Review

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The characterisation and identification of crop cultivars by electrophoresis

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Abbreviations: SGE: Starch gel electrophoresis; PAGE: Polyacrylamide gel electrophoresis; IEF: Isoelectric focusing; UTLIEF: Ultrathin-layer isoelectric focusing; 2-D: Two dimensional; SDS: Sodium dodecyl sulphate; TCA: Trichloroacetic acid; Est: Esterase; AP: Acid phosphatase; Perox: Peroxidase; LAP: Leucine aminopeptidase; ADH: Alcohol dehydrogenase; GOT: Glutamate – oxaloacetate transaminase; PGI: Phosphoglucoisomerase; PGM: Phosphoglucomutase

1 Introduction

Within the agricultural community, it is important to be able to recognise and distinguish between different cultivars (or varieties) of particular crops. This is partly because cultivars often differ in their quality and other important agronomic characteristics. Thus millers and bakers need to be certain of using wheat cultivars of good baking quality or suitable pasta-making quality. Again, brewers and maltsters want to be certain of using only those barley cultivars which are known to be suited for their particular purpose. As there is often a premium paid for good quality cereal crops, or there may be yield benefits from growing cultivars with resistance to particular diseases, farmers need to be assured as to the varietal identity and purity of the crops they are sowing. Seed merchants are legally required to supply seed of the correct cultivar. There are other circumstances in which it is necessary to be able to assess identity. For instance, recent legislation requires that retailers identify potatoes by cultivar. A major need for cultivar characterisation arises from the statutory obligation that all newly produced cultivars be tested against existing ones for their distinctness. Cultivars also have to be shown to be suitably uniform in their characters, and to be stable from one generation to the next (Plant Varieties and Seeds Act, 1964). The breeders of cultivars which meet these requirements are then granted Plant Breeders Rights.

The methods which are traditionally used to assess cultivar identity and purity vary in detail from crop to crop, but generally involve a lengthy and detailed morphological survey of the field-grown plant. This is accompanied in some cases by a microscopic examination of individual seeds. Such methods are very effective in practice, but they do have certain serious drawbacks. The time required for identification is often fairly lengthy, as plants need to be grown before being examined, and plant and seed morphology can be radically affected by the environment and by soil conditions, for instance. Large areas of land are required for the growing of suitable numbers of plants. A detailed and labour-intensive system of data collection is needed as, for example, over 80 separate morphological characters are examined for a barley cultivar. For these reasons, a great deal of attention and effort has been paid to the development of laboratory-based methods for cultivar characterisation. Such biochemical techniques have several potential advantages. Analysis is likely to be much quicker than by traditional techniques and require less personnel. Choice of an appropriate biochemical parameter can eliminate, or at least greatly reduce, any environmental effects. Only a suitably equipped laboratory is required, as opposed to large fields. There are thus compelling reasons to find and use biochemical methods for cultivar identification.

To date, the most widely applied and versatile technique used for laboratory cultivar characterisation has been the analysis of seed and leaf proteins and enzymes by various forms of elec-

Verlag Chemie GmbH, D-6940 Weinheim, 1984

tr oresis. Cultivars of many crops have been analysed and identified by a wide range of gel electrophoretic methods and it is the purpose of this article to review these methods as applied to different species, assess the results and indicate possible future areas of development.

1.1 The rationale for the use of electrophoresis

It may not be immediately apparent why an examination of protein composition enables cultivars to be distinguished. However, the identification of cultivars is in essence an exercise in characterising genotypes, as all cultivars must, by their nature, have differences in their genomes. A protein is a primary product of a structural gene and may be considered to be a marker for that gene. As genes are connected into genetic systems, a protein may become a marker for that system, which may be a chromosome or the genome as a whole. Hence by considering a sufficient number of protein markers, the structure of the genome can be studied to a considerable degree. For analysing intra-species relationships (*i. e.* cultivar differentiation), it is necessary to study those proteins which can exist in multiple forms. The most commonly used proteins for cultivar discrimination are thus (a) seed storage proteins, which are known to be polymorphic with respect to either size, charge, or both parameters in almost all species investigated [1, 2] and (b) seedling or leaf isoenzymes of various types [3]. Electrophoretic techniques are used to detect differences in such polymorphic proteins in different cultivars. As protein composition is genetically determined, it is not affected by environmental conditions. The location of the genes coding for particular proteins is known in several cases, which increases the attraction of this approach.

Hence, provided that there is sufficient heterogeneity in protein composition and provided that our methods of analysis are suitably powerful so as to be able to detect this heterogeneity, considerable insight into the genetic make-up of a plant can be gained from a study of protein composition. Naturally, the portion of the genome which is concerned with determining seed or leaf protein composition is only a minute portion of the total genetic information. It is perhaps not surprising then, that, in many species, all cultivars cannot be individually distinguished by electrophoretic means. Nevertheless, the discriminating power of this approach is remarkable, and is certainly far greater than for any comparable morphological character or marker. This will be illustrated in the consideration of individual species. The wide range of electrophoretic methods which have been used, both singly and in combination, will also be demonstrated. However, before beginning this discussion, a brief outline of the types of seed proteins will be useful.

1.2 Seed proteins

The system of classification of seed proteins is based largely on the pioneering work of Osborne [4], who recognised that seeds contained proteins differing in their solubility properties. Although the classical Osborne scheme can be criticised as being insufficiently rigorous in modern terms [5], it provides a useful way of classifying seed proteins and is still used by most workers in this field. There are thus considered to be four categories of proteins occurring in seeds: 1) albumins, which are soluble in water and compris mostly enzymic proteins; 2) globulins, which are soluble in dilute salt solutions and generally occur in protein bodies (*i. e.* they can be considered storage proteins in the strict sense); 3) prolamins, which are soluble in aqueous alcohol solutions and are also found in protein bodies as true storage proteins; 4) glutelins, which are soluble in alkaline or acid solutions, or in detergents and are probably mainly structural proteins, although some may have metabolic functions.

The proportions of each class of protein present in a seed vary from species to species. Thus in cereals, the major storage protein is usually a prolamin, although oats and rice have high levels of globulin-type and glutelin-type proteins respectively. In leguminous crops, globulins represent the major part of the seed protein. A large part of the work concerning the application of electrophoresis to characterise crop cultivars has thus been concerned with the analysis of prolamins, which are known by their trivial names (gliadin in wheat, hordein in barley, zein in maize, avenin in oats) or globulins. However, there are instances where albumins and glutelins have been analysed with considerable success.

2 Cereals

2.1 Wheat (*Triticum* species)

There is a considerable volume of literature relating to the electrophoretic identification of wheat cultivars, no doubt because of its prime importance in the diet of the Western world. As a result of this concentration of effort, there exist several very well-defined and tested methods for wheat cultivar characterisation, which have been developed in many countries. Unfortunately, there is as yet no consensus as to which of these methods could form the basis of an agreed international testing system, although progress is slowly being made in this area [6]. Most studies have been concerned with analysing the polymorphism of the wheat prolamin (gliadin). It has been known for some time that gliadin exhibits considerable heterogeneity and can in fact be fractionated into some 40–50 components [7]. Different cultivars have different combinations of these components and the gliadin composition is characteristic of a cultivar. The methods used for analysing gliadin composition fall into three main categories: (i) those utilising starch gels containing urea and run at acid pH, usually in aluminium lactate buffer (referred to hereafter as lactate-SGE); (ii) those utilising the above procedure, but with polyacrylamide gels of various concentrations (lactate-PAGE); (iii) those utilising PAGE in the presence of sodium dodecyl sulphate (SDS-PAGE). In addition, methods for wheat cultivar identification have been devised using glutelin composition and the occurrence of certain seed and seedling isoenzymes.

