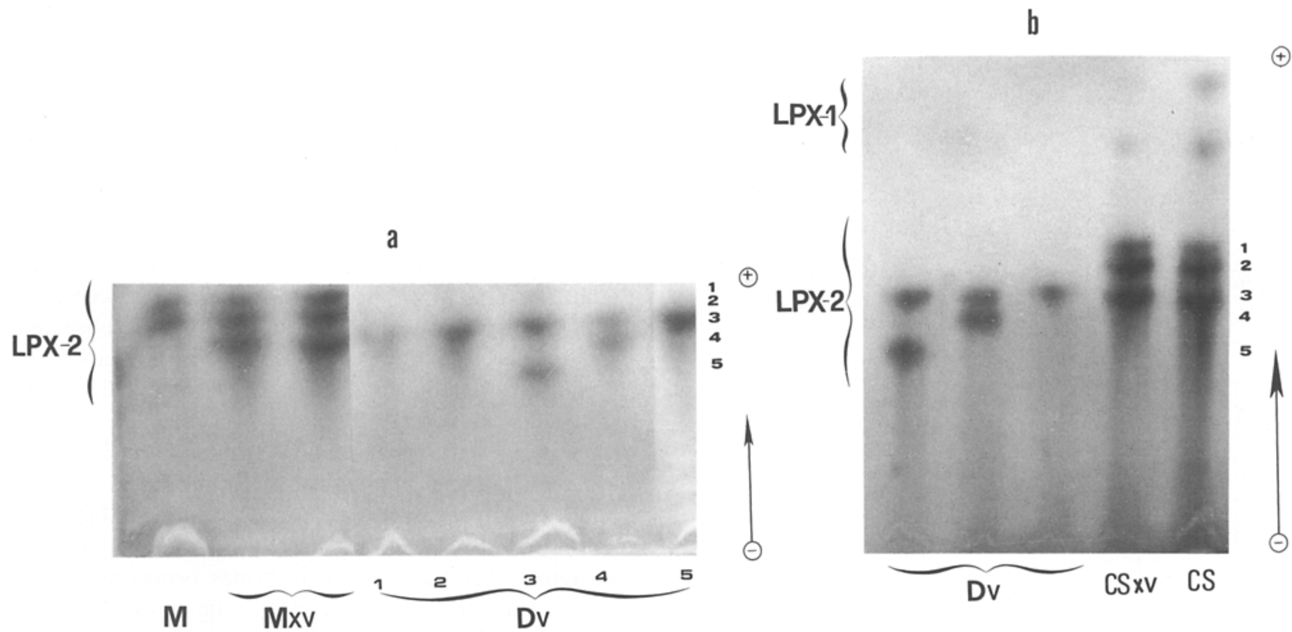

Fig. 7a and b. LPX zymogram phenotypes. Explanation for abbreviations and symbols as in Fig. 1

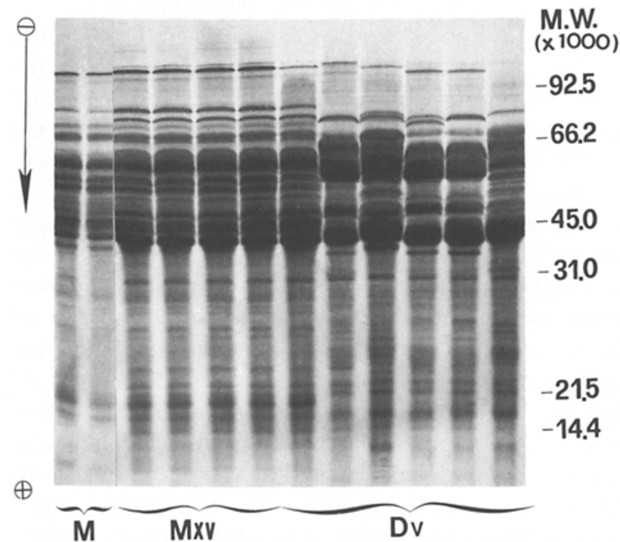

Fig. 8. SDS-PAGE electrophoresis of total seed storage proteins. Explanation for abbreviations and symbols as in Fig. 1

