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D Genome Doners for *Aegilops cylindrica* (CCDD) and *Triticum aestivum* (AABBDD) Deduced from Esterase Isozyme Analysis*

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Summary. Putative D genome donors for *Aegilops cylindrica* (2n = 28, CCDD) and *Triticum aestivum* (2n = 42, AABBDD) were studied with the isoelectric focusing patterns of esterase isozymes. 103 strains of *Ae. cylindrica* were uniform in their isozyme pattern. 30 strains of the putative parent, *Ae. caudata*, showed no zymogram variation, whereas the other parent, *Ae. squarrosa*, comprised 3 phenotypes. Natural *Ae. cylindrica* had an isozyme pattern which corresponded to a mixture of esterases from *Ae. caudata* and type 3 *Ae. squarrosa*. Therefore, it is concluded that the D genome donor of *Ae. cylindrica* is derived from type 3 *Ae. squarrosa*. These results suggest that *Ae. cylindrica* originated with a single amphiploidy event, and the C and D genomes have remained remarkably constant regarding esterase isozyme composition.

On the other hand, *T. aestivum* comprised three zymogram phenotypes. These phenotypes contain bands which can be ascribed to the D genome of type 2 *Ae. squarrosa*. These results suggest that the D genome of *Ae. cylindrica* differs from that of *T. aestivum*. Evolution of the AB and D genomes of *T. aestivum* is indicated by the zymogram polymorphism. The origin of *Ae. cylindrica* is possibly more recent than that of *T. aestivum*.

Key words: D genome donor – *Ae. cylindrica* – *T. aestivum* – Esterase – Polymorphism

Introduction

It is generally accepted that the D genome donor for common wheat (*Triticum aestivum* L., 2n = 42, genome type AABBDD) is *Aegilops squarrosa* L. (2n = 14, DD) (Fig. 1). The D genome is also found in *Ae. cylindrica* Host (2n = 28, CCDD) (Percival 1921; Kagawa 1929; Kihara 1931; Sears 1944). *Ae. cylindrica* is a tetraploid and this species ranges in distribution from the west side of the Black Sea (Bulgaria, Rumania, Hungary and the eastern part of Yugoslavia) to Central Asia (Eig 1929) (Fig. 2). Earlier cytogenetic studies and morphological analysis of *Ae. cylindrica* suggested that the parents of *Ae. cylindrica* are *Ae. caudata* L. (2n = 14, CC) and *Ae. squarrosa* (Kihara 1954; Sears 1941) (Fig. 3). The putative parents, *Ae. caudata* and *Ae. squarrosa*, overlap the area of *Ae. cylindrica*, but have no area in common. *Ae. cylindrica* is frequently found as a weed in wheat fields.

Morphological characteristics of the amphidiploid between *Ae. caudata* and *Ae. squarrosa*, produced by Sears (1941), resembled that of *Ae. cylindrica*. Moreover,

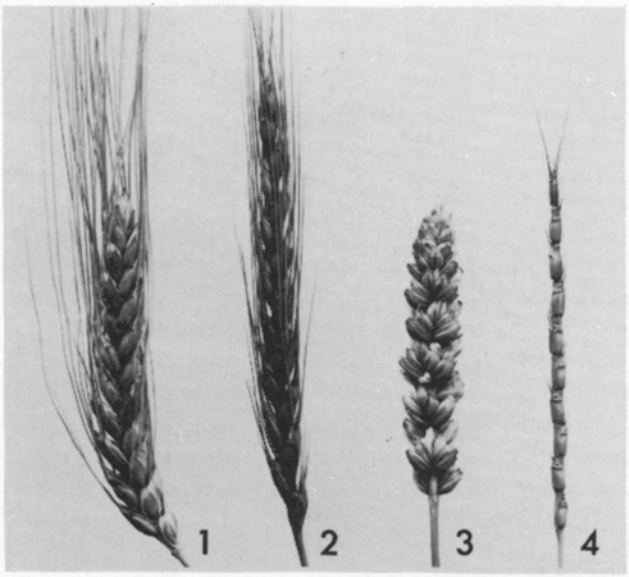

Fig. 1. Spikes of natural and synthesized hexaploid wheat with its parents. (*1*) *T. durum* var. 'reichenbachii'; (*2*) ABD-16a; (*3*) *T. aestivum* cv, 'Chinese Spring'; (*4*) *Ae. squarrosa* var. 'meyeri' KUSE 2144

