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Review

Seed Protein Electrophoresis in Taxonomic and Evolutionary Studies

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Summary. Seed protein electrophoresis is increasingly being utilized as an additional approach for species identification and as a useful tool for tracing back the evolution of various groups of plants. This paper summarizes the main features of the seed protein profile – stability, uniformity and additive nature. In addition, the significance of this approach for resolving specific taxonomic and evolutionary problems is pointed out.

Key words: Chemotaxonomy – Cultivated plants – Germplasm – Polyploidy – Protein profile

Introduction

The use of the seed protein profile, obtained by electrophoresis, for resolving taxonomic and evolutionary problems has been greatly expanded in the last decade. Up to the present, seed protein electrophoresis has been employed in more than 45 different genera belonging to 13 plant families (Table 1). The usefulness of the seed protein profile for taxonomic and evolutionary purposes has been reviewed by several investigators (Boulter and Turner 1966; Johnson 1969). The aim of this paper is to present a more comprehensive assessment of the nature of the seed protein profile and its impact on evolutionary studies. While the significance of this approach has been stressed in many studies, it is important to recognize the limitation of the techniques involved and the pitfalls that might exist in interpreting the results.

Stability is one of the main features of the seed protein profile. For this reason it has been suggested as an additional tool for species identification beside other traditional biosystematic approaches. The uniformity of the profile and its additive nature make seed protein electrophoresis a unique and powerful tool in studies concerning the origin of polyploid plants and the evolution of cultivated plants. Throughout the paper these and some other aspects of seed protein electrophoresis will be discussed.

Stability of the Seed Protein Profile

The seed protein profile obtained by various extraction procedures is conspicuously species specific and a highly stable characteristic. This is particularly true among cultivated plants in which a large number of accessions are available for analysis. Accessions among cultivated plants from different geographical areas and adapted to diverse ecological zones still possess essentially the same profile (Larsen 1967; Ladizinsky 1975a; Ladizinsky and Adler 1975a; Johnson 1972a; Johnson 1975). Furthermore, the composition of seed protein is highly stable and is affected only slightly by environmental conditions or seasonal fluctuations (Dunnhill and Fowden 1965; Lee and Ronald 1967; Adriaanse et al. 1969; Gray et al. 1973). Seed proteins are mainly storage proteins and are not likely to be changed in dry mature seed. Thus, mature seeds of different age still possess the same profile (Robinson and Megarrity 1975). In addition, intrinsic changes in the plant such as chromosomal rearrangements or even doubling of the chromosome numbers have no, or very small, effects on the seed protein profile (Levin and Schall 1970; Bingham and Yeh 1971; Ladizinsky and Johnson 1972; Nakai 1977).

Variation of the Banding Pattern and its Genetic Control

Although uniformity and uniqueness of the seed protein profile are typical of many groups of plants, variation in the number of bands and their position in the profile has been reported, especially where a great number of accessions were examined. Unfortunately, except for a few genera, extensive screening of germplasm to uncover varia-

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Table 1. Genera subjected to seed protein profile analysis

