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# PHYLOGENETIC RELATIONSHIPS AMONG *TRITICUM* AND *AEGILOPS* SPECIES.

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Fifty accessions of Triticum and Aegilops species were studied immunoelectrophoretically for their protein characters and it was found that, in general, the genetic variability in their characters is not related to the level of ploidy, the diploids were found more variable than polyploids. Almost every group was found extremely variable. *Triticum aestivum erythroleucon* and *T. aestivum pseudo hostianum* (hexaploids) showed greater homology with tetraploids.

Substantial evidence was found that several accessions of *Triticum aegilopoides* have close phylogenetic relationship with some of the tetraploids and hexaploids and it is assumed that this species may be the donor of B genome to the tetraploid and hexaploid wheats.

## INTRODUCTION

The genome analysis of hexaploid wheat (AABBDD) has been ascertained from a variety of angles and a considerable understanding of its donors of A and D genomes has been established. The conclusions of Percival (1921) that the hexaploid wheats are the result of crossing between Emmer and *Aegilops squarrosa* was later confirmed by Kihara (1944) and Mcfadden and Sears (1944). There is a reasonable agreement that A genome has been donated by *T. boeoticum*. A lot of confusion prevails regarding the B genome donor. Riley *et al.* (1958), Sarkar and Stebbins (1956) and Johnson (1970) consider that the B genome donor was *Ae. speltoides*, however, Sears (1956) named *Ae. bicornis*, as its donor.

To investigate this issue immuno-electrophoretically, wide ranging accessions of *Triticum aegilopoides*, *T. monococcum*, *Aegilops bicornis*, *Ae. squarrosa*, *Ae. mutica* and *Ae. speltoides* along with Emmer (*T. dicoccoides*) and hexaploid wheat (*T. aestivum*) were studied and compared for their protein characters. The details of the species used are given in Table 1.

## MATERIALS AND METHODS

Fifty accessions of *Triticum* and *Aegilops* (Table 1) were included. Mature and clean seeds in each case were powdered and their proteins extracted

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by soaking the flour in phosphate buffered saline (pH. 7.0) at 5°C for 16 hours and then centrifuged at 2,000 g. for 25 minutes. The extracts were stored at —15°C and preserved with a drop of sodium azide (2.0%), until the extracts were used for raising the antisera.

The antisera were raised in four female New Zealand white rabbits. For this purpose two primitive wheat species namely *Triticum aestivum graecum* 'Grecia' and *Triticum aestivum ethrythro-spermum* 'Nepal' were used.

TABLE 1. *Triticum and Aegilops species or accessions included in the present investigation.*

| Latin name | Accession no. or common name | Source |
|---|---|---|
| *T. aestivum graecum* | Graecia | Reading |
| *T. aestivum ethrythro-spermum* | Nepal | ,, |
| *T. aestivum ethryoleucon* | 8H37 | ,, |
| *T. aestivum pseudohostianum* | Prelude-7 | ,, |
| *T. aestivum* | 32175–70 | Izmir, Turkey |
| *T. aestivum* | Chinese Spring | P.B.I. |
| *T. dicoccoides* | 8 Accessions, nos. 3, 5, 6, 9, 10, 11, 12, 13 | P.B.I. |
| *T. dicoccoides* | 4C2 | Reading |
| *T dicoccoides spontaneonigrum* | 5C10 | ,, |
| *T. timopheevi* | — | P.B.I. |
| *T. monococcum* | — | ,, |
| *T. aegilopoides* | 12 accessions nos. 3, 4, 11, 12, 15, 15A, 16, 17, 18, 19, 20 and 21 | ,, |
| *Aegilops squarrosa* | 9 Accessions Nos. B,C,D,E,F,G,HI,J. | ,, ,, |
| *Ae. squarrosa* | P. 45 | Reading |
| *Ae. bicornis* | — | P.B.I. |
| *Ae. mutica* | — | ,, |
| *Ae. speltoides* | 8 Accessions, nos.* A,D,E,G,H,I,P,R. | P.B.I. |

**The original capitals have been used as small letters in the text, (a, d, e, g, h, i, p, r.)*

**Immuno-electrophoresis and the preparation of slides.**

One per cent lonoagar no. 2 in borate phosphate buffer was used as the medium. 2 ml aliquots were layered on agar-coated microscopic slide and when solid, the agar was cut into one trough (40x3 mm) having two cups on either side.