In comparing methods for cultivar identification, there are several points which may need to be considered in addition to the obvious ones such as gel composition, buffer composition, etc. For instance, in routine analysis it is important that the methods of extraction are suitable for the handling of multiple samples and are not unduly lengthy or technically difficult. The number of samples that can be analysed per gel and the time required for analysis should also be taken into consideration.

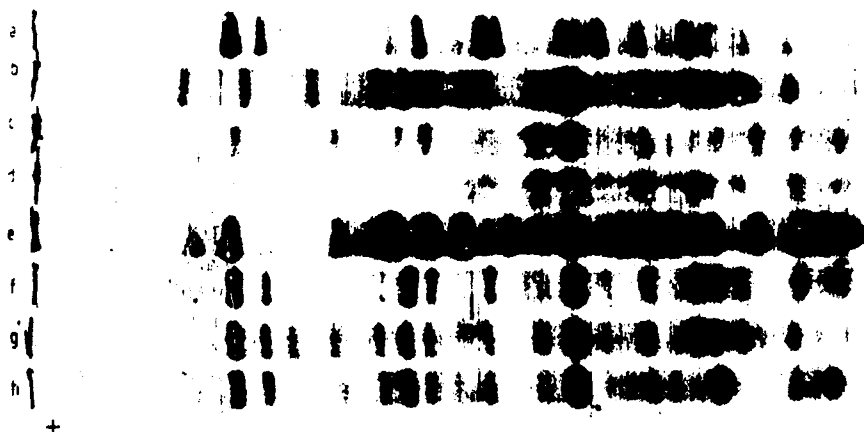

Figure 1. The gliadin banding patterns of a range of wheat cultivars grown in the UK, following lactate-SGE [11]. (a) Minaret, (b) Hotspur, (c) Hammer, (d) Avocet, (e) Mithras, (f) Patron, (g) Mission, (h) Galahad. Unpublished data of Parnell *et. al.*

2.1.1 Lactate-starch gel electrophoresis

The lactate-SGE method is at themoment probably the favoured technique for wheat cultivar characterisation in Europe, although it is rapidly being replaced by PAGE methods (see below). The basic method was devised more or less at the same time by French and Australian workers [8, 9] and has been well described [10]. Briefly, gliadins are extracted from single crushed wheat grains or from wheat flour using 25 % 2-chloroethanol and are then analysed using gels containing 10 % hydrolysed starch, 0.5 M urea and 0.2 % aluminium lactate (buffered with lactic acid to pH 3.2). Following electrophoresis in aluminium lactate buffer containing urea at 8 V/cm of gel, the gels are sliced and stained in a solution of nigrosin and trichloroacetic acid (TCA). This relatively simple method has an impressive degree of discrimination. For example, using a slightly modified and standardised version of the above method [11], it is possible to identify all of the 54 wheat cultivars currently included on the UK National List [12]. Fig. 1 shows some typical electropherograms. A numbering system has been used to identify gliadin bands. The gliadins found in different cultivars have been described and taxonomic keys produced as an aid to the identification of cultivars [6, 10, 12, 13]. This method is currently routinely used in many European countries. There are, however, certain disadvantages to the use of starch gels and, in many laboratories, methods using polyacrylamide gels are gaining favour.

2.1.2 Lactate-polyacrylamide gel electrophoresis

The first use of lactate-PAGE to separate gliadins and hence identify wheat cultivars was described by Bushuk and Zillman [14]. However, several groups of workers have reported modifications and improvements of the basic technique and there have been in addition various derivatives of the method. Also, there are methods which, whilst not directly being related to the Bushuk and Zillman procedure, can nevertheless be conveniently included in a discussion of PAGE techniques carried out at acid pH. Table 1 summarises the salient points of the lactate-PAGE technique and its modifications. Certain features are common to all these techniques, e. g. the extraction of gliadins can be carried out either with single seeds or from a 'flour' of ground wheat and staining is usually achieved with a Coomassie Blue – TCA mixture, to remove the need for de-staining. Again the discriminating power of this method is very impressive and in its various forms it has been used to identify Canadian [22], French [15], American [23], Australian [13], New Zealand [24], Austrian [25], and Italian [26] wheat cultivars. Typical protein patterns produced by a gradient lactate-PAGE system [21] are illustrated in Fig. 2. As with lactate-SGE, a numbering system has been developed to identify the gliadin polypeptide bands, and taxonomic keys produced to aid cultivar characterisation [10, 14, 15]. In addition, various computerised methods of data collection, storage and retrieval have been proposed [27-29]. Future developments in this area will no doubt lead to a system of automatic gel evaluation, by densitometric scanning, and computer-aided matching of this information with the gliadin compositions of all internationally known cultivars. Clearly, a major obstacle to this goal is the existence of so many variations of the basic method. Also, more than one system for gliadin band nomenclature is available [13, 15, 20]. However, the existence of a world-wide wheat cultivar catalogue would be of the

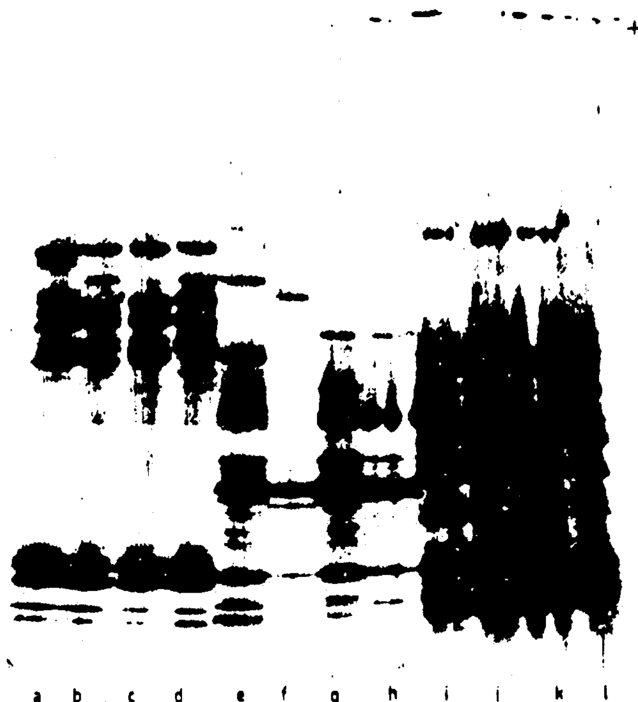

Figure 2. Gradient gel lactate-PAGE protein patterns of 4 cultivars of wheat (a-d), barley (e-h) and oats (i-l). (a) Avalon, (b) Bounty, (c) Broom, (d) Timmo, (e) Carnival, (f) Keg, (g) Igri, (h) Crosier, (i) Leanda, (j) Cabana, (k) Pennal, (l) Trafalgar. Analysis carried out as in [21], using 3-27 % acrylamide gels. The origin and anode are at the top. Unpublished data of Cooke and Cliff.