Family	Genus	Authors
Gramineae	Agropyron	Hunzicker (1967)
	Agrostis	Wilkinson and Bread (1972)
	Avena	Murray et al. (1970); Ladizinsky and Johnson (1972)
	Danthonia	Gray et al. (1973)
	Hordeum	McDaniel (1970)
	Oryza	Nakai (1977)
	Oryzopsis	Hall and Johnson (1963)
	Poa	Wilkinson and Beard (1972)
	Sorghum	Shechter (1975); Schechter and de Wet (1975)
	Stipa	Hall and Johnson (1963)
	Tripsacum	Paulis and Wall (1977)
	Triticum-Aegilops	Johnson (1967a, 1967b, 1968, 1972a, 1972b); Johnson and Hall (1965); Johnson et al. (1967); Shepherd (1968); Waines and Johnson (1975)
	Triticale	Chen and Bushuk (1970); Hristova and Baeva (1972); Yong and Unrau (1964)
	Zea	Paulis and Wall (1977)
Leguminosae	Arachis	Cherry (1975)
	Astragalus	Nakai (1977)
	Caesalpinia	Fox et al. (1964)
	Cicer	Ladizinsky and Adler (1975a)
	Crotalaria	Boulter et al. (1970)
	Glycine	Hymowitz and Hadley (1972); Hymowitz et al (1978); Larsen (1967); Larsen and Caldwell (1968); Mies and Hymowitz (1970); Orf and Hymowitz (1977, 1979); Orf et al. (1978); Pull et al. (1978a, 1978b)
	Lupinus	Fox et al. (1964); Cox (1973)
	Medicago	Przybylska and Hurich (1971a); Bingham and Yeh (1971)
	Melilotus	Przybylska and Hurich (1971b)
	Phaseolus	Adriaanse et al. (1969); Fox et al. (1964)
	Pisum	Przybylska et al (1977); Waines (1975)
	Rhynchosia	Fox et al. (1964)
	Spartium	Fox et al. (1964)
	Stylosanthus	Robinson and Megarrity (1975)
	Trifolium	Przybylska and Hurich (1971)
	Trigonella	Ladizinsky and Porath (1977)
	Vicia	Ladizinsky (1975a)
	Vigna	Carasco et al. (1977)
Chenopodiaceae	Atriplex	Billard (1974)
	Beta	Nakai (1977)
	Chenopodium	Crawford (1974); Crawford and Julien (1976)
	Suaeda	Billard and Boucard (1971); Ungar and Boucard (1974)
Pinaceae	Abies	Clarkson and Fairbrothers (1970)
Aceraceae	Acer	Ziegenfus and Clarkson (1971)
Cruciferae	Brassica	Nakai (1977); Vaughan and Danford (1968); Vaughan and Waite (1966)
Labiatae	Galeopsis	Houts and Hillebrand (1976)
Malvaceae	Gossypium	Cherry et al. (1970); Johnson and Thein (1970); Johnson (1975)
Liliaceae	Hesperaloe	Smith and Smith (1970)
Polemoniaceae	Phlox	Levin and Schaal (1970)
Cruciferae	Sinapis	Vaughan and Denford (1968)
Solanaceae	Solanum	Edmonds and Glidewell (1977)
Liliaceae	Yucca	Smith and Smith (1970)

bility in the seed protein profile is woefully lacking (Table 1). Differences between accessions of the same taxon in darkness and thickness of various bands are the most commonly reported types of variation, suggesting that the formation of many of the bands in the seed protein profile are under control of quantitative gene systems. Caution should be taken in interpreting darkness and thickness of bands as quantitative gene systems. This kind of variation may be due to differential extraction or solubility of seed protein from different accessions. In addition, a third possibility exists that the thickness and darkness of bands may be due to the lack of separation on the gels of several proteins having similar migration rates. In any case, no attempt has been made to estimate the number of genes causing quantitative variation in seed protein bands. In wheat, a study aimed at assigning various bands of the seed protein profile to specific chromosomes using nullisomic lines showed that each band is governed by several genes located on different chromosomes (Shepherd 1968).

There are several reports regarding the genetic mechanism of qualitative variation of the seed protein profile. Larsen (1967) reported two electrophoretic variants in soybeans which he termed 'A' and 'B'. These soybeans had the same number of bands, but differed in the position of one of the bands. Larsen and Caldwell (1968) established that the variation was due to two codominant

alleles of the same locus which have subsequently been designated as $Sp_1{}^a$ and $Sp_1{}^b$ (Orf and Hymowitz 1976). The major trypsin inhibitor present in soybean seed is the Kunitz trypsin inhibitor or soybean trypsin inhibitor A_2 (SBTI-A_2) (Rackis et al. 1962). Four forms of SBTI-A_2 have been identified in the U.S. soybean germplasm collection (Singh et al. 1969; Hymowitz and Hadley 1972; Orf and Hymowitz 1977; Hymowitz et al. 1978). Three of the forms, designated as Ti^a, Ti^b, and Ti^c, are electrophoretically distinguishable from one another. The three forms are inherited as codominant alleles in a multiple allelic system at a single locus. The fourth form is the absence of SBTI-A_2, or null. The gene for the lack of SBTI-A_2 is designated *ti* and is inherited as a recessive allele to the other three SBTI-A_2 forms (Orf and Hymowitz 1979). Recently, soybean accessions were identified as lacking the 120,000 dalton seed lectin (Pull et al. 1978a, 1978b). From data obtained using polyacrylamide gel electrophoresis Orf et al. (1978) established that the presence of soybean lectin is controlled by a dominant allele designated *Le*. The homozygous recessive *le le* results in the absence of soybean lectin and appears as a null in the soybean seed protein profile.