encoded by a set of four paralogous genes. Three of the genes were located on wheat chromosomes of homoeologous group 5 (Hart and Langston 1977) and have been designated *Lpx-A2, Lpx-B2,* and *Lpx-D2* and the protomers they encoded as α_2, β_2, and δ_2, respectively. The fourth gene was probably located on DV chromosome 5V (Montebove et al. 1987), although the phenotype of the CS + 5V disomic addition line was indistinguishable from that of CS (data not shown). The gene coding for LPX-2 isozymes located on the Dv chromosome, will be designated *Lpx-V2* with alleles *Lpx-V2a*, *Lpx-V2b* and *Lpx-V2c*. The protomers they encoded can be indicated as V_{2a} (corresponding to the LPX isozymes visualized in band 3), V_{2b} (visualized in band 4), and V_{2c} (visualized in band 5). Table 8 shows a schematic model for the composition of the LPX-2 isozymes of the studied genotypes. The model assumed that: (1) four types of protomers were produced in equal quantities; and (2) δ_2 and V_{2a} protomers had the same size.

Polymorphism of seed storage proteins

The SDS-PAGE patterns of the total seed storage proteins of M, M × v, and Dv are shown in Fig. 8, and those of CS and CS × v were reported in Fig. 7 of Montebove et al. (1987). M and M × v have a common band in the zone of HMW glutenins with mol. wt. around 95,000. Furthermore, in M × v two additional bands of slightly different molecular weights from that of M were found, but with molecular weight analogous to those of the HMW proteins of Dv.

The variation for seed storage protein within CS × v and M × v was almost nil. And this was in accordance with the expectation that in amphiploids the alleles of the parental gametes were fixed at all loci. The intraspecific variability in Dv for glutenin subunit weight was high (De Pace 1987; and data not shown) compared to that found in other Triticeae (Preston et al. 1975). The molecular weight variability of the Dv HMW glutenin-like subunits was within the range of the molecular weight variability of glutenin subunits coded by genes on the

Table 8. Expected quantitative distribution (I), diagrams of zymogram phenotypes (II) and schematic model for the protomer (III) composition of the LPX-2 isozymes produced by *D. villosum* (Dv), the hexaploid amphiploid *T. turgidum* var *durum* cv 'Modoc' × *D. villosum* (M × v), the octoploid amphiploid *T. aestivum* cv 'Chinese Spring' × *D. villosum* (CS × v), *T. turgidum* var *durum* cv 'Modoc' (M) and *T. aestivum* cv 'Chinese Spring' (CS)

Band no.	Isozyme	Dv			M × v			CS × v			M			CS		
		(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
1	Lpx-2a				1/3	—	α_2	1/4	—	α_2	1/2	—	α_2	1/3	—	α_2
2	Lpx-2b				1/3	—	β_2	1/4	—	β_2	1/2	—	β_2	1/3	—	β_2
3	Lpx-2c	1	—	V_{2a}	1/3	—	V_{2a}	2/4	▬	V_{2a}, δ_2				1/3	—	δ_2

Table 9. Average values for some morphological characters of wheat × *Dasypyrum* amphiploids and their parents

Genotypes	No. individuals	100 kernel weight, g	No. kernels/ plant	Kernel weight/ plant, g	Chaff weight/ plant, g	Biomass, g	Harvest index	N (% dry weight)	
								Kernels	Plant at booting stage
CS	80	3.73 ± 0.142	388.5 ± 5.2	14.49 ± 1.73	33.41 ± 0.74	47.90 ± 1.03	0.303 ± 0.011	3.123 ± 0.016	
CS × v	8	2.18 ± 0.315	201.6 ± 10.1	4.39 ± 3.21	29.25 ± 4.26	33.64 ± 3.61	0.131 ± 0.041	3.314 ± 0.027	
M × v	20	3.42 ± 0.291	373.0 ± 8.6	12.76 ± 1.89	30.92 ± 1.04	43.68 ± 1.21	0.292 ± 0.016	3.171 ± 0.032	3.899 ± 0.335
M	80	4.30 ± 0.183	400.0 ± 3.8	17.20 ± 1.94	31.43 ± 1.13	48.63 ± 1.32	0.354 ± 0.011	2.527 ± 0.021	2.981 ± 0.269
Dv16a 19.9	31	0.64	65.0	0.42	4.78	5.20	0.081	2.960	2.734
Dv16a 20.14	6	0.75	59.0	0.44	10.46	10.90	0.040	3.179	2.684
Dv16a 20.15	25	0.57	157.0	0.89	10.58	11.47	0.078	2.981	2.515
Dv16b 23a	14	0.76	174.2	1.32	12.26	13.58	0.097	3.310	2.512
Dv16b 24	20	0.65	49.6	0.32	7.74	8.06	0.040	4.270	2.747
Dv16b 26	10	0.61	38.2	0.23	7.38	7.61	0.030	3.245	2.023
Dv, weig.av.	106	0.64 ± 0.006	95.3 ± 5.1	0.61 ± 0.04	8.26 ± 0.24	8.88 ± 0.25	0.067 ± 0.002	3.298 ± 0.047	2.586 ± 0.020
Dv16a, l.k.[a]								2.944	
Dv16a, d.k.[b]								2.546	

