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The Early Application of Electrophoresis of Protein in Higher Plant Taxonomy

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ABSTRACT

The aims of this research are firstly, to study the advantages of electrophoretic techniques. Secondly, to look at the usefulness of a few mediums support of electrophoretic proteins especially the acrylamide gel. Thirdly, to examine the number of plant organs which could be used as the sources of plant proteins, and how these plants protein should be applied in the medium support that has been selected. Besides, the staining and detection procedures would be described, while the application of electrophoretic approach in higher plant taxonomy will also be evaluated. In this study we recorded that a number of taxonomic problems usually caused by morphological complexity within species can be solved using this experimental approach of electrophoresis. This method has been considered very useful in helping taxonomists making decisions.

Key words: electrophoresis, proteins, taxonomy.

INTRODUCTION

Some thirty years ago, the activities of plant taxonomists were particularly concerned with observations of morphological characters. Recently they have tended to use a new additional experimental method called electrophoresis. This method has been regarded to be most useful for resolving taxonomic problems**,** if comparisons about morphological characters are felt not adequate in helping taxonomists to make decisions.

By using electrophoretic methods, taxonomic activities have increased rapidly, both in animal systematics (Gottlieb, 1978; Evans, 1985) as well as in plant taxonomy. In plant taxonomy, more applications have been in higher plants rather than in lower plant taxonomy, even though in the study of origin of species (Gastony, 1986) or in taxonomic relationships among species within the genus *Polyporus* (Shannons *et al.,* 1973), electrophoretic data have been considered very helpful.

In electrophoretic techniques, only a few kinds of medium supports have been employed. Three commonly used for taxonomic purposes are paper, cellulose acetate and gels. The latter, in recent years has been the most commonly employed and can provide more accurate data because of better resolution of protein mixtures.

Most applications of electrophoretic techniques in plant classifications use gel medium supports. This has resulted from the reliability of data produced by gel electrophoresis, which have been accepted widely, particularly in studies of plant population genetics (Brown, 1978). Accompanying reliability is the sensitivity of electrophoresis to detect single amino acid substitutions in proteins that may not be shown by other experimental methods other than total amino acid analysis. This technique however is expensive and beyond reach of most taxonomists. So undoubtedly electrophoresis will play an increasingly important role in taxonomy, and in monitoring for example the manipulations of crop genetic resources (Brown, 1978).

In accordance with employing gel supports for taxonomic purposes, plant protein samples

are noted to have many advantages. Besides, the great numbers of individual proteins, which can be detected, plant proteins are usually present universally in all types of tissue and even a single organ like a leaf may contain several thousand proteins (Harborne and Turner, 1984). Moreover, they noted that variations in the total complement of seed proteins have a close correlation with the tribal and sub family classifications in for example Leguminosae (van Sumere *et al.,* 1976).

Using plant protein samples combined with gel electrophoretic procedures, identification, and monitoring of wheat in USA and England has been both more rapid and efficient. Harborne and Turner (1984) noted this method has also been used in cultivar analysis, for example in *Phaseolus vulgaris* (Boulter *et al.,* 1966). It may be important to note that the use of electrophoresis for taxonomic purposes is not restricted to taxonomists, but biochemists too have made a great contribution in resolving problems of species delimitation. It has been suggested that this method is more precise and objective in recording and evaluating the mobility and characteristics of protein than most (Gottlieb, 1978).

In this, examples of the application of gel electrophoresis using polyacrylamide gel in higher plant taxonomy especially related to the origin of species, variety identifications and population variations would be discussed.

