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Studies of isozymes in oat species

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Summary. Starch and polyacrylamide gel electrophoresis of seven isozyme systems was investigated as a means of identifying wild and cultivated species of *Avena* with different ploidy levels. By examining the characteristic isoenzymatic patterns, it was shown that there was considerable variability within the different species. However, it was nevertheless possible to unequivocally identify the species of wild oats and to distinguish between the different species belonging to the same genomic set, thus providing a definitive reference technique for the identification of *Avena* species in seed-testing laboratories. The relationships between *Avena* species were inferred from the electrophoresis data. The divergence of the *A. maroccana* – *A. murphyi* complex and its contribution to the AACC genomes are emphasized.

Key words: *Avena* – Isozyme patterns – Phylogenetic relationships

Introduction

Hexaploid oats probably originated in the Mediterranean and Middle Eastern countries. However, the more extensive use of this type of oat is confined to more nothern habitats. The concentration of protein in the groat is considerably higher than in other cereals (Peterson and Brinegar 1986). In recent years, considerable interest has been shown in developing new strains of oats with greater protein contents. The genus *Avena* includes a large number of cultivated and wild species of different ploidy levels. The wild species that are progenitors of the cultivated hexaploids are a potential source of new genetic variation which could be used in breeding. Optimum utilization and conservation of this natural material depends on our knowledge of its genetic variation and of the relationships between the wild species and their cultivated relatives.

Rajhathy and Thomas (1974) and Baum (1977) have made extensive studies on the taxonomy of the genus *Avena* mainly based on morphological and cytological analysis and on interspecific crossability. Two different genomes, AA and CC, can be distinguished among the diploid species. Genome A has been further subdivided into five sets (AcAc, A1A1, AdAd, AsAs and ApAp) on the basis of additional chromosome alterations, while genome C has diverged in two directions (CpCp and CvCv). A and C genomes represent groups reproductively isolated. At the tetraploid level, two large groups have been described, AABB and AACC, while all hexaploids are characterized by the genome formula AACCDD. Karyotypic and morphological studies have revealed significant differences, making it possible to identify the different genomes (Rajhathy and Thomas 1974; Baum and Rajhathy 1976; Fominaya et al. 1988a, b). At the cytological level it is, however, difficult to distinguish between different species with the same genomic constitution. This was also a determining factor in the present search for discriminating criteria between the species.

Both isozymes and proteins have been widely used in studies on the variability existing in populations of different species (Symeonidis and Tsekos 1984; Sanz et al. 1987). It has been suggested that the electrophoretic profiles might allow strains to be identified, especially when certain improvements are to be patented (Tanksley and Jones 1981; Moore and Collins 1983; Lookhart 1985; Cooke and Draper 1986). In species where addition lines are available, studies to locate genes in specific chromosomes have been carried out, such as in rye (Salinas and

Benito 1985), wild barley (Fernández and Jouve 1987) and *Datura* (Carlson 1972).

It is obvious that electrophoretic sorting is more or less useful. Understanding the patterns of isoenzymatic variation in a taxonomic group adds to phylogenetic information as well as to species classification. Biochemical distinctions offer another dimension to the classical morphological criteria, thus deepening our knowledge.

In the work described herein, a comparative study has been made of isoenzymatic systems in 12 species of oats having different ploidy levels. A discussion of their phylogenetic relationships based on the electrophoretic results is included.

Material and methods

This study includes six diploid, five tetraploid and one hexaploid species of the genus *Avena*. Nomenclature and genome designation are based on the work of Rajhathy and Thomas (1974), (Table 1). All accessions were kindly provided by Dr. Rolland Loiselle of the Ottawa Research Station, Canada, and developed by self-pollination.

For biochemical investigations, 15 plants from each species were analyzed following the suggestions of Nielsen (1984) for isozyme polymorphism studies in self-pollinating plants.