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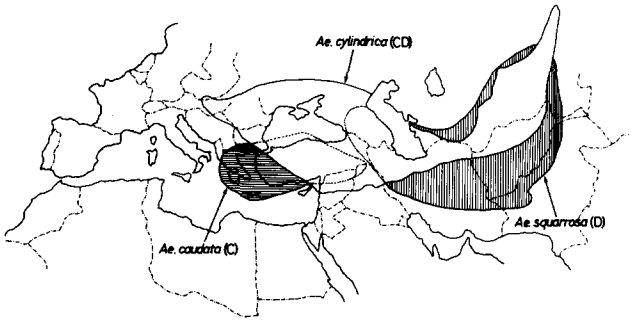

Fig. 2. Geographical distribution of *Ae. cylindrica* and its putative parental species, *Ae. caudata* and *Ae. squarrosa* (Eig 1929; Zohary 1965)

Fig. 3. Spikes of *Ae. cylindrica* and its putative parents. (*1*) *Ae. caudata* var. 'polyathera'; (*2*) *Ae. cylindrica* var. 'pauciaristata'; (*3*) *Ae. squarrosa* var. 'typica'

McFadden and Sears (1946) confirmed that the amphidiploid between *Ae. caudata* and *Ae. squarrosa* corresponded to *Ae. cylindrica* cytologically, in agreement with the proposal that the D genome of *Ae. cylindrica* derived from *Ae. squarrosa*. Johnson (1967) supported this conclusion with results from electrophoretic analysis of protein polymorphisms. After the discovery of the occurrence of these zymogram phenotypes among *Ae. squarrosa* lines, I reported that *T. aestivum* had a D genome found in type 2 *Ae. squarrosa* (Nakai 1979). In continuation of these previous investigations, the esterase isozyme patterns in *Ae. cylindrica* strains from different localities and those in the putative parental strains, *Ae. caudata* and *Ae. squarrosa*, were analyzed. Esterase isozymes of the three artificially synthesized strains of hexaploid wheats and their parents have also been re-examined in comparison with *Ae. cylindrica*.

Studies on esterase isozyme patterns in the present work confirm that the D genome of *Ae. cylindrica* derived from *Ae. squarrosa*. However, the D genome donor of *Ae. cylindrica* differed from that of *T. aestivum*. Based on the results obtained, the relationship of the D genome donors in *Ae. cylindrica* and in *T. aestivum* is discussed.

Materials and Methods

Materials

In this experiment, strains of *Aegilops cylindrica* Host (2n = 28, genome type CCDD) were used together with its putative parental species, *Ae. caudata* L. (2n = 14, CC) and *Ae. squarrosa* L. (2n = 14, DD). Of a total of 103 strains of *Ae. cylindrica* examined, 7 strains were collected in Afghanistan by Dr. S. Sakamoto, Plant Germ-plasm Institute, Kyoto University, in 1978; 31 strains in Iran by the Kyoto University Scientific Expedition to the Karakorum and Hindukush (abbreviated as KUSE) in 1955; and 53 strains in Turkey by the Botanical Mission of the University of Kyoto (BMUK) in 1959 and the Kyoto University Scientific Expedition to Eastern Turkey (KUET) in 1976. These materials were all obtained from Prof. Dr. M. Tanaka, Plant Germ-plasm Institute, Kyoto University. The remaining 12 strains from Bulgaria were collected in 1978 by Mr. Y. Mukai, Osaka Kyoiku University. Of the 30 strains of *Ae. caudata* re-examined, 20 strains were collected in Turkey, one strain in Syria, and 9 strains in Greece by BMUK and KUET. These materials have been stocked at the Plant Germplasm Institute, Kyoto University. Of 128 strains of *Ae. squarrosa* also re-examined, 56 strains were from Afghanistan and 45 strains were from Iran. In addition, 21 strains from the Transcaucasus and 6 strains from Turkey were collected by the Botanical Expedition to the Caucasus (BEC) in 1966 and the Northern Highland of Mesopotamia (BEM) in 1970, respectively.