Table 1. Lactate-PAGE techniques used for wheat cultivar identification

	Extraction	Gel composition	Buffer composition	Horizontal (H) or vertical (V)	Electrophoresis conditions	Approx. time per analysis	No. of samples per gel	Equipment	Reference
1	70 % Ethanol, 1 h, room temperature (+ sucrose)[a]	6 % Acrylamide, 0.3 % Bis	8.5 mM Aluminium lactate + lactic acid to pH 3.1	H	72–74 mA Constant current, 21 °C	6.5 h	10	Custom-made	[14]
2	As [14]	As [14]	3.5 mM Sodium lactate + lactic acid to pH 3.1	H	As [14]	5 h	10	As [14]	[10]
3	25 % Chloro-ethanol + saccharose, 1 h, room temperature	As [14]	As [10]	H	As [14]	5 h	10	As [14]	[15]
4	70 % Ethanol, 1 h, room temperature (+ glycerin)[a]	As [14]	As [14] in gels; diluted × 2 in tank	V	290 V, Constant voltage	5.25 h	8	E-C 6052	[16]
5	As [14]	As [14]	As [10]	V	75 mA, Constant current	5 h	10	E-C 470	[17]
6	As [14]	As [14]	As [14]	V	570 V[b)] 1000 V 1400 V, 7 °C	1.3 h[b)] 0.6 h 0.3 h	20	Bio-Rad 220	[18]
7	As [14]	7.5 % Acrylamide, 0.37 % Bis	8.5 mM Sodium lactate + lactic acid to pH 3.1	V	20 mA, Constant current, 21 °C	4–5 h	6	Pharmacia GE-2/4	[19]
8	As [14]	As [19]	13.0 mM Acetic acid/sodium acetate buffer, pH 3.1	V	–[c)]	–[c)]	–[c)]	–[c)]	[20]
9	1.0 M Urea, 1 h, room temperature	Gradient of 2.5 %–13 % acrylamide	4.25 mM Sodium hydroxide + lactic acid to pH 3.1	V	200 V Constant voltage, 25 °C	1 h pre-run + 2 h	12	Pharmacia GE-2/4	[13, 21]

a) added to samples prior to electrophoresis
b) depending on gel thickness and number of gels in parallel
c) information not supplied

greatest importance and significance and every effort should be made to reach agreement on the methods adopted.

2.1.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Although SDS-PAGE is not routinely used to identify wheat cultivars, it can be useful in certain situations, such as resolving two cultivars which are apparently very similar following lactate-SGE or-PAGE. Most of the systems employed are variations of the discontinuous Laemmli procedure [30], although there are certain refinements. Shewry *et al.* [31], used SDS-PAGE in 17.5 % acrylamide gels to fractionate the proteins extracted from single wheat grains by isopropanol-mercaptoethanol. Other workers have used a total SDS-soluble fraction of wheat grains to discriminate between cultivars [10, 13, 32–34]. The most useful portion of this 'total' protein is the high-molecular weight glutelin fraction, known as glutenin in wheat [10, 23, 34], although gliadins, which co-extract, also show differences between cultivars. An interesting approach is the use of SDS-PAGE in gradient gels (7.5 %–16 % acrylamide) [10, 35]. In general, a combination of lactate-PAGE of gliadins and SDS-PAGE of glutenin subunits ought to be sufficient to discriminate between most cultivars [10].

2.1.4 Other methods

Although much of the early development work for gel isoelectric focusing (IEF) was carried out by Wrigley's group using wheat seed proteins [36], the technique has not proved popular for wheat cultivar identification, even though its potential for this purpose has long been recognised [37]. The probable reasons for this have been suggested as (i) expense - the technique is too costly for multiple routine application; (ii) comparative lack of discriminating power, since the solvents used for extraction of wheat proteins for IEF, such as 1.0 M urea, also extract albumins in addition to gliadins and cultivar differences in gliadin patterns tend to be obscured [10, 21]. However, IEF as the first stage of two-dimensional (2-D) mapping procedures has been shown to be capable of discriminating between cultivars which were otherwise identical [10, 21, 32]. Thus IEF in combination with lactate-SGE [10, 21] or with PAGE in Tris-borate buffer, pH 8.9 [32] or SDS-

PAGE [38] has been used to characterise wheat genotypes. Although such procedures could not yet be recommended for routine cultivar identification, they can be a useful supplement to the simpler methods of electrophoresis and provide information in difficult cases.

The objection to the routine use of IEF on the grounds of expense can be considerably overcome by the use of ultrathin-layer IEF (UTLIEF) as described by Radola [39]. The potential applications of this method for cultivar identification in wheat and other species have recently been described [40] and will be discussed further in subsequent sections.

2.1.5 The use of isoenzymes

The use of gliadins or glutenins to identify wheat cultivars involves staining gels with a general protein stain, to visualise as many polypeptide components as possible. These proteins are genetically coded at multiple loci [38, 41] and so electrophoretic techniques are examining variability at many or all of these loci. However, it is also possible to get information about genome variability by selectively staining for specific enzymes, which may be coded at more than one locus or, although encoded at a single locus, nevertheless exhibit polymorphism. This approach, investigating the isoenzyme variation between wheat cultivars, has been used with some success. Thus Almgard and Clapham [42] were able to distinguish Swedish cultivars on the basis of both the gliadin composition and the esterase (Est) and acid phosphase (AP) patterns of seeds revealed by lactate-SGE or IEF. Similar work using gradient gel PAGE has been reported [32]. Peroxidase (Perox) and other enzymes from seeds or seedlings have been used for the characterisation of Spanish wheat cultivars [43]. The use of β-amylase and Perox patterns can distinguish cultivars with closely similar gliadin composition [10]. Such isoenzyme methods have also been used with great success in other species.

2.1.6 Electrophoretic uniformity of cultivars

Although the majority of cultivars characterised by gliadin composition are electrophoretically uniform, there are a small number, in all countries, which contain electrophoretic variants or biotypes [12, 13, 15, 22, 23, 26, 44]. Clearly, if the techniques are to be of any use in varietal identification or purity assessment, it is necessary to have knowledge of the existence of such biotypes. This can only be achieved by analysis of a sufficient number (50-100) of single seeds, a fact not always appreciated by authors. The existence of biotypes is only to be expected when there is no selection pressure for gliadin uniformity in breeding programmes. The extent of the non-uniformity depends on the degree of genetic linkage between gliadin pattern and the characters used in breeding. As long as the variant electrophoretic patterns are recognised and catalogued, they present no serious problems to the use of gliadin composition for cultivar identification.

2.1.7 Effect of environment

It was mentioned in the Introduction that one of the advantages of the use of biochemical methods for cultivar identification was that environmental effects could be largely eliminated. The available evidence suggests that this is certainly true for gliadin composition, and probably true for isoenzyme patterns. Thus it has been well established that the gliadin composition of a given cultivar is genetically determined and is not affected by growth conditions, fertiliser treatments, husbandry practice or storage conditions [7, 8, 23, 42, 45, 46]. Again, provided that standard conditions are used, seed and seedling isoenzyme composition is also largely independent of environmental influences [42]. Only conditions of severe sulphur deficiency, which would never occur in normal agricultural practice, have been shown to alter seed protein composition of a given cultivar is genetically determined and is not affected by growth conditions, fertiliser treatments, many morphological markers.

2.1.8 Wheat quality

A final point worth considering briefly with regard to gliadin composition concerns the possible use of electrophoresis to predict quality characteristics and morphological attributes of wheat cultivars. By the application of computer analysis, Wrigley's group has been able to demonstrate associations, not necessarily causal, between certain combinations of gliadin components and quality characters, such as dough strength, in both bread- and pasta-wheat [28, 35, 48, 49]. Various morphological features can also be predicted from cultivar gliadin patterns [48]. There is a well-established correlation between certain glutenin sub-units and bread-making quality [50]. It will thus be appreciated that electrophoresis can be of great value in breeding and testing programmes, in addition to cultivar discrimination.