Individual samples of plant leaf tissue were crushed and immersed in 15 µl of distilled water. The crude extract obtained was soaked up using a paper wick (Whatman 3 MM) and placed in the gel. Horizontal starch (Connaught, 10% w/v) gel electrophoresis was used for analysis of acid phosphatases (ACPH, E.C. 3.1.3.2.) cathodal peroxidases (CPX, E.C. 1.11.1.7), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), phosphoglucose mutase (PGM, E.C. 2.7.5.1), 6-phosphogluconate dehydrogenase (6-PGD, E.C. 1.1.1.44) and malate dehydrogenase (MDH, E.C. 1.1.1.37). The aspartate aminotransferase isozymes (AAT, E.C. 2.6.1.1.) were separated using horizontal polyacrylamide gels (8% w/v).

For the analysis of the ACPH, CPX, 6-PGD and MDH isozymes, gels were run at 175 v for 5 h (except MDH, which was run at 3 h) with 0.005 *M* histidine-HCl acid pH 7.0 gel buffer and 0.043 *M* tris-citric acid pH 7.0 electrode buffer. The electrophoresis conditions for the rest of the enzymatic systems were 275 v for 3 h with 0.015 tris-citric acid, at pH gel buffer 7.75 and with a 0.01 *M* NaOH boric acid pH 8.6 electrode buffer. The methods of enzyme staining used have been reported by Shaw and Koen (1968), Brewer and Sing (1970) and Rao and Rao (1980).

Phylogenetic relationships were established by comparing the isoenzymatic patterns of all the species. The presence or absence of a certain isoenzymatic band was considered as a differentiating feature. Similarities between species have been estimated by means of the "band counting" method. (S m = number of band of common mobility/maximum number of bands observed; Ferguson 1980). Beginning with the similarity matrix, we employed the cluster analysis of variables using BMDP statistical software (Hartigan 1983), which consists of an amalgamating process based on the minimum distance method. The program was run on a Data General Eclipse MC 4,000 computer, from which the phylogenetic tree was obtained. The suggestions made by Ferguson (1980), who considers it necessary to use a minimum of ten loci in interspecific comparisons, have been taken into account.

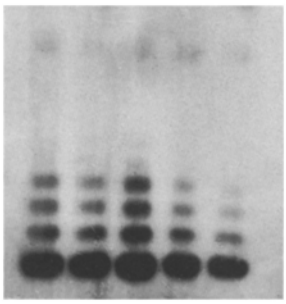

Fig. 1. Photograph of electrophoretic patterns of PGI isozyme of *A. damascena*

Table 1. Examined species of *Avena* and some proposed genome relationships

Species	No. of accession	Chromosome no.	Genome formula
A. canariensis	CAV3873	14	AcAc
A. longiglumis	CAV3922	14	AlAl
A. damascena	CAV0258	14	AdAd
A. strigosa	CAV2836	14	AsAs
A. maroccana	CAV4388	28	AACC
A. murphyi	CAV2832	28	AACC
A. sativa	Var Pandora (La Guardia)	42	AACCDD
A. abyssinica	CAV3280	28	AABB
A. vaviloviana	CAV3278	28	AABB
A. barbata	CAV0140	28	AABB
A. pilosa	CAV0063	14	CpCp
A. clauda	CAV0001	14	CpCp

Results and discussion

In order to determine the degree of homogeneity of the base population, a study on the different systems was carried out, using 5 plants selected at random by accession (Fig. 1). In all cases, the plants analyzed for each accession showed the same electrophoretic profile for each system and retained the differences observed. Photographs of gels showing the isozyme phenotypes of the different species and schematic representation of their mobility variants are shown in Fig. 2 and 3. The band set of each species is summarized in Table 2.

In accordance with Singh et al. (1973), who suggested the use of unique characteristic isoenzymatic patterns for the identification of varieties, we have considered that this criterion might also be valid at the species level and especially useful in relation to those species whose morphological and cytological differences are difficult to appreciate.