Three strains of synthesized hexaploid wheat and their parents, i.e., tetraploid wheats and *Ae. squarrosa*, were used in this study. ABD-1 was synthesized as a hybrid between *T. dicoccoides* var. 'spontaneonigrum' and *Ae. squarrosa* var. 'typica' No. 2 by Dr. H. Kihara and coworkers in 1944 (Kihara 1947). ABD-16a was synthesized as a hybrid between *T. durum* var. 'reichenbachii' and *Ae. squarrosa* var. 'meyeri' KUSE 2144 by Dr. M. Tanaka in 1959 (Tanaka 1961). RL 5406 was produced as a hybrid between 'Tetra-Canthatch' (2n = 28, AABB), in which the D genome was removed from 'Canthatch' (2n = 42, AABBDD), and *Ae. squarrosa* by Dr. E.R. Kerber, of the Canada Agriculture Research Station, Winnipeg.

Methods

Isozymes were analyzed by isoelectric focusing in gels as described previously (Nakai 1973). About 20 mg (one grain) of seed which had been kept in a germination chamber for 24 hours at 23°C was homogenized in a glass morter in 1 ml of 0.05 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at about 20,000 × g for 15 minutes at 0°C. The supernatant was placed on a polyacrylamide gel containing a carrier ampholite (LKB) with a pH range of 6.0 to 8.0. The anode vessel on the top and the cathode vessel on the bottom were filled with 0.02M hydrochloric acid and 0.02M ethylendiamine, respectively. The electric current

was stabilized at 200 volts and was passed for three hours. Gels were removed from the tubes, stained with 0.2% Fast Blue RR salt and 0.02% α-naphtyle acetate (w/v) in phosphate buffer (pH 7.0, 1/15M) for 20 min. The homology of the esterase bands was judged by a mixture (1:1 by weight) of esterase extracts. Also, cytochrome C_2 (pI = 6.2) from *Rhodospirillum rubrum* and horse myoglobin (pI = 7.1, 7.3) were used as marker proteins for estimation of pH values at different points on the gels.

Results

1 Zymogram Phenotypes of Ae. cylindrica and the Putative Parental Species

(i) *Ae. cylindrica*: All of the 103 strains of *Ae. cylindrica* showed identical zymogram phenotypes which consisted of seven major bands (5′E, 6E, 8E, 8′E, 10E, 11E and 12E) and five minor bands (1E, 3E, 4E, 5E and 6′E) (Fig. 4). The zymogram phenotype of *Ae. cylindrica* was quite uniform.

(ii) *Ae. squarrosa* and *Ae. caudata*: It has previously (Nakai 1979) been reported that *Ae. squarrosa* comprised three zymogram phenotypes. These were designated as type 1, type 2 and type 3, respectively (Fig. 5). Type 1 had three major bands, 3E, 5E and 6E (Fig. 5-1). Type 2 had one extra major band, 8E-B (Fig. 5-2) and type 3 had four major bands, 6′E, 8′E, 10E and 12E, and five minor bands, 1E, 3E, 4E, 5E and 6′E (Fig. 5-3). Bands 10E and 12E (identical to 10E of Nakai 1979) of phenotype 3 are newly designated in the present study. Phenotype 3 differed greatly from the two other types. Phenotype 2 occurred in 20% and phenotype 3 in 79% of the strains studied.