2.2 Barley (*Hordeum vulgare*)

2.2.1 Analysis of hordein

Although the biochemical identification of barley cultivars has received less attention than wheat, there are now several methods available. Many of these methods are concerned with the fractionation of the barley prolamin, known as hordein. Early attempts at using lactate-SGE-type systems to analyse hordein were only partially successful [51, 52], probably due to the use of unsatisfactory extraction methods. However, Miflin's group has published a series of papers dealing with the extraction, solubilisation and analysis of hordein [53, 54] and has shown how such analysis can be used for cultivar identification [55]. Their method utilises a discontinuous SDS-PAGE system. Other systems which have been used for barley cultivar identification include variations of lactate-PAGE [56, 57] and IEF of hordein [58, 59]. Some of the main features of the various systems are described in Table 2. From the point of view of routine application, the best methods would appear to be (i) SDS-PAGE, although with an extraction schedule somewhat simplified from that originally proposed by Shewry *et al.* [55]; (ii) lactate-PAGE (see Fig. 2 and 3) which has been successfully used in both Canada and the UK, where catalogues of cultivar hordein patterns and taxonomic keys have been produced [61, 63]. The IEF methods suffer from the disadvantage of relatively high expense, although again recent work has indicated that UTLIEF can be useful for identifying barley cultivars [40] and can reduce the cost element.

Table 2. Some of the electrophoretic techniques used for barley cultivar identification

	Extraction	Type of method	Gel composition	Buffer composition	Approx. time per analysis	No. of samples per gel	Reference
1	55% Propan-2-ol + 2% mercaptoethanol, sonicated 0.5 h, 20 °C[a)]	SDS-PAGE	17.5% Acrylamide, 0.18% Bis + SDS in 125 mM Tris-borate, pH 8.9[b)]	125 mM Tris-borate, pH 8.9 + SDS	3 h	15	[55]
2	62.5 mM Tris-HCl buffer, pH 6.8 + SDS + glycerol + mercaptoethanol + dimethylformamide, 2 h, room temperature, boiled 2.5 min.	SDS-PAGE	10.0% Acrylamide, 0.08% Bis + SDS in 375 mM Tris-HCl, pH 8.8[b)]	25 mM Tris, 0.19 glycine, pH 8.8 + SDS	8 h	12	[60]
3	55% Propan-2-ol + 2% monothioglycerol, 2 h, room temperature (+ sucrose in buffer)[c)]	Lactate-PAGE	6% Acrylamide, 0.3% Bis	8.5 mM Aluminium lactate + lactic acid to pH 3.1	3 h	20	[56, 61]
4	55% Propan-2-ol + 2% mercaptoethanol 2 h, room temperature (+ sucrose in buffer)[c)]	Lactate-PAGE	9% Acrylamide, 0.45% Bis in aluminium lactate-lactic acid, pH 3.1	4.25 mM Sodium hydroxide + lactic acid to pH 3.1	0.5 h pre-run + 2 h	16	[57]
5	1.0 M Urea + 1% mercaptoethanol, 1 h room temperature	Gradient gel lactate-PAGE	Gradient of 3-27% acrylamide	4.25 mM Sodium hydroxide + lactic acid to pH 3.1	1 h pre-run + 1 h	12	[21]
6	50% Chloroethanol, room temperature	PAGE	7% Acrylamide, 0.18% Bis in acetic acid/potassium hydroxide, pH 2.9[b)]	Acetic acid/glycine, pH 4.0	3.5 h	18	[62]
7	50% Propan-2-ol + 0.3% mercaptoethanol, 2 h, room temperature, evaporated to dryness and resuspended in 6.0 M urea	IEF	6.25% Acrylamide, 0.17% Bis + carrier ampholytes, pH 5-9, + 4.5 M urea	d)	2 h	15	[58, 59]
8	1.0 M Urea + 1% mercaptoethanol, 1 h, room temperature	IEF	8.25% Acrylamide, 0.27% Bis + carrier ampholytes, pH 3.5-10 + 2 M urea	d)	3.5 h	10	[10, 21]

a) samples are dried, alkylated and reduced prior to electrophoresis
b) stacking gels are used in these methods
c) buffered sucrose added to samples prior to electrophoresis
d) not applicable to these techniques

With any of these methods, the degree of discrimination possible is not as great as with the comparable systems applied to wheat. For instance, Cooke and Cliff [63] were able to divide 68 UK barley cultivars into 19 groups on the basis of their hordein composition following lactate-PAGE. Similarly Shewry *et al.* [64] investigated 164 European barley cultivars and could divide these into 32 groups following SDS-PAGE. Using IEF, it was possible to divide 30 common French cultivars into 7 main groups, with some additional discrimination possible within each group [58]. In several cases, the use of other electrophoretic methods can further discriminate between some of the grouped cultivars. Thus IEF or urea-PAGE at pH 4.6 of hordein can subdivide some of the groups produced following SDS-PAGE [53, 55]; gradient gel lactate-PAGE or UTLIEF can discriminate between certain cultivars identical by uniform-pore lactate-PAGE [63, and R. J. Cooke, unpublished observations]. 2-D Separations, either urea-PAGE + SDS-PAGE or IEF + SDS-PAGE have also proved useful in certain cases [53, 65].

There seem to be two reasons for the slightly restricted degree of barley cultivar identification possible by electrophoresis of hordein: (i) the closely related ancestry of most modern barley cultivars; (ii) the location of the genes coding for hordein. It is now well established that the two principal types of hordein, known as hordein – B and hordein – C [53], are encoded by two separate, but linked, multigenic loci, *Hor-2* and *Hor-1* respectively, located on the short arm of chromosome 5 (see [64]). A third type of hordein, the D-type which exhibits a limited polymorphism [60] is also coded by a locus (*Hor-3*) near the centromere of the same chromosome [66]. By contrast, the genes coding for gliadins in wheat have been mapped at several loci on chromosomes 1 and 6 of genomes A, B and D [41]. The more restricted genetic basis for hordein expression leads to the occurrence of fewer polypeptide bands and a limited number of combinations of these bands. To enhance the level of discrimination between barley cultivars, the use of various combinations of electrophoretic methods (see above), or electrophoresis in conjunction with simple seed morphological characters, has been suggested [53, 55, 62, 63, 67].