METHODE ON ELECTROPHORETIC TECHNIQUES

Medium support

In dealing with the electrophoretic medium support, the gel is considered as very suitable and widely used in plant taxonomic purposes. Generally, gels that are quite often employed in recent years have been starch and polyacrylamide gels. Gel electrophoretic systems, were used successfully for separation of-protein. The starch gel as an electrophoretic medium and spectacular gains in resolution of 20-25 serum components could be recognized as compared to 5-6 components by conventional methods. Subsequently this technique in recent years has gained immense importance through the introduction of the synthetic polyacrylamide gel, which was employed in a specific method for disc electrophoresis. The gel of polyacrylamide is considered as one of the best medium supports for resolving larger number of protein bands and is clearer than with cellulose acetate for paper. The percentage of polyacrylamide in the electrophoresis medium commonly used is 7 %, usually in a tris-glycine buffer at pH 8.1. In certain cases, the proportions of polyacrylamide and the pH are varied. Davis (1965 cited in Johnson, 1970). Polyacrylamide gel support without doubt, at the present times has attracted most of the plant taxonomists who are interested in protein approaches to chemotaxonomy using electrophoresis, and these have covered a wide range of higher plants.

Sources of plant proteins

The source of protein from plant organs is varied, depending on the sorts of plants. The most important of plant organs for storing proteins are the seeds. The use of plant seeds as a source of proteins especially in taxonomic works, at the present times has been widely adopted. Generally speaking, the reliability of' seeds as source of proteins is due to the fact that their compositions are only little affected by environmental conditions or seasonal factors (Harborne and Turner, 1984).

Most investigations of plant proteins have used seeds, but in certain taxa of plants pollen grains have been used as a good protein source for taxonomic purposes, for instance in*: Lasthenia* (Altosaar *et al.,* 1974); *Cassia* (Todaria *et al.,* 1983); *Triticum* and *Aegilopsis* (Johnson, 1972). Leaf and other vegetative material are of less general use because of susceptibility to environmental and growth conditions, but in certain cases these other organs have been employed.

Seed extractions and application

In *order* to obtain protein extracts of a good quality, several extractants can be used depending, for example on the type of seed coats or tissue conditions (*Conkle et al.,* 1982). Water solution can be regarded as an effective solute for example in extractions of *Gossypium* and *Bulanisia* seeds (Johnson, 1970; Cosmas and Hunziker, 1979). The proportions between weight and volume ranging from 1 to 6 and the extraction time 15 -30 minutes.

Another solvents, which are considered to have a good application for seed extractions,

are buffer, alcohol and K_2SO_4 (Johnson, 1972; Todaria *et al.,* 1983). They noted the proportion between weight and volume in the latest solvent is around 5 to 100. Before applying the sample in medium support, the seed of each sample is usually ground separately in a pre-chilled mortar and pestle and protein extracts are often centrifuged at about 1000-2000 *I* g, the supernatants are then applied in the gel.

After application of the sample, the voltages are than applied, depending on the form of column or slabs. In the first case, the gels are run at 3-4 m A per column for 45-60 minutes, but in the slab forms the diameter or volume of slabs need to be taken into account, after that staining the gel can be achieved.

Staining and detection

To localize the proteins, the gel can be stained with certain stains, such as Amido Black, Coomassie Blue, Azocarmine, and Naphthol Blue Black. At the present time, Amido Black is regarded as the best stain to obtaining a clearer background, satisfaction quantitative response and more sensitivity especially at low protein concentrations Blue Black substance has higher sensitivity than Amido Black, Meanwhile, other authors have reported, gels can also be stained successfully by using 0.5 % Aniline Blue Black (Johnson and Fairbrothers, 1975).

Before staining the gels fixation is carried out in 7 % acetic acid solution a- methyl alcohol -water -acetic acid at 5 : 5 : 1 and background staining can be made in 4 : 1 : 1 with the same solvents (Cosmas and Hunziker, 1979). The bands that have been stained can be assigned with the Rf values according to their mobility relative to the buffer front. Based on the assumption that a band of identical mobility in two related taxa is the same protein, the results of electrophoretic analysis can be presented as the number of bands, that are common to two a- more species and the number, which are species-specific. Each band then represents a single taxonomic character (Harborne and Turner, 1984).

The number of protein bands detectable by electrophoresis in storage tissue such as seed is relatively unpredictable, but Harborne and Turner (1984) noted an average between 10 and 30 have been found. In finding the homologous bands, they can be clustered based on the Rf values, and from that data index similarities between the species examined can be obtained. The Coefficient of similarity (Cs) can be calculated as follow s: Cs =2W / (A + B), where W = number of homologous bands, A = number of band in species a, and B= number of bands in species b (Backer cited in Todaria *et al.,* 1983).