[a] Light kernels
[b] Dark kernels

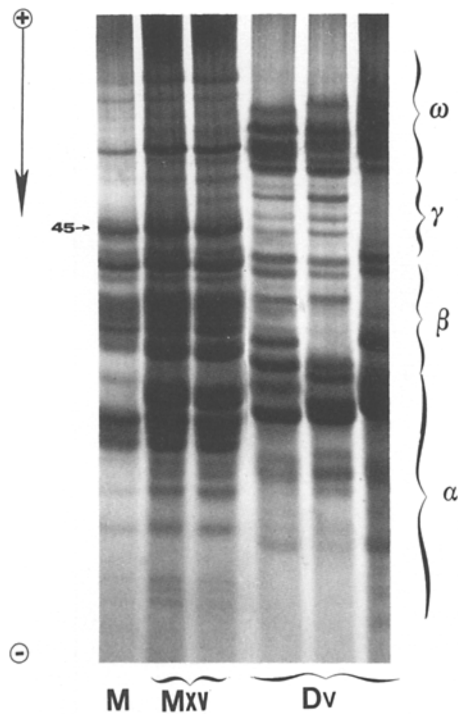

Fig. 9. Al-lactate PAGE electrophoresis of dimethylformamide extract of seed storage proteins. Explanation for abbreviations and symbols as in Fig. 1

chromosome of genome B of wheat. In fact, results reported by Payne et al. (1984) and du Cros et al. (1983) on molecular weight variability of glutenins coded by genes of genome B (*Glu-B1*) in bread wheat and durum wheat, respectively, indicated that the HMW glutenin subunits coded by *Glu-B1* genes was around 95,000. These glutenins were visible in bands that occupied the same position of the HMW glutenin-like bands of Dv in the 17% SDS-polyacrylamide gel.

CS × v, when compared with CS, revealed an additional HMW glutenin-like band (band 8v) coded by Dv chromosome 1V, which was present between the HMW glutenin bands 7 and 8 of CS (Montebove et al. 1987) coded by the *Glu-B1* complex locus on wheat chromosome 1B.

The electrophoretic pattern for gliadins of all studied genotypes (Fig. 9) showed that M × v was richer in bands than M in the region of α and β gliadins; these additional M × v bands could be specified by Dv genes.

CS × v showed additional bands in the ω and α regions when compared with the banding patterns of CS and M × v. In general, the two amphiploids showed all the bands found in the gliadin electrophoretic pattern of the wheat parent, plus additional bands which were coded by the Dv genes present in the amphiploids.

The gliadin band 45, correlated with the good quality of the durum wheat gluten (Damidaux et al. 1978; Kosmolak et al. 1980), was present in both M and M × v gliadin polypeptide pattern. This aspect together with the high protein content (see following paragraph) makes the M × v seed storage proteins very interesting for further study.

Morphophysiologic characters

Morphological characters. The overall phenotypes of the CS × v and M × v amphiploids were similar to the respective wheat parents. In particular, the values recorded for kernel size, kernel number, total biomass and harvest index of CS × v and M × v plants were much closer to those of the wheat parent than the Dv parent (Table 9). This indicated that for the expression of characters related to the gross plant and kernel morphology, there was partial dominance of the wheat genes over the action of the Dv genes.

M × v and CS × v showed up to 15% and 60%, respectively, of floret sterility. This sterility contributed to the lower grain yield of CS × v and to the lower HI of the amphiploids compared with the parents.

M × v had a brittle rachis and rigid glume traits of Dv and CS × v had a nonbrittle rachis.