Electrophoretic Characterization of a Taxon

When the seed protein profile of a given taxon has only a single pattern, such as in *Agropyron* (Hunziker 1967), hexaploid wheat (Johnson 1972a), chickpea (Ladizinsky and Adler 1975a), and soybeans (Savoy 1977), the electrophoretic characterization is a simple task. When variation exists in the number of bands and particularly in their position in the profile, it is necessary to determine that the variation observed is not due to experimental error. To ascertain whether two bands with similar migration velocities in different accessions indeed represent different proteins, it is necessary to create an artificial hybrid on a single gel. That is, seed protein solutions of both accessions are placed on the same gel. In the case of true variation, two bands will appear in the gel; otherwise they will form a single band (Johnson and Thein 1970).

When variation in the number or the position of bands does exist it can be expressed by three different methods. Hymowitz and co-workers (Hymowitz and Hadley 1972; Orf and Hymowitz 1977; Hymowitz et al. 1978) used Rf values to express the location of the variant forms of the SBTI-A_2 protein in soybean seed. The three forms, designated as Ti^a, Ti^b, and Ti^c, are electrophoretically distinguishable from one another by their different Rf values of 0.79, 0.75, and 0.83, respectively. The Rf values refer to the relative mobility of the SBTI-A_2 forms to a bromophenol blue dye front in a 10% polyacrylamide gel anodic system using a pH 8.3 Tris-glycine buffer (Fig. 1). The bromophenol blue dye front (+) at the bottom of the gel is arbitrarily given the value of 1, while the top of the gel (−) is given a value of 0. The Rf value of a particular variant band is proportional to the distance between the two reference standards.

Johnson and Thein (1970) and Johnson (1972a) used the optical density values of bands in the gel for calculating the correlation coefficient for any two gels analyzed. Thus, for example, the mean correlation coefficient within the genomic groups of the diploid cottons was 0.70 while between groups it was only 0.47.

The index of similarity used by Vaughan and Denford (1968) and many others is the third way of expressing variation in the banding patterns between two gels. It is calculated as follows:

$$\text{S.I.} = \frac{\text{No. of pairs of similar bands}}{\text{No. of different bands} + \text{No. of pairs of similar bands}} \times 100$$

Using this index, Ungar and Boucaud (1974) found very close similarity (92%) between three subspecies of *Suaeda maritima*. Close similarity also was reported between three subspecies of *Acer saccharum-nigrum, saccharum* and *floridanum* (Ziegenfus and Clarkson 1971). On the other hand, Shechter and de Wet (1975) and Shechter (1975) reported high similarity indices between 4 subspecies of *Sorghum bicolor-durra, caudata, guinea* and *bicolor* but low similarity between these subspecies and *kafir*. These and many other examples suggest that taxonomic categories below the species level, despite morphological and ecological differences, still possess basically the same profile and justify further use of the seed protein profile in attempts to delimit species boundaries.

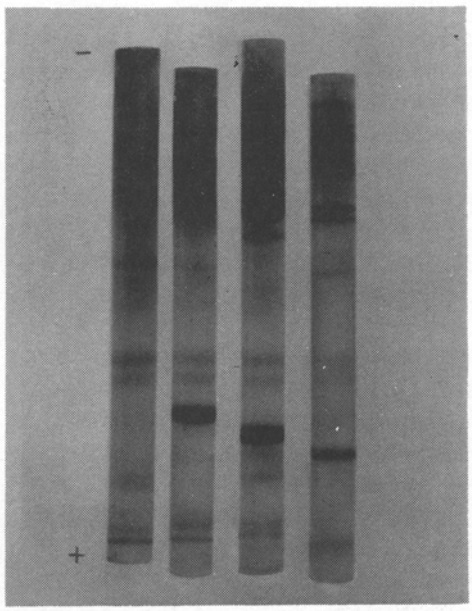

Fig. 1. Electrophoretic patterns of SBTI-A_2 proteins in soybean seed extracts. From left to right no SBTI-A_2 band (*ti*), Rf 0.75 (Ti^b), Rf 0.79 (Ti^a) and Rf 0.83 (Ti^c)

Assessment of Species Relationships by Similarity of Protein Profiles

Measurement of the degree of similarity of the profile of conspecific categories by the methods described is legitimate from the biological point of view since most of the variation observed probably can be attributed to a small number of genes that cuase the different migration rates of various protein bands. By applying the same techniques for comparing categories above the species level, one might face a situation in which bands with the same migration rate in profiles of two species do not necessarily represent similar proteins (Mies and Hymowitz 1973).