TAXONOMIC APPLICATIONS

The electrophoretic separations of proteins now has an established place in modern chemotaxonomic practice (Harborne and Turner, 1984), most applications have been within groups of closely related taxa, both at the population level or in comparison to sympatric species within the same genus. The following text explains some example of taxonomic applications based on electrophoresis of protein, i.e. origin of species and taxonomic relationship.

Origin of species

In a study on the origin of subspecies *Triticum aesticum* (hexaploid), Johnson (1972) reported that all protein profiles show a very uniform appearance in the gel that could be simulated by the patterns produced by a protein mixture (2 : 1) from specific profile types of the ancient tetraploid cultivar *T. dicoccum* and the wild diploid *Aegilops squarrosa*. The protein patterns covered the range of variability observed in each species.

Among diploids of the Tritidae, *A. squarrosa* has an albumin pattern highly consistent among accession with dense bands centered at -9.0 cm that tend to fuse with narrower one at -9.0 cm. All accession has less dense bands at -8.1, -6.7, and -4.4 and all has dense bands leading band at -10.4. The ancient cultivar *T. dicoccum* (10 -15) is relatively uniform with respect to entire protein profile. The albumin pattern comprises eight or nine bands at -9.7, -9.0, -8.3 sometimes being double. While *Aestivum* hexaploid wheat present remarkably uniform albumin pattern similar in its main features to that of *A. squarrosa* with leading band at -10.4 double bands centered at -9.7 and conspicuous bands at -8.1, -6.7 and 4.3. Johnson reported, closer examination shows the present of fait dicoccum homologous at -7.6. -6.9, and -5.3. The dicoccum bands at -9.7 and -9.0,

expected in the *Aestivum*, are completely masked by the broad squarrosa band. In general, the *Aestivum* pattern shows dense bands where parental homologous reinforces one another at -4.3. It may be the reason to confirm that using the advantages of gel electrophoresis, the *Aestivum* protein profile roughly represents the addition of the patterns of the presumed parental species so that the parentage of the *Aestivum* hexaploid in general *as T. dicoccum* and *A. squarrosa*.

Taxonomic relationship

An investigation to see the albumin and globulin bands of 20 taxa of *Lasthenia* shows homologous bands. There were 61 homologous bands of albumins and 20 of globulins was carried out by Altosaar *et al.,* (1974) *L. microglossa* possesses the greatest number of albumin bands (13) while the least number of albumin bands (4) appeared in *L. glabrata* subsp. *glabrata*. Albumin band (8) was the most common (Rf 0.135 -0.145) and occurred in 11 of the taxa. *L. minor* subsp*. maritima* showed the most globulin bands (5), while both *L. fremontii* and *L. microglossa* showed the fewest (1). At the same time, the number bands of globulin and albumin of *L. burkei* and *L. conjugens* were noted.

Both of those species have 2 globulin bands, while for albumin, 8 bands occur in *L. burkei,* and 9 bands in *L. conjugens*. The most common globulin band (Rf 0.3 -0.314) occurred in only 6 of the taxa (Altosaar *et al.,* 1974). They also noted that the average coefficients of variation for all albumin and globulin bands that appeared in this study were 0.028 and 0.044 respectively.

It is become apparent that gel electrophoresis of crude seed-protein extracts has been extremely useful in elucidating evolutionary relationship in many genera (Johnson, 1972; Payne *et al.,* 1973 cited in Altosaar *et al.,* (1974). At least in the present study disc-electrophoresis of albumin and globulin seed fractions have provide some additional information about genus *Lasthenia*. Although the number of clear relationship that arose are few Altosaar *et al.* (1974) noted nearly every species of *Lasthenia* has an unique array of seed proteins, and further sampling of additional population may reveal the existence of intraspecific affinities variation.