The wells were filled with extract and then subjected to electrophoresis at 5.0 V/cm (35 V across each slide) and 48 m A through 8 slides in Shandon Universal Electrophoresis apparatus containing borate phosphate buffer. After an optimum run of 75 minutes, the trough was emptied and filled with ABS 2-3 times and were later incubated for 24 hours for developing.

The developed slides were washed in a stirred alkaline saline solution for 24 hours and were dried, covered with a piece of paper. The slides were washed in tap water after removing the paper. On drying they were stained with 2% Ponceau S and worked with 2 per cent of acetic acid for 5 minutes. The major precipitin arcs were traced on a sheet of paper while examining them on illuminated perspex sheet. For detailed studies the arc patterns were magnified. (Lester, 1965).

Depending upon the immuno-electrophoretic mobility, shape, size and density, a total of 25 arcs have been identified throughout the taxa. Each arc has been assigned a number showing its relative position in the master plan, (Fig. 1).

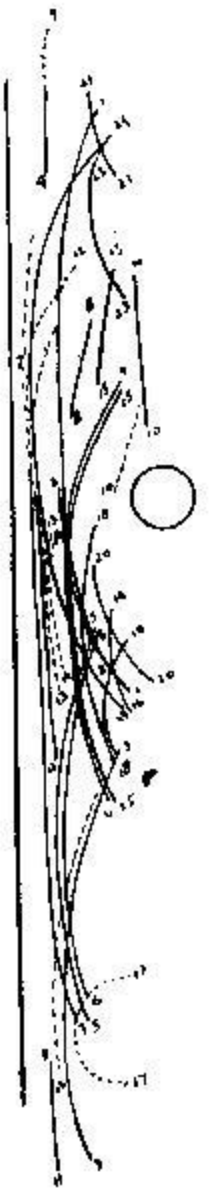

FIG 1. MASTER PLAN OF ARCS OF ALL SPECIES WITH BOTH ANTI SERA

## RESULTS

The mean number of protein characters recorded in the various taxa is given in Table 2. The survey of the number of protein spectra among taxa indicates that each taxon in itself is a composition of quite diverse group of plants and indebted with a vast amount of variation. Accessions of *T. aestivum* show a range of 7.0 to 12.0 arcs in their protein spectra and similar magnitude of variation is noticeable in the remaining enteries.

TABLE 2. *Average number of arcs in different species.*

| Name of species | Antiserum 'Grecia' | Antiserum 'Nepal' |
|---|---|---|
| *T. aestivum* | 9.78 | 10.99 |
| *T. dicoccoides* | 8.11 | 9.08 |
| *T. timopheevi* | 12.0 | 6.0 |
| *T. monococcum* | 11.0 | 5.0 |
| *T. aegilopoides* | 8.2 | 8.9 |
| *Ae. squarrosa* | 10.65 | 10.22 |
| *Ae. speltoides* | 11.4 | 10.6 |
| *Ae. bicornis* | 8.0 | 10.0 |
| *Ae. mutica* | 9.0 | 11.0 |

Table 2 shows that on an average *T. aestivum* and *T. dicoccoides* produced 9.78 and 9.08 arcs respectively with ABS 'Grecia' whereas some diploids produced a higher number than the former. The arcs produced by *Aegilops squarrosa* accession G is the highest. On an average, this species produced more arcs than *T. dicoccoides* and is not strikingly lower than the arcs observed in *T. aestivum*.