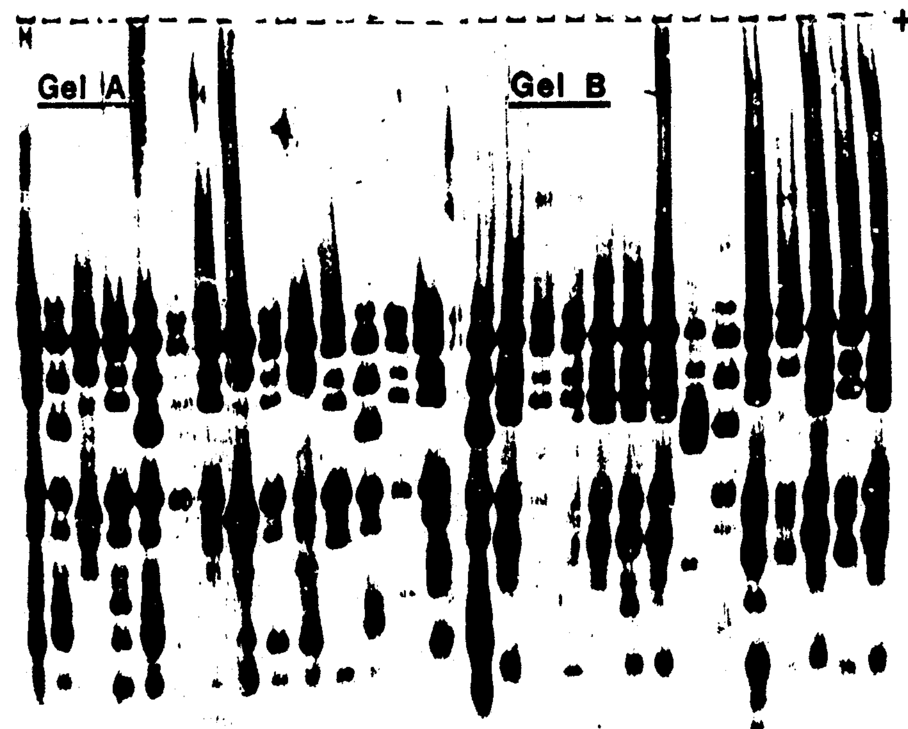

Figure 3. The hordein banding patterns of a range of spring (A) and winter (B) barley cultivars grown in the UK, following lactate-PAGE [57]. The origin and anode are at the top. Cultivars are as listed in Cooke and Cliff [57].

2.2.2 The use of isoenzymes

Several authors have reported on the usefulness of isoenzymes as an aid in barley cultivar identification. Thus Nielsen and Frydenberg [68] analysed the distribution of α-amylase and five Est isoenzymes in seeds and seedlings of 107 European barley cultivars using SGE and agar gel electrophoresis. Almgard and Landegren [69] used four isoenzyme systems (6-phosphogluconate dehydrogenase, ribonuclease, AP and Est) from seedlings and roots to classify Swedish cultivars. Both of these groups also used the response of cultivars to DDT as an additional character in their discrimination schemes. A combination of rachilla hair length and the polymorphism of six isoenzymes from seeds or seedlings can distinguish Canadian cultivars [67]. By electrophoresis in agar or starch gels, Andersen [70] was able to classify 47 European cultivars on the basis of α- and β-amylase and Est patterns. Again, the use of simple grain morphological characters was used for increased discrimination. Another combination of characters which has been evaluated involves hordein patterns, Giemsa C-banding patterns of chromosomes from root tips, Est and α-amylase isoenzymes and DDT response [71]. It is clear that no single biochemical marker will differentiate between all barley cultivars. However, a high degree of discrimination can be achieved by combining methods.

2.2.3 Barley quality

Whether or not hordein composition is linked to the suitability of cultivars for brewing and malting is a topic of some concern. The taxonomic groups revealed by electrophoresis of hordein do tend to collect together those cultivars which have good malting characteristics (see, for example, [61, 63]) but unsuitable cultivars are also included in some of these groups. Using PAGE at acid pH in 7.5 % acrylamide gels, it was claimed that the hordein-B pattern could be used as a predictive character for malting quality [72]. However, analysis by SDS-PAGE argued against this [73] and recently an extended study of 84 cultivars by the same acid PAGE technique has failed to reveal a significant correlation [74]. However, it is not yet possible to rule out a direct involvement of hordein in malting [75].

What has been conclusively demonstrated is that the resistance of barley cultivars to certain races of powdery mildew is related to hordein polypeptide composition as revealed by SDS-PAGE or PAGE at acid pH [64, 74]. This arises because of the proximity of the loci coding for certain resistances to the *Hor-1* and *Hor-2* loci. It is thus possible to predict disease resistances in cultivars on the basis of hordein composition, again indicating the potential value of electrophoresis in breeding programmes.

2.3 Oats (*Avena sativa*)

The identification of oats cultivars by electrophoresis has received some attention, although only a limited amount of information is so far available. Oats generally have lower amounts of prolamin (avenin) and larger amounts of globulin-type proteins than wheat or barley grains. Both avenin and oat globulin have been shown to demonstrate polymorphism. Thus Mosse and co-workers used lactate-SGE to separate avenin polypeptides [76] and demonstrated the potential usefulness of this technique for both species and cultivar discrimination [77]. Similar work has been carried out using PAGE at acid pH [20]. Oat globulin has been fractionated by SDS-PAGE and IEF and been shown to possess considerable heterogeneity [78]. There is thus considerable scope for using electrophoretic methods for oats cultivar identification.

2.3.1 Lactate – polyacrylamide gel electrophoresis

In addition to the foregoing studies using lactate-SGE, it has been demonstrated that oat proteins, extracted in 2.5 M urea, can be separated by gradient gel lactate-PAGE in sodium lactate buffer, pH 3.1 [10, 21, Fig. 2]. The use of wide-range gradient gels (2.5–27 %) is recommended. No details of the discrimination between cultivars that is achieved by this method are available. Work in the author's laboratory (R. J. Cooke and E. M. Cliff, manuscript in preparation) has demonstrated how a modified version of the lactate-PAGE system used for barley cultivar identification [57] can be used to differentiate between oats cultivars. Avenin, extracted from single de-husked oats grains in 25 % chloroethanol, can be separated into 13 polypeptide components by lactate-PAGE using 12 % acrylamide gels. Cultivars have different combinations of these components and 35 UK cultivars can be classified into 12 groups on the basis of the avenin composition. Further discrimination within these groups in possible using other electrophoretic methods (see below).

2.3.2 Isoelectric focusing

IEF has been demonstrated as being potentially useful for discriminating between oats cultivars and species [10]. However, the most comprehensive study of IEF for this purpose to date has been carried out in the author's laboratory. Oat seed protein was extracted in 1.0 M urea and separated on 0.1 mm thick gels containing 5 % acrylamide, 0.15 % BIS, 3 % ampholytes (pH 4–10) and 2.0 M urea. This UTLIEF method resolves the seed protein (albumin, prolamin and globulin) into at least 60 components (Fig. 4). Moreover, cultivars which are identical following lactate-PAGE analysis of avenin can often be separated and identified (R.J. Cooke and E. M. Cliff, in preparation). The use of ultrathin gels in this and other areas of cultivar characterisation will no doubt increase, as they offer considerable financial and technical advantages. The improvement in speed of analysis, the increased resolution possible because of the use of higher field strengths, the much decreased staining and destaining times and the reduced volumes of carrier ampholyte required are all very attractive features of UTLIEF [39, 40]. In addition, the gels are ideally suited for automatic evaluation by densitometric scanning, as they dry perfectly flat, do not noticeably shrink and variations in background colour and gel composition are minimised. Thus UTLIEF, plus densitometry of the protein patterns and computer-aided evaluation, storage and retrieval of the data offers a very promising avenue for cultivar identification work.

2.3.3 The use of isoenzymes

As with wheat and barley, it is possible to identify oats cultivars by investigating the distribution of isoenzymes of various types. Indeed, some of the first work describing the use of such techniques was reported using oats [79–81]. It is now clear that several isoenzymes are useful for differentiating between oats cultivars, including Est. Perox. leucine aminopeptidase (LAP), AP, alcohol dehydrogenase (ADH) and glutamate-oxaloacetate transaminase (GOT) [82–84]. Various parts of the seed and young seedling have been used as a source of the isoenzymes and either PAGE [84] or SGE [82, 83] has been used to separate them. As in all such studies, it is essential to use clearly defined tissue, grown under carefully standardised conditions, in order to obtain the best separations. Not all cultivars can be identified, but the degree of discrimination is usually very good and greater than can be achieved by comparable morphological markers [84].