On the other hand, all of the 30 strains of *Ae. caudata*, which is the other putative parent, showed the same zymogram phenotype. No new zymogram phenotype of *Ae. caudata* was found in the present work. The zymogram of *Ae. caudata* consisted of four major bands (3E, 5′E, 8E and 11E) (Fig. 6-1).

(iii) Zymograms of Extract Mixtures: Phenotype 1 and 2 of *Ae. squarrosa* lacked two major bands (10E and 12E) which were present in *Ae. cylindrica*. The zymogram of an extract mixture of type 1 or type 2 of *Ae. squarrosa* and *Ae. caudata* differed greatly from the zymogram phenotype of *Ae. cylindrica*. On the other hand, a zymogram of a one to one mixture by weight of *Ae. caudata* and type 3 *Ae. squarrosa* extracts resembled that from natural *Ae. cylindrica* (Fig. 6-3). For example, four bands, 3E, 5′E, 8E and 11E (Fig. 6-1), of *Ae. caudata* correspond to four bands of *Ae. cylindrica* (indicated by arrows in Fig. 6-2) and eight bands of *Ae. squarrosa* (1E, 4E, 5E, 6E, 6′E, 8′E, 10E and 12E) correspond to the eight bands of *Ae. cylindrica* (Fig. 6-2).

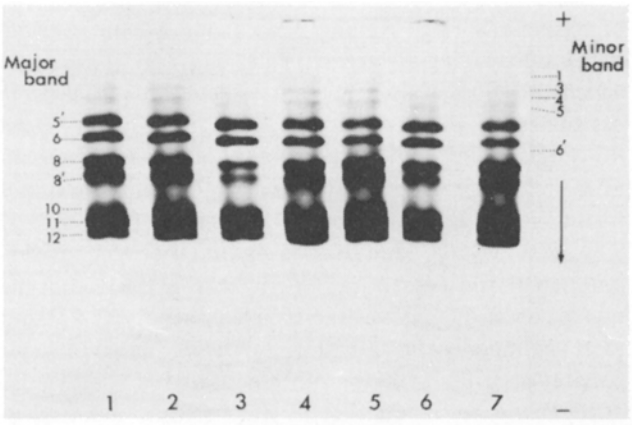

Fig. 4. Isoelectric focusing of esterase isozymes of *Ae. cylindrica*. (*1*) var. 'typica' KUET 4653 (Turkey); (*2*) var. 'pauciaristata' KUET 4655 (Turkey); (*3*) var. 'pauciaristata' KUET 4656 (Turkey); (*4*) var. 'pauciaristata' KUET 4657 (Turkey); (*5*) var. 'typica' KUSE 2401 (Iran); (*6*) var. 'typica' (Afghanistan); (*7*) var. 'typica' (Bulgaria)

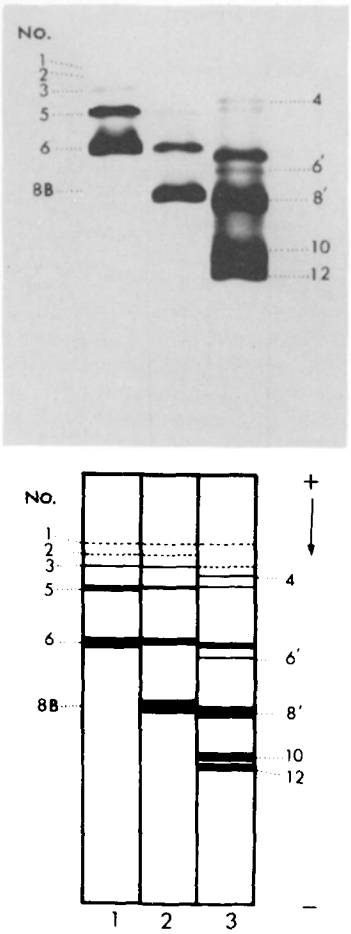

Fig. 5. Photograph of esterase zymograms and a schematic drawing of the bands for *Ae. squarrosa*. (*1*) *Ae. squarrosa* var. 'strangulata' KUSE 2135 (type 1); (*2*) var. 'meyeri' KUSE 2144 (type 2); (*3*) var. 'typica' No. 2 (type 3)