In all the isoenzymatic systems analyzed, two zones of activity could be differentiated, depending on the greater or lesser degree of mobility. An exception was

Table 2. Electrophoretical band set of each *Avena* species investigated

Species	CPX	ACPH	PGM	PGI	MDH	6-PGD	AAT
A. canariensis	2, 8, 9, 10, 11	2, 16, 18, 21, 25, 28, 33	1	4, 8, 10, 11, 12	3, 5, 10, 12, 15	3, 6	1, 4, 8, 9
A. longiglumis	2, 7, 9, 10, 11	1, 2, 18, 22, 27, 33, 35	1	3, 11, 12, 13, 14	2, 6, 10, 15, 18	3, 8	1, 6, 7
A. damascena	1, 3, 5, 7, 9, 10, 11	2, 12, 16, 18, 23, 34	1	1, 5, 6, 7, 9	2, 6, 10, 15, 18	3, 6	1, 3, 6, 7
A. strigosa	1, 3, 7, 9, 10, 11	2, 13, 18, 23, 34	1	1, 8, 10, 11, 12	4, 10, 15, 18	3, 8	1, 2, 7
A. maroccana	1, 3, 5, 7, 9, 10, 11	2, 20, 24, 26, 30, 33	1, 2	1, 2, 8, 10, 11, 12, 13, 14	1, 4, 7, 10, 14, 16	1, 6	1, 3, 5, 6, 7
A. murphyi	3, 5, 7, 9, 10, 11	2, 3, 4, 5, 12, 16, 19, 26, 30	1	1, 2, 8, 10, 11, 12	1, 4, 7, 9, 11, 15	2, 6, 7, 9	1, 3, 5, 6, 7
A. sativa	1, 3, 4, 5, 7, 9, 10, 11	2, 16, 19, 31	1	1, 2, 8, 10, 11, 12, 13, 14	1, 4, 7, 8, 9, 12, 15, 18	2, 6, 7	1, 3, 5, 6, 7
A. abyssinica	1, 3, 5, 7, 9, 10, 11	1, 2, 8, 10, 15, 17, 24, 29	1	1, 8, 10, 11, 12	1, 8, 9, 12, 15, 18	3, 4, 5, 6, 7	1, 3, 6, 7
A. vavilioviana	1, 3, 5, 7, 9, 10, 11	2, 8, 10, 15, 17, 24, 31	1	1, 8, 10, 11, 12	1, 8, 9, 12, 15, 17	3, 4, 5, 6, 7	1, 3, 6, 7
A. barbata	1, 3, 5, 7, 9, 10, 11	2, 8, 10, 15, 17, 20,31	1	1, 8, 10, 11, 12	1, 8, 9, 12, 15, 17	3, 4, 5, 6, 7	1, 3, 6, 7
A. pilosa	1, 3, 4, 5, 6, 9, 10, 11	1, 2, 6, 7, 9, 11, 14, 28, 30, 32, 35	1	1, 8, 10, 11, 12	1, 3, 9, 11, 13	3, 7	1, 5
A. clauda	1, 3, 4, 5, 6, 9, 10, 11	1, 2, 6, 7, 9, 11, 14, 28, 30, 32, 35	1	1, 8, 10, 11, 12	1, 3, 9, 11, 13	3, 7	1, 5

found for phosphoglucose mutase, where only one region of isoenzymatic activity could be observed.

The cathodic peroxidases proved to be the enzymatic system showing the smallest interspecific differences. Eleven bands could be observed, which were able to sort out eight different phenotypes. The zymogram pattern of CPX- 1, 3, 5, 7, 9, 10 and 11 was present in all the CC diploid species. It is of interest to note that band 8 was only present in *A. canariensis,* suggesting the possibility of using this isoenzymatic system as an identifying feature.

Zymograms ACPH-1 and -2 showed the greatest degree of interspecific variability. The presence of three bands exclusively in zone 1 in *A. murphyi* is particularly noteworthy. In contrast, the electrophoretic PGM pattern was homogeneous with the appearance of a band in all of the analyzed species except for *A. maroccana,* in which two distinct bands could be resolved. The two isoenzymatic systems allow *A. murphyi* to be distinguished from *A. maroccana.* Both are considered to have the same genomic constitution and certain morphological and karyotypical characteristics in common, and have been grouped as the *A. maroccana – A. murphyi* complex (Rajhathy and Thomas 1974).