The diploid cottons *Gossypium arboreum* and *G. herbaceum* are members of the A genome group of species. These two species are fully interfertile and their seed protein profiles are very much alike (Johnson and Thein 1970; Cherry et al 1970). The reflection of cytogenetic affinities between species with incomplete reproductive barriers and the seed protein profile was demonstrated in the genus *Pisum*. The four main taxa in *Pisum* – *P. sativum, P. elatius, P. humile* and *P. fulvum* are cytogenetically closely related and can exchange genes on large scale (Ben-Ze'ev and Zohary 1973). Spontaneous hybrids between the latter two species also occur in nature. All the *Pisum* species have more or less similar patterns of albumin and globulin proteins and the ranges of variation are parallel (Przybylska et al. 1977; Waines 1975; Ladizinsky, unpublished data). Similarly, the seed protein profiles of three species of *Phlox* were in good accordance with the morphological and cytological evidence as well as with that of flavonoid compounds (Levin and Schaal 1970). Furthermore, in *Brassica*, although based on one or two accessions from each species examined, Vaughan and Denford (1968) found that each of the following species, *B. campestris, B. oleracea*, and *B. nigra*, could be distinguished from one another by a unique band or bands in their seed protein profile. On the other hand, the profiles of *B. carniata, B. juncea*, and *B. napus* represented different combinations of the profiles of the last three species. This was taken as additional evidence that the latter three *Brassica* species are of hybrid origin.

The Origin of Polyploids as Indicated by the Protein Profile

Additiveness is another typical feature of the seed protein profile. When proteins of two electrophoretic variants are mixed, the uncommon bands will persist in the gel. The common bands will merge but no new bands will be formed. Furthermore, the individuality of the uncommon bands will be expressed in the F_1 hybrids of the two variants. In other words, one can detect the parents of a specific hybrid by comparing its protein profile with a profile obtained by a protein mixture of the suspected parents.

Allopolyploids are permanent interspecific hybrids perpetuating themselves following a doubling of their chromosome number. Information accumulated thus far indicates that the seed protein profile of synthetic allopolyploids represents an exact summation of the number of bands of their diploid parents (Hall and Johnson 1963; Shephard 1968; Murray et al. 1970; Chen and Bushuk 1970; Houts and Hillebrand 1976). This apparently does not hold in high ploidy levels where many genomes are combined together. While the profile of the hexaploid *Triticale* represents the exact summation of its tetraploid wheat and rye parents, some of the parental bands were missing in the profile of octoploid *Triticale* originated from hexaploid wheat and rye (Hristova and Baeva 1972). Similar results were reported in highly polyploid *Solanum* (Edmonds and Glidewell 1977).

The additive properties of the seed protein profile has successfully utilized in studies aiming at elucidating the origin and the evolution of polyploid plants. In an excellent study by Johnson (1972a), an electrophoretic confirmation regarding the origin of *Aegilops cylindrica* was obtained. *Aegilops cylindrica* is an allotetraploid, genome constitutive CCDD, which originated from the diploids *Ae. caudata* (CC) and *Ae. squarrosa* (DD). While the profile of the tetraploid is quite uniform, some variation exists in the diploids. By matching the appropriate variant in each diploid, the profile of *Ae. cylindrica* could be synthesized from the protein mixtures of these diploid variants. Johnson concluded that *Ae. cylindrica* originated in an area where the specific electrophoretic variants of *Ae. caudata* and *Ae. squarrosa* grew, or still grow, side by side. In a similar manner, Edmonds and Glidewell (1977) demonstrated that the hexaploid *Solanum nigrum* originated from the tetraploid *S. villosum* and the diploid *S. americanum*. Following genome analysis, Kihara (1954) proposed that the tetraploid *Aegilops ovata* originated from the diploids *Ae. umbellulata* and *Ae. comosa*. By comparing the seed protein profile of the above three *Aegilops* species and of *Ae. squarrosa* Waines and Johnson (1975) believed that *Ae. squarrosa* is the better candidate as the diploid progenitor of *Ae. ovata*. These investigations suggested that chromosome rearrangement and introgression from other species blurred the genomic constitution and the protein profile of *Ae. ovata*.