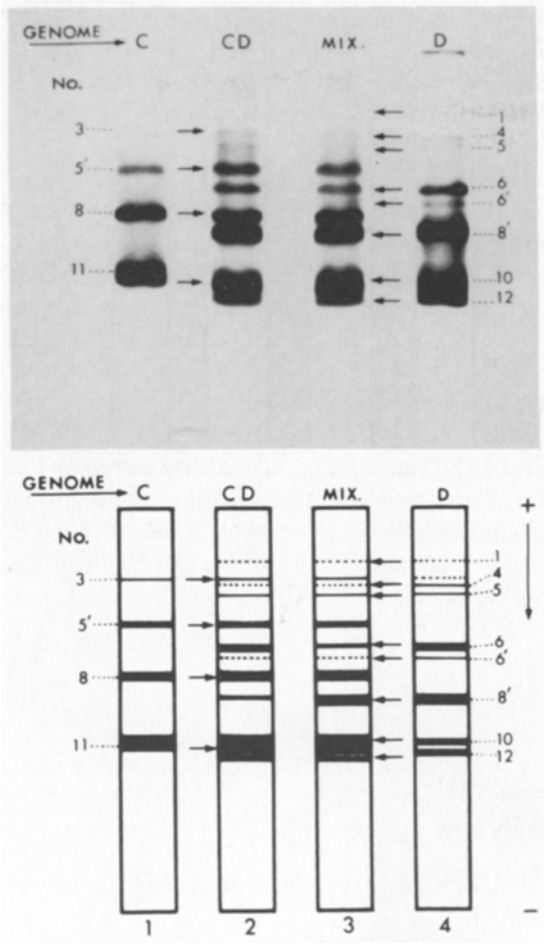

Fig. 6. Isoelectric focusing and diagrams of esterase isozymes of *Ae. cylindrica* and parental species. (*1*) *Ae. caudata* var. 'polyathera' (Ankara); (*2*) *Ae. cylindrica* var. 'typica' G 406 (arrows indicate the homology of each band which was identified by protein mixtures of *Ae. caudata* and *Ae. squarrosa*); (*3*) 1:1 mixture by weight of extracts of *Ae. caudata* and type 3 *Ae. squarrosa*; (*4*) *Ae. squarrosa* var. 'typica' No. 2

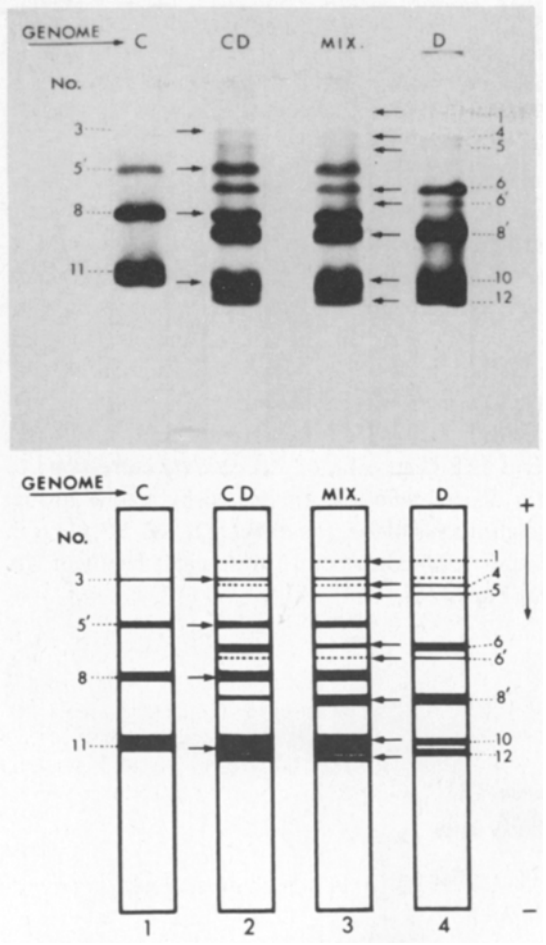

Fig. 7. Isoelectric focusing and diagrams of esterase isozymes of ABD-1 and parental species. (*1*) *T. aestivum* cv. 'Chinese Spring'; (*2*) *T. dicoccoides* var. 'spontaneonigrum'; (*3*) ABD-1; (*4*) 1:1 mixture by weight of *T. dic. spontaneonigrum* and type 3 *Ae. squarrosa*; (*5*) *ae. squarrosa* var. 'typica' No. 2