2.4 Maize (*Zea mays*)

The prolamin of maize seed (zein) consists of a number of polypeptides of similar molecular weight but with considerable heterogeneity in isoelectric point [85, 86]. Thus the

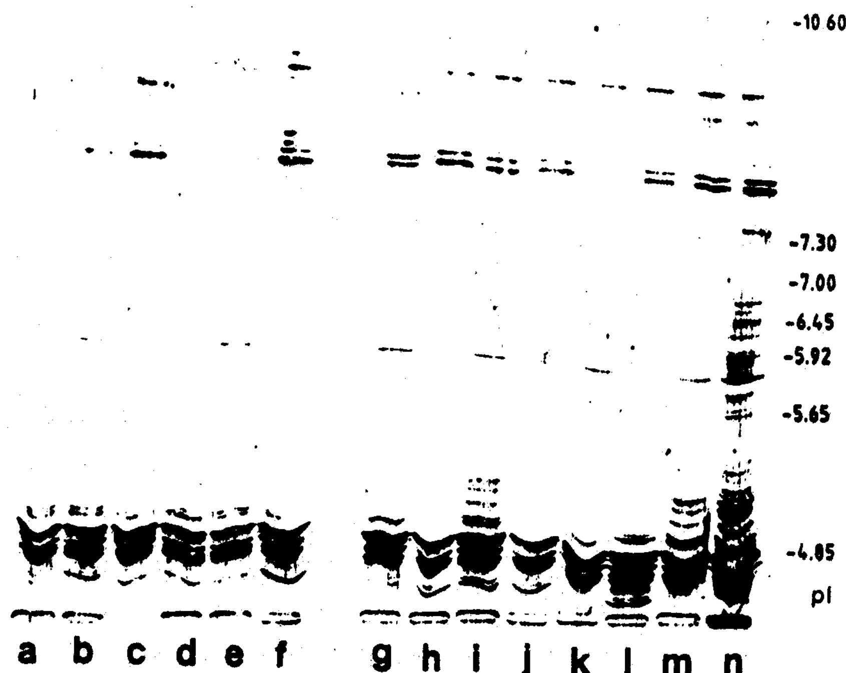

Figure 4. The protein patterns of a range of oats cultivars, following UTLIEF of the urea-soluble proteins from single seeds: (a) Stormont Sceptre, (b) Saladin, (c) Matra, (d) Fyne, (e) Bulwark, (f) Avalanche, (g) Siluria, (h) Rosette, (i) Orlando, (j) Mostyn, (k) Leanda, (l) Cabana, (m) Blyth, (n) Astor. Analysis carried out as in [40]. Unpublished data of Cooke.

favoured system for the fractionation of zein is IEF and this technique has been used to identify different genotypes of maize.

2.4.1 Isoelectric focusing of zein

When analysed by IEF in gels containing a pH gradient from 6 to 9, zein can be fractionated into 28 components, differing in their p*I* value [86, 87]. Different genotypes (cultivars, lines or hybrids) contain between 8 and 15 components and can be identified by their zein banding pattern [87, 88]. The zein IEF pattern is independent of environmental effects [86]. An important use of the zein IEF pattern is in evaluating the genetic purity of hybrid maize cultivars, as an alternative to traditional growing-on tests. By analysis of zein, it is possible to differentiate between hybrids and those seeds derived from self-pollination or from out-crossing with foreign pollen [86, 89]. The IEF pattern of various mutant stocks has been used to locate the structural genes coding for zein [86, 90]. No doubt the use of UTLIEF for zein analysis would further increase the attraction of this method for identifying maize cultivars and checking genetic purity.

2.4.2 Other methods

Stegemann [91] has reported that SDS-PAGE can be used to analyse proteins extracted from several parts of maize seeds or seedlings and that differences between maize lines can be detected. He also showed how the protein pattern could be affected by the composition of the buffer used for SDS-PAGE, emphasising the need to use several conditions of electrophoresis in order to obtain the maximum benefits from the analyses. 2-D (IEF and SDS-PAGE) separations of maize endosperm proteins have also been reported [92, 93] and could be used for cultivar discrimination in certain cases.

2.4.3 The use of isoenzymes

As with other cereals, isoenzyme polymorphism can be a useful way of distinguishing maize cultivars. Thus the use of Est and Perox patterns following PAGE or IEF has been shown to identify certain cultivars and lines [92]. Either dry seeds or young seedlings can be used. A comprehensive survey of isoenzyme variability in maize inbred lines and hybrids has recently been reported [94]. Using SGE to fractionate proteins from 5-day-old coleoptile tissue and staining for 12 isoenzymes (equivalent to 22 loci), it was possible to distinguish between 88 inbred lines and 146 hybrids of maize. Such an approach clearly has considerable potential for cultivar identification.

2.5 Rice (*Oryza sativa*)

It is perhaps surprising that the proteins of rice seeds have been studied less than any of the other major cereals. However, techniques for their extraction and characterisation have been described [95] and there are two reports of the use of electrophoresis to differentiate between rice cultivars. Park and Stegemann [96] investigated a range of extraction and analytical methods. Gradient-PAGE analysis of the water-soluble proteins (albumins) in gels ranging from 5 % to 30 % of acrylamide at pH 8.9 proved to be the best approach, with more than 25 protein components being separated and substantial differences between cultivars. Staining of such gels for Est activity was also recommended. IEF of albumins was useful, especially if Est activity was assessed and a wide-range pH gradient (2–11) used. Considerable variation in Est isoenzymes in rice has been reported previously [98]. Different gradient-PAGE and IEF methods have been shown to be useful for rice cultivar discrimination by other workers [10, 97]. Extraction of rice grains with 3.0 M urea containing 1 % mercaptoethanol was followed by analysis using gradient gel lactate-PAGE (as in [21]) or IEF at pH 4–9 in gels containing urea. There seems little reason to suppose that the methods applied to wheat, barley and oat prolamins and glutelins would not be successful for analysing proteins from rice seeds, and hence be useful for identifying cultivars.

2.6 Other cereals

The gradient gel lactate-PAGE techniques applied to wheat, barley, oats and rice can be applied to the analysis of rye (*Secale cereale*) and *Triticale* seed proteins [10, 21]. PAGE at acid pH has been reported as being useful for distinguishing Polish rye cultivars [99]. Rye seed proteins have also been separated by 2-D methods (IEF and PAGE) into about 20 components, which vary between genotypes [100]. The extraction and analysis of rye prolamin (secalin) has recently been described [101]. SDS-PAGE, lactate-PAGE and various 2-D methods were used to demonstrate the polymorphism of secalin. However, because rye is largely an outcrossing species, there is considerable variation in secalin pattern within a cultivar, limiting the value of such techniques for cultivar identification.

3 Grain legumes

In general, the seed and leaf proteins of grain leguminous species have been studied less than those of cereals. Nevertheless there is an extensive body of literature relating to the structure, synthesis and genetics of legume seed storage proteins and knowledge in these areas is increasing rapidly (see [2] for review). Briefly, the major seed proteins found in legumes are globulins (salt-soluble), which occur as large, multimeric molecules of at least two types – legumin (sedimentation coefficient 11S) and vicilin (7 S). Different species also contain various other classes of globulin. Each of these types of protein consists of a 'family' of closely related molecules displaying considerable heterogeneity in both molecular weight and charge. Examination of this polymorphism forms the basis of many of the electrophoretic methods for cultivar identification. Compared with cereals, there are relatively few reports of the direct use of seed protein profiles to characterise legume cultivars. There are, however, plenty of indications of potential applications.