Two homologous arcs 1 and 2 are of universal occurrence throughout the taxa listed and show wide separation in *T. dicoccoides* and *Ae. speltoides*. Arc 3 is the fastest protein spectrum and is also of universal presence except about 50% accessions each of *Ae. squarrosa* and *Ae. speltoides*. Arc 4 is universally found in polyploids but rarely seen in diploids, however, some accessions of *T. aegilopoides* possess it. Sister arcs 5 and 6 hardly separate from each other and are nearly homologous. The absence of arc 5 in *T. aestivum erythroleucon* among hexaploids, 5C10, 4C2, and accession 6 of *T. aeglopoides*, was a very peculiar one of its own nature. Arc 7 is a lengthy and somewhat variable one of common occurrence but missing only in acession e of *Ae. speltoides*. Arc 8 a small and faint one occurs in about 50% diploids and tetraploids but rare among hexaploids such as *T. aestivum*

*erythroleucon* and *T. aestivum pseudohostianum*. Arc 9 is common among hexaploids and of fair distribution among other ploidy tiers. Arc 10 is a specific reaction of ABS 'Nepal' and is not shown by the Aegilops species. Arc 11 is common among polyploids except 'Nepal' but missing from all accessions of *T. speltoides* and *Ae. squarrosa*.

Arc 12 is comparable to arc 7 in many ways and is present in *T. aegilopoides*, *Ae. speltoides*, a couple of *Ae. squarrosa* accessions and 4C2 (*T. dicoccoides*). Arcs 13 and 14 were observed only in *T. aestivum erythroleucon*. Arcs 15, 16 and 17 are of faint density and occur in nearly 50% entries. Arc 18 is well developed and is occuring commonly except some accessions of *T. aegilopoides*, however, arc 19 occurs in several accessions of *T. aegilopoides*, one *Aegilops squarrosa* and the *T. aestivum erythroleucon* only.

Arc 20 is characteristically missing from all accessions of *T. dicoccoides* and few diploids but is present in all *T. vastivum* accessions. Arc 21 is a characteristic of some *T. aegilopoides* only. Arc 22 is specific to *Ae. mutica* otherwise rare among other taxa. Arc 23 is primarily confined to diploids and does not occur in polyploids.

Arc 24 is a special feature of *Ae. mutica* only. This is a small arc, bisects arc 1 at one end and remains parallel to it for the remaining length. It is of rare occurrence among other taxa. Similarly arc 25 is the special feature of one *Ae. squarrosa* accession only.

## DISCUSSION

The immunological studies, pertaining to Triticum and Aegilops species show an enormous amount of variation, not only at the intergeneric and interspecific levels but also within each species.

The view point of Konarev *et al.* (1970a) that hexaploids and tetraploids are more heterogeneous than *Ae. squarrosa* and other diploid wheats cannot be held valid in its entirety, because the number of ares is much variable among the accessions of *Ae. squarrosa* and *T. aegilopoides*. The next conclusion of Konarev and his colleagues (1970b) to judge or predict genome complexity from such evidence does not seem to be valid either, because the number of protein spectra in accession 6 of *Ae. squarrosa* exceeds all the haxaploids and tetraploids However, the results are in agreement with Johnson (1972).

The universal presence of arc 1 and 2 among the taxa belonging to genome A,B,C and D shows that these ares are the donations from a common

and ancient ancestor. However, it is interesting to note that all Triticum and Aegilops entries are maintaining their inheritance intact, hence, this fact establishes a direct phylogenetic link between Triticum and Aegilops species. The immunological properties and their immutability since antiquity suggests that these protein spectra are controlled by multigenes and they will stay stable unless a long series of mutations change majority of the contributing genes.