On the other hand, in species relationships of *Bulnesia* (Cosmas and Hunziker, 1979), reported that 84 protein bands were identified, in seven species tested. Characteristic "marker" band of each of the species is always present in the electrophoregams. These are Nos. 40 for *B. arborea* Nos. 40. 671 75 and 79 for *B. carraqo*; 29, 34, 37 for *B. bonariensis*; 241 251 41, 42 for *B. schiekendantzii* and *B. foliosa*; 30, 47 for *B. sarmientoi* and 22, 48, 72 for *B. retama.*

There were no constant differences between geographic races of *B. arborea* from Colombia and Venezuela. The bands that are present in the population from Venezuela but are absent in the one from Colombia, are not constant and result from inter population variability. The difference between electrophoregram *B. carrapo* and *B. arborea* has been considered by Cosmas and Hunziker (1979) to give support the idea that both taxa are separate allotropic species.

In this study, an attempt to detect intra population variation has been made. These species of *B. arborea, B. bonoriensis B.sarmientoi* and *B. retama* show variability in their protein fractions so that could be attributed to genetic polymorphisms (Cosmas and Hunziker, 1979). Smaller variation in the individual patterns was noted in *B. arborea* from Colombia and Venezuela and in *B. bonareinsis.* Todaria *et al.,* (1983) in the study of seven species of the genus *Cassia* reported no common band found in all species. However, band 4 was common in four species (*C. occidentalis, C. laevigata, C. glauca* and *C. dimidiat*a) and bands 2, 3, 5. 17. 21' 27 and 29 were shared by three species each.

Based on the coefficient of similarity, *C. glauca* seems to be closely related to *C. occidentalis* on the one hand and *C. absuson* the other, exhibiting the similarity of coefficient 47 % and 42.1 % and sharing 4 out of a total 13 bands, and 4 out of a total of 15, respectively. These facts indicate their genetic affinity with each other and justify their placement near to one another Baker (1879 cited in Todaria *et al.,* 1983). Accordingly*, C. dimidiata* that has maximum similarity (coefficient of similarity 33.3 %) with *C. glauca* have also been placed nearer to one another. Another result that has also been noted in this study is the frequency of protein bands. The highest number (10) of bands, and the lowest

number (6) protein bands occur in *C. glauca* and *C. laevigata* respectively.

Another study in protein profiles of 14 varieties of *Narcissus* has been worked out and compared. The results indicated that the variety identification is positive either on the basis of distribution of typical protein bands or band combinations (Bhargava *et al.,* 1987). This data support the classification of the genus, proposed on the grounds of chromosome studies.

Because of its reliability, in providing data for intra specific compatibilities and species relationships at the present time polyacrylamide is widely used. In the study of intraspecific compatibilities the seed proteins of 17 wild species of *Phaseolus* have been identified. They reported that three of them show very little variation in the protein pattern within most species, while considerable variation among species was evident. They concluded that the data agreed with previous research on morphological characteristics and also generally agrees with current information on intra specific similarities based on hybridization studies.

CONCLUSION

Modern experimental techniques in higher plant taxonomy nowadays are likely to be more accurate than orthodox taxonomy in classification. Data which are used are not only based on the morphological characters, but other additional evidence for instance pollen and chromosomal features have also been taken into consideration, because of the fact they provide great contributions in assisting the work of Taxonomists. Using the above additional evidence, many taxonomic works in recent years have become more significant after new experimental techniques namely electrophoresis has been introduced. Using the advantages of electrophoretic methods, study about relationships of *Bulnesia* species have been noted. That both species of *B. arborea* from Venezuela and Colombia have been detected no constant different between their protein pattern, while the difference between electrophoregram of *B. arborea* and *B. carrapo* has been considered the result of separate allotropic species. Another study in seven species of *Cassia,* the common protein bands were found, while the lowest protein bands to be in *C. glauca* and *C. laevigata.* Accordingly in the variety studies of *Narcissus,* the data supply the position of this genus proposed on the ground of chromosome studies. Meanwhile, in the *Phaseolus* the results agreed with the previously research on intraspecific relationship in *Phaseolus,* based on morphological characters. From the above facts, it is not surprisingly that in future more and more applications of experimental techniques especially electrophoresis will play an important role in higher plant taxonomy because of the failure observation methods may only be solved by this approach.

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