3.1 Peas (*Pisum sativum*)

Heterogeneity of the globulin fraction from different lines of peas was first demonstrated using SGE at pH 8.6 [102]. This technique was also used to analyse different fractions of the seed proteins of a range of legumes [103]. The fraction assumed to contain globulins was particularly useful for distinguishing pea cultivars. This early work has been confirmed

and extended and it is now well established that there exist electrophoretically distinct sub-units of vicilin and legumin and of a third type of globulin known as convicilin. SDS-PAGE, IEF, 2-D techniques (SDS-PAGE under non-reducing and reducing conditions and IEF followed by SDS-PAGE) and various immuno-electrophoretic methods have been used to demonstrate the heterogeneity of pea seed proteins [104–108]. Different pea cultivars and lines have been shown to contain different sub-units of legumin, vicilin and convicilin. That these differences are due to genetic factors has been demonstrated and the location of the structural genes for the major globulin proteins has been determined [109, 110]. All of this evidence suggests that seed protein composition should be very useful for identifying pea cultivars. This has been confirmed recently by Cooke [111] who reported on the use of SDS-PAGE to distinguish between 25 pea cultivars (Fig. 5). More recent work (R. J. Cooke, unpublished) has shown that 45 pea cultivars can be divided into at least 20 groups on the basis of the seed protein composition revealed by SDS-PAGE. The considerable charge heterogeneity of pea globulin means that UTLIEF can also be used to distinguish between pea cultivars [40]. An exciting recent development is the application of immobilised, ultra-narrow pH gradients to separate pea proteins. It has been shown that 22 pea cultivars can be distinguished if the buffer-soluble seed proteins are analysed by IEF in a gradient of pH from 5.0 to 5.7 in the presence of 9.0 M urea [112]. Such very narrow gradients are known to give extremely high resolution and will surely prove very useful for analysing genetic variability. If such gradients can be made in ultrathin gels, the combined advantages of these two new methods could lead to some considerable advances in cultivar identification.

There are also brief reports indicating that certain isoenzyme systems can be useful for discriminating between pea cultivars. Thus, using SGE, it has been shown that the Perox, Est and LAP patterns of shoots and roots differ in some Swedish cultivars, allowing the identification of morphologically similar cultivars [113, 114].

3.2 Broad (field) beans (*Vicia faba*)

As with peas, the major storage proteins of *V. faba* seeds have also been shown to be composed of sub-units which display considerable molecular weight and charge heterogeneity [2, 115]. The variation in sub-unit composition between cultivars is due to genetic factors and is not affected by the environment, at least within the limits normally encountered in agriculture [116]. Early work using SGE indicated that several protein fractions could be useful for distinguishing between a limited number of cultivars [103]. This has been confirmed and SDS-PAGE has been used to characterise some UK *Vicia* cultivars [117]. Variation in both legumin and vicilin polypeptides was useful. This technique could also be used to check the homogeneity of batches of seed, a point of some interest since broad beans are both self- and cross-pollinating, to a variable and unpredictable degree, when grown in the field. This limits the use of SDS-PAGE of globulins for identifying field-grown bean cultivars, as there is substantial variation in sub-unit composition within a cultivar (R. J. Cooke, unpublished). SDS-PAGE of water – and buffer – soluble proteins has also been used to distinguish between certain German and Egyptian cultivars [118]. Urea-soluble seed proteins can be analysed by UTLIEF and show differences between cultivars [40]. The use of isoenzymes to differentiate between broad bean cultivars has been applied with some success. Thus the Est and Perox patterns obtained from young leaves were found to be useful for identifying 40 cultivars [119]. Esterases from seeds have also been separated by PAGE [118–120] and, in conjunction with GOT isoenzymes, provide a very useful system for genotype identification and the measurement of out-crossing [120]. The same enzymes from pollen have also been used for these purposes [121]. It is likely that a combination of simple seed morphological characters and electrophoretic examination of seed proteins or isoenzymes would provide a very discriminating scheme for *Vicia* cultivar identification.

3.3 French (common) beans (*Phaseolus vulgaris*)

The seed proteins of *P. vulgaris* have been classified into three fractions on the basis of their solubility properties: phaseolin (or G1), globulin – 2 (G2) and albumin [122]. By the use of IEF, SDS-PAGE and 2-D IEF + SDS-PAGE, it has been demonstrated that there is substantial molecular weight and charge heterogeneity, both in phaseolin [123, 124] and in the other fractions [125]. This heterogeneity has a genetic basis and can therefore be used for identifying cultivars. Early work used lactate-SGE of the water-soluble proteins to characterise 32 cultivars [126]. More recently, SDS-PAGE and 2-D IEF + SDS-PAGE have been used to map linkage relationships between genes controlling seed protein composition [127]. A reference catalogue of 107 cultivars, giving the phaseolin and G2/albumin patterns revealed by 2-D IEF + SDS-PAGE and the agglutination ratio (a measure of lectin content), has been published [125], and should allow cultivars to be identified.

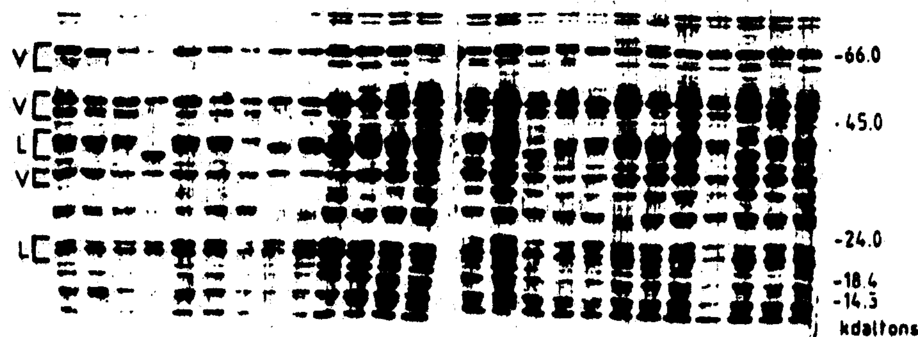

Figure 5. The protein patterns of 25 cultivars of peas, following SDS-PAGE. Analysis and list of cultivars as in Cooke [111]. V and L indicate positions of vicilin and legumin-type polypeptides.

The seed proteins of *P. coccineus* (runner beans) have been separated by UTLIEF and by an ultrathin-layer 2-D technique (UTLIEF + ultrathin-layer PAGE at pH 8.9) [128]. Different cultivars have different protein maps. Such ultrathin-layer 2-D analysis holds great promise for cultivar characterisation, increasing even further the resolving power of 2-D techniques and offering advantages of reduced price and reduced analytical time. As with other crop species the use of isoenzymes for *Phaseolus* cultivar identification has been proposed. Thus 34 major US common bean cultivars can be characterised on the basis of the Est and Perox protein patterns of young leaves, stems or roots following SGE [129]. AP activity was also useful in certain instances.