PGI allowed seven phenotypes to be sorted out, with PGI-1, 8, 11 and 12 found in *A. strigosa,* the three AABB species and the two CpCp species. The pattern PGI- 1, 2, 8, 10, 11, 12, 13 and 14 was present in *A. maroccana* and *A. sativa.* PGI- 1, 5, 6, 7 and 9 was unique for *A. damascena* and differentiates this species from the others with the same genome (AA). Karyotypical studies using conventional dyes (Rajhathy and Thomas 1974) as well as banding techniques (Fominaya et al. 1988a) have not been able to clearly differentiate between the AA species in this way.

The two zones of MDH activity showed similar electrophoretic profiles for species belonging to the same genomic group except for *A. canariensis* and *A. maroccana.* The isoenzymatic system 6-PGD exhibited two zones of electrophoretic mobility. Region 1 showed a band in the species examined except for the tetraploid group AABB which showed a pattern with three bands in its zymogram. However, in region 2, the greatest number of bands was detected in *A. murphyi,* which showed two bands in common with the AABB species and *A. sativa,* and a single band in common with the diploid species. AAT showed six different isoenzymatic phentoypes without intragenomic variation, with the exception of *A. canariensis* whose zymogram was very different from that of the other *Avena* species.

The systematic analysis of related species with different levels of ploidy occupies an important place in evolutionary studies with the advantage of using well-established biochemical characters (Lewontin 1974; Gottlieb 1977).