The presence of arc 3 sharply categorises the accessions of *Ae. speltoides* and *Aegilops squarrosa*. The accessions E, G and $H_1$ of *Ae. squarrosa* and d, e, f, p and r of *Ae. speltoides* do not exhibit this arc showing that the bearers and non-bearers have a closer phylogenetic link among themselves. The universal presence of arc 4 except accessions 17, 18, 19 and 20 of *T. aegilopoides* shows that this diverse group of diploids possesses two distnct groups.

The case of 5 and 6, two iso-proteins, is suggestive of a new link among three taxa viz. accession 11 of *T. aegilopoides*, 5C10, 4C2, another accession from *T. dicoccoides* and *T. aestivum erythroleucon*. The remaining enteries showing their absence also depict a relationship among themselves.

In case the protein characters 5 and 6 are considered a very stable character, which evidently is their nature, then one would to think of a new concept of the evolution of polyploid wheats. On this basis, it is thought that one Aegilopoides (accession 11), three Dicoccoides (accessions 5C10, 4C2 and 6) and one hexaploid (*T. aestivum erythroleucon*) form one separate and distinct phylogenetic group and are not very closely relating to the other polyploid wheats. It has not been possible to identify any accession of *Ae. squarrosa* showing the protein spectra under discussion, otherwise, one would have concieved a parallel and entirely new evolution of hexaploid wheat. It is also certain that the total genetic variability of *Ae. squarrosa* encounterable in nature has not been included in the present investigations. Therefore, it necessitates further study of natural variability of *Ae. squarrosa* to establish the new and independent evolution of hexaploid wheat.

Next four protein spectra being of universal occurrence further strengthen the close relationship among the taxa, but, on the contrary, do not yield evidence of diagnostic nature. The absence of arc 10 from *Ae. bicornis* and *Ae. mutica* sharply identifies these taxa from rest of them and shows that these species have a loose phylogenetic link with rest of them This work therefore, negates Sears' finding (1959) but, on the contrary,

supports Johnson and Hall (1966) who do not consider *Ae. bicornis* as a donor of B genome to the hexaploid wheat. The group of protein characters from 11 to 17 are faint and blurreds being of sporadic occurrence and as such their data are not considered reliable.

Arc 18 is homologous to arc 4 in many respects. As pointed out above, arc 4 is a major one and is shown by the accessions 17, 18, 19 and 20 of *T. aegilopoides* while arc 18 is present in accessions 17, 19, 20 and 21 of the same species. Momentarily neglecting accessions 18 and 21, an interesting picture emerges. Out of a dozen accessions of *T. aegilopoides* only 17, 19 and 20 possess both the arcs 4 and 18, moreover most of the tetraploids and hexaploids possess both of them. This shows, that owing to the presence of these two major arcs, the accessions 17, 19, and 20 of *T. aegilopoides* have a direct phylogenatic link with the higher polyploids. This point can further be supported by arc 21 which is once again a unique feature of exactly the same accessions of *T. aegilopoides* namely 17, 19, 20 and 21 hence there is mounting evidence that the B genome has been donated by *T. aegilopoides*, rather than by *Ae. speltoides* which is mostly suspected as the donor of B genome.

In addition to this, on the basis of these three protein spectra namely 4, 18 and 21, it is believed that the listed accessions of *T. aegilopoides* form two distinct groups and should, therefore, be reclassified on the basis of cyto-morphological data.

*Ae. mutica*, the only brearer of arc 24, does not seem to be closely related to any of the A,B, or D genomes as none of the other species shows this arc. Arc 25 seems to be a single major gene mutation and is the characteristic feature of *Ae. squarrosa* F, being only a solitary case of its type it does not afford any diagnostic criterion except that it makes its bearer distinct from other accessions.

The present investigations reveal that the diploids are equally heterogeneous as that of tetraploids and hexaploids. This becomes clear when the diversity of any one of the diploids is taken into consideration individually. *T. aestivum ethrythrospermum*, a hexaploid, lacks some very specific and major arcs which normally are present in rest of the *T. aestivum* species, showing closeness towards some *T. dicoccoides* accessions like 4C2 and 5C10.

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