2 *Zymogram Phenotype of Hexaploid Wheat*

In a previous paper (Nakai 1979), I reported that common wheat has three zymogram phenotypes. Phenotype 1 ('Chinese Spring' type) was similar to phenotype 2 (S-615 type). Phenotype 3 ('Elgin' type) differed from phenotype 1 and 2. Among these, phenotype 1 seemed to be the most primitive type.

In the present study, ABD-1 differed greatly from present-day hexaploid wheat (Fig. 7). The phenotype of *Ae. squarrosa* which was used in the synthesis of ABD-1 was phenotype 3. ABD-16a was exactly the same as the phenotype of 'Chinese Spring' (Fig. 8). For example, nine bands, 1E, 2E, 3E, 5E, 5'E, 7E, 8E-A, 8E-C and 9E of *T. durum* in Fig. 8-1, and three bands of type 2 *Ae. squarrosa* (4E, 6E and 8E-B in Fig. 8-4) corresponded to three bands of 'Chinese Spring'. Among these bands, 8E (no. 8) consisted of two highly active bands and one minor band which had close isoelectric points. These three bands were designated as 8E-A, 8E-B and 8E-C, respectively. Band 8E-A (controlled by the gene on chormosome 3A) and 8E-C (chromosome 3B) were derived from tetraploid wheat, and 8E-B (chromosome 3D) was from *Ae. squarrosa.* RL 5406 was produced as a hybrid between 'Tetra-Canthatch' and type 2 *Ae. squarrosa* (Fig. 9). Phenotype of 'Tetra-Canthatch' was the same as phenotype 4 found most commonly in tetraploid wheats. The zymogram phenotype of RL 5406 was the same as that of 'Canthatch'. The phenotype of 'Canthatch' was basically identical to that of ABD-16a. The strains of *Ae. squarrosa* which were used by Tanaka and Kerber for synthesis of amphiploids showed phenotype 2. Two bands (10E and 12E) of type 3 *Ae. squarrosa* were lacking in the present-day hexaploid wheat.