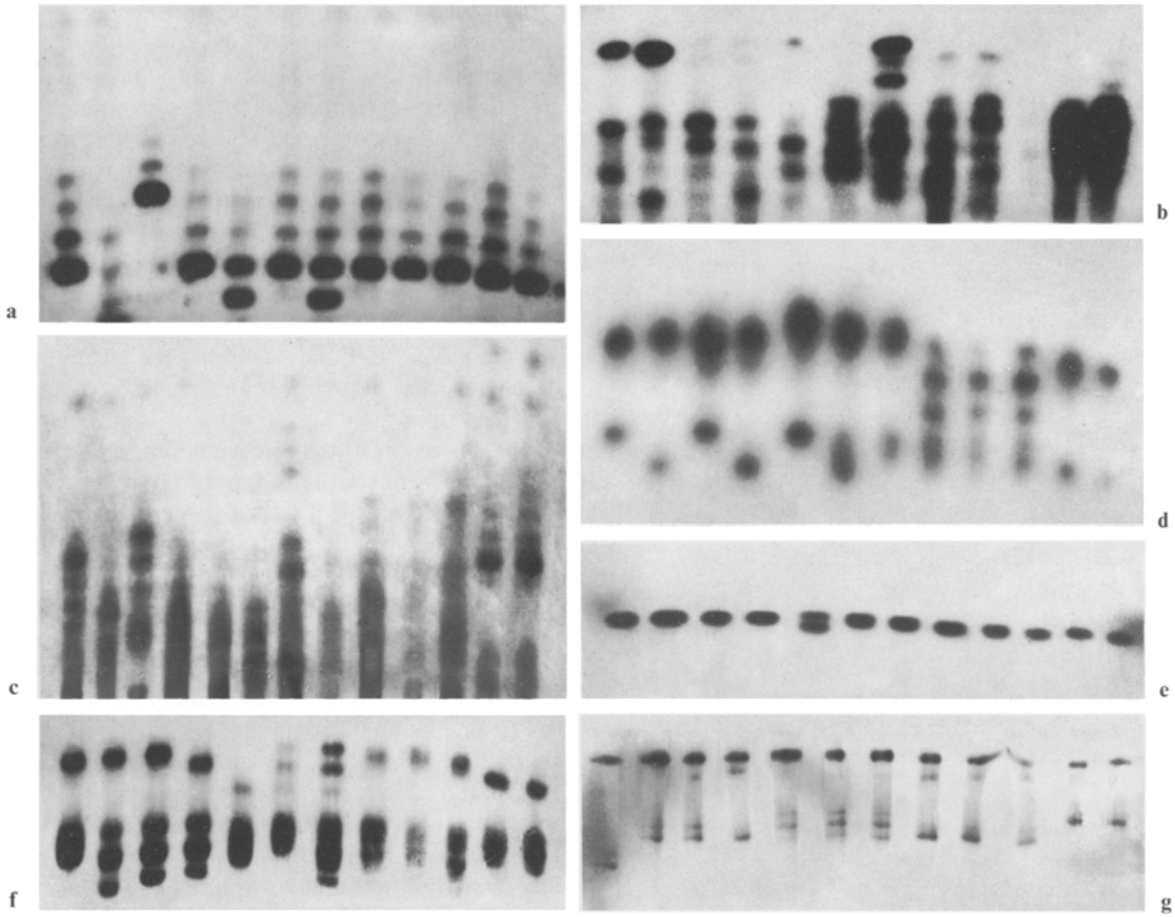

Fig. 2a–g. Photographs of gel showing PGI **a**, CPx **b**, ACPH **c**, 6-PGD **d**, PGM **e**, MDH **f**, AAT **g** zymograms. From left to right: *A. canariensis, A. longiglumis, A. damascena, A. strigosa, A. maroccana, A. murphyi, A. sativa, A. abyssinica, A. vaviloviana, A. barbata, A. pilosa* and *A. clauda*

Table 3 shows the similarity matrix inferred from comparison of electrophoretic patterns between species pairs, as previously described. The values of similarity varied from 0.32 (*A. pilosa, A. clauda, A. longiglumis* and *A. damascena*) to 1 (*A. pilosa* and *A. clauda*), which suggests greater divergence between species having different genomes and greater similarity between species having the same genomes. Such a conclusion agrees with data on the geographical, morphological and cytogenetical analysis carried out by Ladizinsky and Zohary (1971), who demonstrated that diploid species possess characteristics that confer upon them an adaptive divergence. Checking the degree of similarity between species with similar or different levels of ploidy (Tables 3 and 4) reveals that the individual isoenzymatic systems contributed only slightly to the final value of the coefficient of similarity (Sm). Certain trends could, however, be observed: (1) the number of bands increases with the level of ploidy ($2\times=30.2$; $4\times=35.8$; $6\times=37$); (2) diploid species, with the exception of genome C carriers, are more divergent amongst themselves than are tetraploid species.

Such conclusions basically agree with those obtained from previous studies based on the morphology, compatibility (Rajhathy and Thomas 1974) and isoenzymatic variation (Jain and Singh 1979). The present taxochemical observations support the opinion of a clear differentiation and speciation already at the diploid level. The results are also in agreement with those of Craig et al. (1972) who analyzed the esterases of ten species of *Avena* and found a minor degree of similarity between diploid species having the A genome.

Meiotic studies of tetraploid species having the genomic formula AABB, along with morphological evidence and geographical distribution, have suggested a possible autotetraploid origin from diploid species carrying the genome formula AsAs (Holden 1966; Ladizinsky and Zohary 1968; Sadasivaiah and Rajhathy 1968). However, the present results hardly support this idea (Table 5). Pattern relationships between genomes Ad, As and C on the one hand and the AABB species on the other place the autotetraploid origin of these species in doubt.