The ligules and the edges at the base of the leaf lamina of the amphiploids showed long (3 to 4 mm) bristles. These bristles were absent in the wheat parents and were useful morphological markers to distinguish the seedlings of the amphiploids from the CS and M seedlings.

Seed and plant nitrogen content. The N content of the M × v kernels was 25.5% higher than that of the M kernels; CS × v kernels showed an N content that was 6.1% higher than that recorded in CS kernels (Table 9). DV also had higher mean N content than CS and M.

Records taken from the shooting to the milk stage, indicated that the highest N content in the whole plant occurred at the booting stage. During this stage M × v showed a 30.8% higher nitrogen content than M and a 23% higher N content than that in its mature grains.

Powdery mildew resistance. Seedlings of CS and M were highly susceptible to a local strain of *Erysiphe graminis* f.s. *tritici.* Twenty days after inoculation the upper surface of the leaf blades and sheaths were 98% covered by large pustules. Dv, CS × v and M × v were immune to the infection at both the seedling and adult stages (Table 10, Fig. 10).

Only seedlings and adult plants of Dv were highly susceptible to infection by *E. graminis* f.sp. *haynaldiae*, while all the other genotypes (M, M × v, CS, and CS × v) were immune.

These results indicated that the wheat genotypes (CS and M) and the Dv genotypes were highly susceptible to infection by their respective 'forma specialis' of

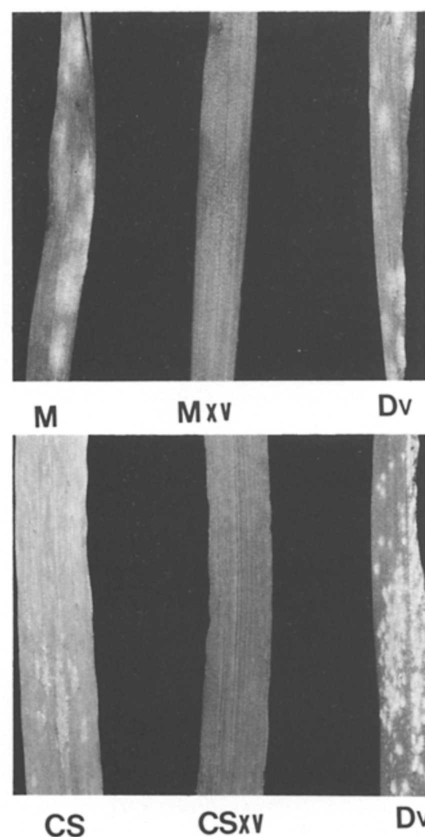

Fig. 10. Presence (M, CS, Dv) and absence (M × v, CS × v) of powdery mildew pustules on lower leaf lamina surface 20 days after inoculation with *Erysiphe graminis* conidia. Explanation for abbreviations as in Fig. 1

Table 10. Powdery mildew symtoms on wheat and wheat × *Dasypyrum* amphiploid plants after 20 days from infection with two 'forma specialis' of *Erysiphe graminis*. R = resistant, no pustules; S = susceptible, 98% of the upper leaf surface covered by pustules

Genotypes	Reaction to the infection with conidia of:	
	E. graminis f.sp. *tritici*	*E. graminis* f.sp. *haynaldiae*
CS	S	R
CS × v	R	R
M × v	R	R
M	S	R
Dv	R	S

E. graminis, while the octoploid (CS × v) and hexaploid (M × v) amphiploids were immune to both *E. graminis* f.sp. *tritici* and *E. graminis* f.sp. *haynaldiae*. Therefore, it appears that the wheat genotypes carry genes for resistance to *E. graminis* f.sp. *haynaldiae* and Dv carry genes for resistance to *E. graminis* f.sp. *tritici*. Both wheat and Dv resistance genes were expressed in the wheat × *Dasypyrum* amphiploids.

Resistance to the powdery mildew disease in the amphiploids may be explained by a biochemical mechanism based on the protein-for-protein hypothesis proposed by Vanderplank (1978). This hypothesis implies that in the gene system involved in the amphiploid-pathogen interaction there are genes that specify proteins that do not polymerize with the *E. graminis haynaldiae* pathogen proteins, and Dv genes that specify proteins that do not polymerize with the *E. graminis tritici* pathogen proteins. This failure to polymerize means resistance to disease (Vanderplank 1982). On the other hand, the production of secondary products specified by wheat and Dv genes that interfere with the growth of both strains of pathogen on the amphiploids could also be postulated.