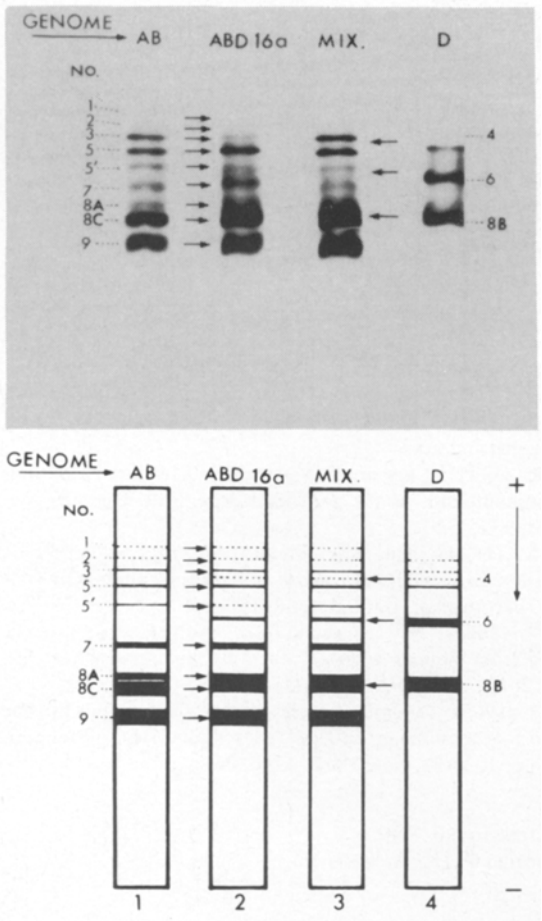

Fig. 8. Isoelectric focusing and diagrams of esterase zymograms produced by *T. durum* var. 'reichenbachii' (*1*); ABD-16a (*2*); 1:1 mixture by weight of *T. durum* and type 2 *Ae. squarrosa* (*3*) and *Ae. squarrosa* var. 'meyeri' KUSE 2144 (*4*)

Fig. 9. Spikes of synthesized hexaploid wheat and its parents. (*1*) Tetra-Canthatch in which the D genome was removed from present-day hexaploid, Canthatch; (*2*) RL 5406; (*3*) *T. aestivum* cv. 'Canthatch'; (*4*) *Ae. squarrosa* var. 'meyeri' RL 5289

Discussion

The D genome donor type to *Ae. cylindrica* in the polymorphic populations of *Ae. squarrosa* was found to be phenotype 3 of *Ae. squarrosa*. The tetraploid *Ae. cylindrica* comprises two varieties, i.e., var. 'typica' (lemma and glume awns occur throughout the spike) and var. 'pauciaristata' (awns develop on top of the spike). Nevertheless, 103 strains of the two varieties examined showed qualitatively the same uniform zymogram phenotypes. These results show that the C and D genomes of *Ae. cylindrica* have remained unaltered with respect to the esterase isozyme pattern since establishment of the amphidiploid. In this study, no zymogram variation was found in *Ae. caudata*, but three zymogram phenotypes of esterase are characteristic for *Ae. squarrosa*. The phenotype of a mixture by weight of extracts from *Ae. caudata* and phenotype 3 of *Ae. squarrosa* corresponded perfectly to extracts of natural *Ae. cylindrica* (Fig. 6). This results indicates that a type 3 *Ae. squarrosa* contributed the D genome to *Ae. cylindrica*.

Johnson (1967) suggested that *Ae. cylindrica* originated by a single amphidiploidy event based on the observed uniformity of electrophoretic patterns of proteins. From the present study, a similar conclusion can be drawn.

On the other hand, I reported previously (Nakai 1978) that seven different phenotypes of esterase zymograms were found in tetraploid wheat, type 4 being distributed most widely. This phenotype was similar to that of common wheat. Moreover, the phenotype of Tetra-Canthatch (2n = 28, AABB) in which the D genome was removed from the present-day hexaploid, 'Canthatch' (2n = 42, AABBDD), had a characteristic type 4 zymogram. Therefore, the donor of the AB genome to common wheat should have this zymogram. Fifteen hexaploid wheats synthesized from type 4 tetraploid wheats and type 2 *Ae. squarrosa*, had all zymograms identical to 'Chinese Spring' (Nakai 1979). These results indicate that type 2 *Ae. squarrosa* donated the D genome to *T. aestivum*. This D genome can be distinguished from the D genome of *Ae. cylindrica*.

Common wheat has three zymogram phenotypes. Phenotype 1 (Chinese Spring type) clearly had band 4E which was controlled by gene(s) on chromosome 3D, together with 5'E band which was controlled by the gene(s) on chromosome 3A or 3B (Nakai 1973). However, the activities of these two bands, i.e., 4E and 5'E, in type

2(S-615 type), were lower than those in 'Chinese Spring'. Moreover, phenotype 3 lacked these two bands and one major band (9E) which was controlled by the gene on chromosome 3A. These results suggests that the AB and D genomes of *T. aestivum* have not remained unaltered since the origin of the allohexaploid.

Further electrophoretic studies on ABD-16a, RL 5406 and its parental species, tetraploid wheat and *Ae. squarrosa* were conducted. The findings obtained indicated that type 2 *Ae. squarrosa* presented the D genome to *T. aestivum* (Fig. 8).

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