3.4 Soybeans (*Glycine max*)

Given its importance as an oil crop, it may be surprising that research on the seed proteins of soybeans and their possible use for cultivar identification has lagged behind that on other legumes. However, it is established that glycinin, the major seed protein fraction, displays a certain amount of molecular weight and charge heterogeneity in sub-unit composition in different cultivars [130]. There are reports indicating that PAGE of seed proteins, and certain isoenzymes *e.g.* AP, urease, ADH, Est and various oxidases can achieve a degree of discrimination between cultivars [131–135]. In addition, the seed protein composition, plus Perox, phenolase and trypsin inhibitor patterns following IEF has been shown to distinguish between 12 cultivars [136]. The distribution of the alleles for seed protein and for trypsin inhibitor has been reported in 447 cultivars, using non-denaturing PAGE [137]. This information could presumably be used for identifying cultivars or at least dividing them into groups. A combination of seed characteristics, such as hilum colour, and protein or isoenzyme analysis following PAGE or SGE has also been suggested for laboratory-based cultivar differentiation [138, 139]. The seed proteins of another important oil crop, peanuts or groundnuts (*Arachis hypogaea*) have been investigated by various electrophoretic procedures, including SDS-PAGE [140] and cultivar differences have been demonstrated by 2-D procedures [141].

4 Potatoes (*Solanum tuberosum*)

Potato tubers have fairly high protein levels, comparable in many instances to cereal grains. Early workers fractionated these proteins by agar or paper electrophoresis [142] or PAGE [143] and demonstrated differences in protein patterns between cultivars. This work has culminated in the compilation of an 'Index of European Potato Varieties', comprising the total tuber protein and Est patterns of over 1000 cultivars, following PAGE at pH 8.9 [144, 145]. Potato proteins have a limited molecular weight distribution, but considerable charge heterogeneity, in an analogous manner to maize storage proteins [86]. Thus IEF is also a useful way of separating potato proteins [146]. The protein patterns are genetically determined and not affected by environmental factors. Both proteins and trypsin inhibitors have been analysed by IEF and have been shown to be characteristic of cultivars [147]. Undoubtedly, UTLIEF of tuber proteins could prove to be a very useful way of identifying and classifiying potatoes. Various 2-D techniques (PAGE + IEF) have also been used to map potato proteins and to identify cultivars [148].

5 Other species

There are many other species in which electrophoresis has been used to characterise cultivars. Some useful information relating to the most well documented of these studies is given in Table 3. With several of these species, electrophoresis of seed or leaf proteins has been used for reasons other than straightforward identification. For example, much of the work on the *Brassica* crops has been directed towards devising systems for identifying siblings in hybrid cultivars, i.e. checking the genetic purity of batches of seed [152–155]. However, there is little reason to doubt that similar methods could be adapted for cultivar identification, at least in certain cases.

The work relating to *Lolium* species is a particularly well-documented example of the application of electrophoresis to cultivar characterisation in a crop other than cereals or legumes. The genetic basis for the variation in the PGI and GOT isoenzymes has been established [156, 158]. As with all out-crossing species, cultivars are identified on the basis of genotype frequencies, rather than the presence or absence of polypeptide components. Nevertheless, electrophoresis proves to be a very discriminating technique. For example, it has recently been shown that of 23 cultivars of *L. perenne* grown in the UK, it is possible to distinguish 9 from all but two or three others and 16 from all but five others, using SGE analysis of just one enzyme (PGI) [159]. Similar careful work establishing the genetic basis of the isoenzyme differences is required with other species and enzymes before electrophoresis can be used with real confidence in such situations.

In addition to the species given in Table 3, there are many reports of the use of electrophoresis to identify cultivars of various minor crops, fruits and even flowers. Thus both PAGE and IEF of proteins and various isoenzymes has been used to distinguish cultivars of grapes [168, 169] and

Table 3. Other species in which electrophoresis has been used for the characterisation of cultivars

Species	Tissue	Type of electrophoresis	System analysed	Reference
Coffea spp.	Beans	SDS-PAGE; 2D IEF + SDS-PAGE	Protein	[149]
Brassica spp.	Leaves Seeds	PAGE	PGM Protein	[150] [151]
Brassica oleracea	Seeds	SGE	PGM PGI AP	[152, 153]
Brassica oleracea	Seeds Cotyledons	PAGE	AP	[154, 155]
Lolium perenne, *Lolium multiflorum*	Leaves	SGE	PGI GOT	[156, 157] [158, 159]
Lolium perenne, *Lolium multiflorum*	Seeds	PAGE	Est, Protein	[160, 161]
Agrostis palustris	Leaves	PAGE	Protein	[162]
Poa pratensis	Leaves	PAGE	Protein, Perox.	[162, 163]
Medicago spp.	Seeds	PAGE	Protein	[164, 165]
Medicago sativa	Seeds, roots, leaves	SGE	Perox, LAP, Est, ADH	[166, 167]

strawberries [170, 171]. Perox isoenzyme patterns can identify pear cultivars [172]. Using SGE of extracts of pollen and staining for malic enzyme, Est and 14 other enzymes, Pontikis *et al.* [173] were able to distinguish cultivars of olives. PAGE of seed proteins has been used to differentiate between some *Allium* (onion) species and cultivars, Est and other isoenzymes being the most useful markers [174, 175]. Isoenzyme differences have also been reported in fescue (*Festuca arundinacea*) cultivars [176]. Separation of leaf proteins by PAGE and staining for 7 isoenzymes allows rose cultivars to be distinguished [177, 178]. The proteins of cherries, tomatoes, cucumbers and apples have also been examined by various electrophoretic procedures. This and other work concerning plant and food proteins has been reviewed recently [86].

6 Conclusions

The purpose of this article has been to demonstrate the wide range of electrophoretic techniques which have been applied to the analysis of the proteins and enzymes of different crop species and the use that is made of such techniques in identifying and classifying cultivars of these species. Clearly, no single technique can be of universal application to all crops and to all situations. The results that have been achieved with what might be called 'traditional' techniques (SGE, PAGE, IEF) are often remarkable and allow for considerable discrimination between cultivars. As further research reveals more about the genetic aspects of seed and leaf proteins and enzymes, it can be predicted that more crops will be investigated and more powerful techniques employed. Methods utilising ultrathin gels in either one-dimensional or 2-D separations [40, 128, 179] seem to offer considerable scope in this area, not only because of the decreased cost of such analysis but also because of the increased resolving power that is often attainable. IEF in narrow, immobilised gradients [112] also seems likely to prove very useful, especially as part of 2-D techniques. It has recently been shown that the seed proteins and trypsin inhibitors of several legume species and cultivars can be excellently and reproducibly resolved by a 2-D method involving IEF in an immobilised pH gradient in the first dimension and SDS-gradient-PAGE in the second [180]. Such gel systems have not been widely used to date for cultivar identification, but will surely become routine for certain purposes. More sensitive staining methods, *e.g.* the silver staining techniques, have also not yet been used in the area of cultivar characterisation, but could prove to be valuable in particular circumstances. Whatever methods are used, it is important that a standardised system for a particular species or for particular cultivars of a species is established, to facilitate collaboration between laboratories and the checking of results.

The increasing complexity of modern methods of genetic improvement in crop plants ensures that electrophoresis will be applied to an increasing extent for the purposes of distinguishing and identifying crop cultivars. The use of these powerful biochemical techniques will improve the possibility of discriminating between cultivars and will also assist plant breeders in the initial rapid screening of their new cultivars for uniformity, distinctness and certain quality characteristics. Thus, interest in and use of electrophoretic methods in agriculture is likely to expand considerably in the future.

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