Discussion

The results of the isozyme and seed storage protein electrophoretic analyses in wheat × *Dasypyrum* amphiploids indicated that the combination of the V genome of the diploid species *D. villosum* with the A and B genomes of tetraploid (durum) wheat, and with the A, B and D genomes of hexaploid wheat, to form hexaploid (M × v) and octoploid (CS × v) amphiploids, respectively, produced phenotypes having both parental proteins and novel proteins, specified by all the alleles at the homologous loci inherited from the parental species.

The isozymes and seed storage proteins detected in each amphiploid were, in fact, accounted for by simple additivity (for LPX monomeric enzyme system) or combination (for dimeric isozyme systems) of all the protomers coded by the parental alleles. The novel proteins were heterodimeric forms of the oligomeric GOT, ADH, GPI and SOD isozymes not produced in either one of the parents.

Since the alleles at the replicated loci controlling isozyme and storage protein synthesis in the amphiploids were all functional, it seems that these *D. villosum* alleles in wheat × *Dasypyrum* amphiploids were not suppressed, as it has been observed for the rRNA genes in *T. dicoccum-D. villosum* hexaploid amphiploid by Friebe et al. (1987).

Although for some morphological characters there is evidence of a partial dominance of the wheat alleles on homologous Dv alleles, the characters for which the wheat × *Dasypyrum* amphiploids carry all the parental alleles in a functional state may be high and they may involve characters of practical value, such as disease resistance and seed storage protein content. The powdery mildew resistance and polypeptide composition of seed storage protein in M × v and CS × v, and the high seed nitrogen content of M × v can be explained only by assuming that the alleles from the parental species at paralogous loci are functional.

Bennett (1984) reviewed the documented use in wheat of powdery mildew resistance known to be controlled by single genes. Nine genes causing powdery mildew resistance were known in Triticinae: five on A genome chromosomes (*Pm1, Pm3, Pm4, Pm7* and *Pm9*), three on B-genome chromosomes (*Pm6, Pm8* and *Mld*), and one on a D-genome chromosome (*Pm2*). Two genes *Pm7* and *Pm8*, were derived from *Secale cereale*. In tetraploid and hexaploid wheats, therefore, the powdery mildew resistance was due to the action of many Mendelian genes. It was possible that *D. villosum*, which showed immunity to our isolate of *E. graminis* f.sp. *tritici*, carried homologous (=orthologous) alleles to those present on the A and B wheat genome chromosomes causing resistance to powdery mildew forma specialis. Vice versa, the homologous alleles on A and B genomes were effective in controlling the pathogenicity of *E. graminis* f.sp. *haynaldiae*. Because M × v and CS × v carry all the alleles on A, B, D and V genomes, they are expected to be resistant to both forma specialis of *E. graminis* as was found in our controlled experiments. If the immunity to powdery mildew of M × v and CS × v was due to the occurrence of many Mendelian genes, then it seems improbable that a disomic addition and/or recombinant lines with the same immunity as in the amphiploids can be derived. A preliminary screening of Sears' disomic addition lines revealed, in fact, that none of them has the same high level of powdery mildew resistance as CS × v, although the Sears' CS + 1V addition line showed a lower proportion of pustules than the other disomic addition lines (C. De Pace and P. Magro, unpublished data). However, the Dv used by Sears is not the same one used to produce the CS × v amphiploid used in this study. So, it is perhaps not too surprising that none of the CS + Dv addition lines were immune to powdery mildew.

Although the high seed protein content of M × v may be due to its lower HI compared with M, nevertheless there may be genes on Dv chromosomes that promote increased kernel nitrogen content in wheat × *Dasypyrum* amphiploids. In fact, other alien species (*Agropyron* spp.) have been used by a number of workers to transfer genes that increase seed protein content into wheat (see Fedak 1985 for a review).

The high N content of the M × v plants at the booting stage and the immunity to powdery mildew suggests that this amphiploid can be of some use for producing green matter for animal feeding.

Despite the high N content in the M × v kernels, these kernels are not easily harvested because of the brittle rachis and rigid glumes. Recent mutagenic results, however, provided the possibility to obtain M × v with tough rachis and good fertility.

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