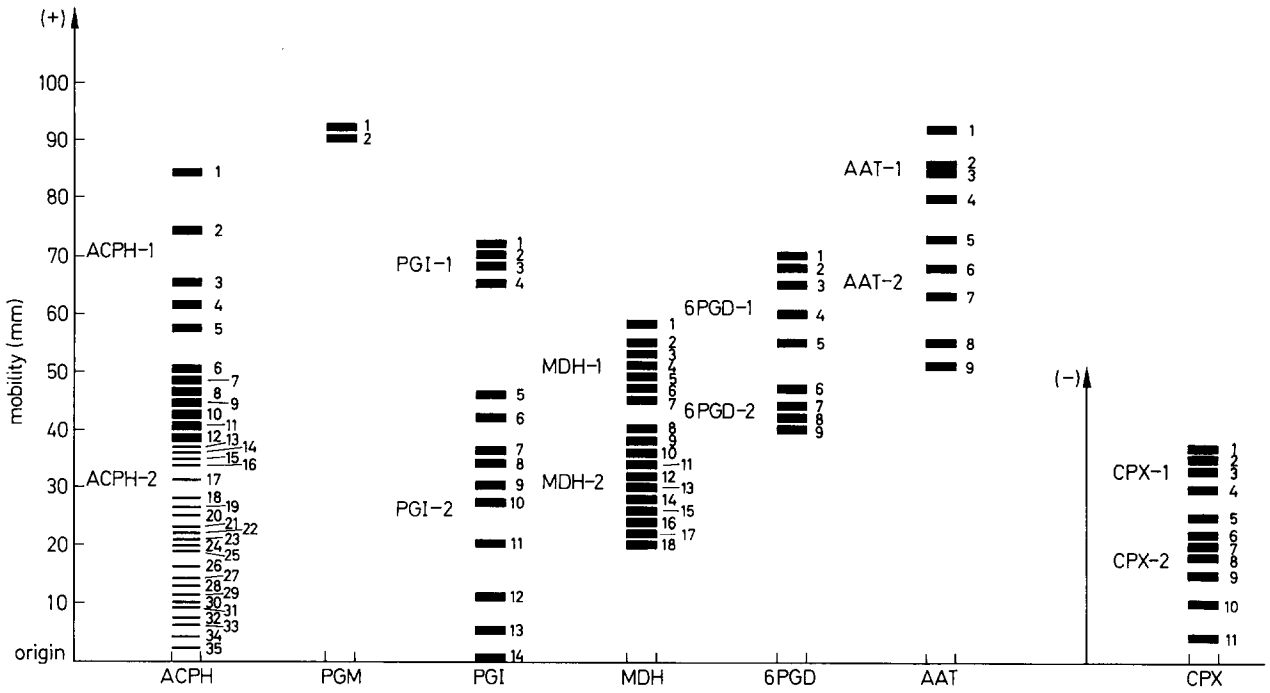

Fig. 3. Electrophoretical mobility variants observed in the isozyme analysis

Table 3. Similarity matrix calculated by the Ferguson coefficient for the *Avena* species investigated

	A. canariensis	*A. longiglumis*	*A. damascena*	*A. strigosa*	*A. maroccana*	*A. murphyi*	*A. sativa*	*A. abyssinica*	*A. vaviloviana*	*A. barbata*	*A. pilosa*	*A. clauda*
A. canariensis	1.00											
A. longiglumis	0.48	1.00										
A. damascena	0.40	0.53	1.00									
A. strigosa	0.48	0.57	0.60	1.00								
A. maroccana	0.36	0.44	0.44	0.47	1.00							
A. murphyi	0.35	0.35	0.46	0.43	0.68	1.00						
A. sativa	0.38	0.43	0.49	0.49	0.70	0.78	1.00					
A. abyssinica	0.39	0.36	0.50	0.50	0.58	0.59	0.70	1.00				
A. vaviloviana	0.40	0.37	0.49	0.49	0.58	0.59	0.70	0.92	1.00			
A. barbata	0.40	0.37	0.49	0.49	0.58	0.59	0.70	0.89	0.97	1.00		
A. pilosa	0.35	0.32	0.32	0.41	0.47	0.51	0.51	0.53	0.51	0.51	1.00	
A. clauda	0.35	0.32	0.32	0.41	0.47	0.51	0.51	0.53	0.51	0.51	1.00	1.00

Table 4. Similarity matrix between different genomes based on allozyme variation

	AA	AACC	AACCDD	AABB	CC
AA	0.33				
AACC	0.49	0.68			
AACCDD	0.47	0.67	1.00		
AABB	0.42	0.52	0.68	0.92	
CC	0.33	0.42	0.51	0.50	1.00

Table 5. Relationships measured by similarity matrix between different 2x genomes and the two higher ploidy groups

	AACC	AACCDD	AABB
AcAc	0.31	0.38	0.37
AlAl	0.35	0.43	0.39
AdAd	0.40	0.49	0.47
AsAs	0.38	0.49	0.47
CpCp	0.42	0.51	0.50
CpCp	0.42	0.51	0.50