Repeatedly, it has been pointed out that the protein profile of polyploids is much more uniform in comparison with their diploid progenitors (Johnson 1978a, 1972a, 1975; Ladizinsky and Johnson 1972). This might suggest, that at least in the *Triticum-Aegilops* group, in *Avena* and *Gossympium* the polyploid species perhaps have monophyletic origins or nearly so, or alternatively, that the

polyploids derived from hybridization between specific genotypes were more successful than others.

Seed Protein Electrophoresis and the Origin of Cultivated Plants

The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants. A cultivated plant and its immediate wild progenitor still form a common gene pool (Harlan and de Wet 1971) and can be considered from the genetic point of view, as members of the same species. Therefore, despite conspicuous morphological differences between them they will share, more or less, the same protein profile. Indeed similarity between the seed protein profile of wild species and their cultivated counterparts has been reported in wheat (Johnson et al. 1967; Johnson 1967b, 1968), cotton (Johnson and Thein 1970; Cherry et al. 1970; Johnson 1975), soybean (Mies and Hymowitz 1973); peanut (Cherry 1975), and corn (Paulis and Wall 1977). Employing these principles Ladizinsky and Adler (1975a) identified *Cicer reticulatum* as the wild progenitor of the chickpea. This conclusion was supported later by breeding experiments (Ladizinsky and Adler 1975b). Contrariwise, the hypothesis that the broad bean *Vicia faba* evolved from the *Vicia narbonensis* group of species (Zohary and Hopf 1973) was disproved by comparing their seed protein profiles (Ladizinsky 1975a). The profile of *V. faba* possesses 7 bands which are missing in all of the *V. narbonensis* group of species. In addition, the hypothesis regarding the genetic affinities between the *V. faba* and the *V. narbonensis* group of species was tested by breeding experiments. While the species in the *V. narbonensis* group – *V. narbonensis, V. galilaea*, and *V. hyaeniscyamus* – are cross compatible, they are cross incompatible with *V. faba* (Ladizinsky 1975b).

The usefulness of seed electrophoresis has been demonstrated in attempts to elucidate the origin and evolution of cultivated plants which are members of a polyploid series. The hexaploid wheat *Triticum aestivum* is composed of three different genomes AABBDD. Cytogenetic studies revealed that the AABB genomes were derived from the tetraploid wheat *T. dicoccum* and the DD genome was donated by the wild species *Aegilops squarrosa*. These genomic relationships were fully supported by seed protein electrophoresis. The profile of *T. aestivum* was simulated by a synthetic protein mixture of *T. durum* and *Ae. squarrosa* (Johnson et al. 1967; Johnson 1972a). Cytogenetic studies also showed that the A genome of the tetraploid wheat was contributed by the diploid wheat *T. boeoticum*. For several years it was believed that the B genome of wheat was contributed by *Aegilops speltoides*. However, comparison of the seed protein of this wild species with that of tetraploid wheats revealed that the prominent albumin bands of *Ae. speltoides* are missing in the profile of the tetraploid wheats (Johnson 1972b). Thus *Ae. speltoides* could not be considered as one of the diploid progenitors of hexaploid wheat. A similar conclusion was reached by cytogenetic investigations (Kimber and Athwal 1972).

Seed protein electrophoresis has also corroborated the cytogenetic evidence regarding the origin of tetraploid cotton, *Gossypium hirsutum* (Cherry et al. 1970; Johnson 1975). Tetraploid cotton is composed of two different genomes AADD. Protein mixtures of *Gossypium arboreum*, the donor of the A genome, and *G. raimondii*, representative of the D_β genome, are identical to that of the tetraploids *G. hirsutum, G. barbadense* and *G. tomentosum*. On the other hand, the profile of the tetraploid *G. palmeri* could be simulated by protein mixtures of the diploids *G. arboreum* and *G. trilobum* (D_ϵ genome). These data suggest a polyphyletic origin of the tetraploid cottons.

The highly uniform protein profile of cultivated polyploid plants not only permits a relatively quick identification of their diploid progenitors but is also of practical value for plant breeders. Uniformity of the protein profile suggests that these polyploids evolved from a few diploid genotypes and consequently represent only a small segment of the genetic variability. If this is true, introduction of additional variability existing in the diploid species to polyploid cultivated plants could be of great economic value.

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