The origin of the A genome in the hexaploid species has been a centre of controversy. Work carried out by Baum et al. (1973) and Craig et al. (1974), based on morphological and isoenzymatic observations, respectively, pointed to *A. canariensis* as the likely donor. It should, however, be observed from the present taxochemical approach that the five different diploid genomes show different degrees of homology with the tetraploid and hexaploid groups (Table 5). A greater level of similarity can be observed between *A. canariensis* and the 6× oats than between this 2× species and the 4× AACC and AABB groups. In addition, a close similarity can be observed between *A. damascena* and *A. strigosa* on the one hand and the AACC species on the other. Such observations suggest that the last-mentioned 2× species could be the possible donors of the A genome in tetraploid oats with the constitution AACC, and that *A. canariensis* could be donor to the third genome of the hexaploid species. Such ideas coincide well with the low chromosome pairing found in interspecific hybrids between genome A and the tetraploid AACC species when the diploid parent is *A. canariensis* (Leggett 1980) and also coincide with the large differences between the mitochondrial genomes found here (Murai and Tsunewaki 1986).

Figure 4 shows a phylogenetic tree based on the present enzymatic studies. A pronounced branching suggests a complex phylogenetic differentation within *Avena*. The results agree with a classification based on cytological and morphological criteria (Rajhathy and Thomas 1974; Baum 1977). Thus, the diploid species *A. damascena* and *A. strigosa* of genome formula AA show a close relationship but are clearly divergent from *A. canariensis*. Similarly, *A. pilosa* and *A. clauda* show a high degree of homology and could be considered as an offshoot group separate from the other diploids. With respect to the AABB tetraploids, *A. barbata* and *A. vaviloviana* show a greater degree of mutual homology than with *A. abyssinica*.

Rajhathy (1971a, b) has proposed the designation AC for genomes of *A. maroccana* on the basis of karyotypic analysis, electrophoresis (Murray et al. 1970; Rajhathy et al. 1971) and studies on ultrastructure of chloroplast (Steer et al. 1970), and has suggested that both genomes were involved in the hexaploid constitution. After the discovery of a new 4× species, *A. murphyi* (Ladizinsky 1971 b), and its established close relationship with *A. maroccana* it has been proposed that the *A. maroccana* – *A. murphyi* complex should rather be looked upon as the donor of the AC genomes in the hexaploid species (Ladizinsky and Zohary 1971; Craig et al. 1972; Rajhathy and Thomas 1974; Leggett 1980). The similarities and differences found in the zymograms of *A. maroccana* and *A. murphyi* agree with this interpretation and are also supported by other biochemical analyses (Craig et al. 1972; Kim and Mosse 1979; Jain and Singh 1979).

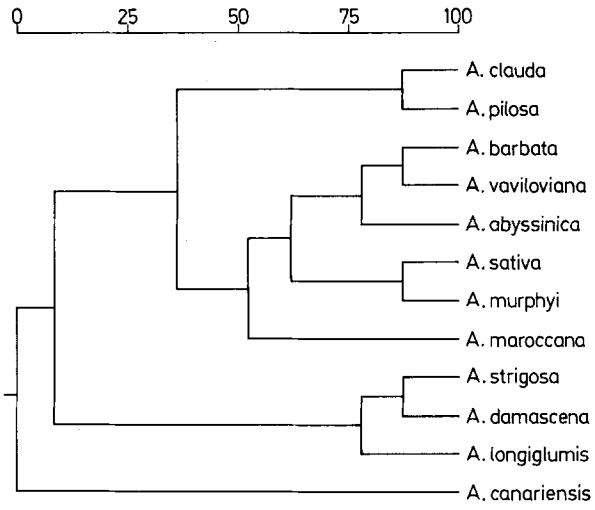

Fig. 4. Phylogenetic tree inferred from biochemical analysis

A certain divergence within the *A. maroccana* – *A. murphyi* complex can also be biochemically supported with some preference for *A. murphyi* as the AACC donor to hexaploid oat.

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