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# SEED PROTEIN GEL ELECTROPHORESIS IN GENETIC RESOURCE MANAGEMENT

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## ABSTRACT

The usefulness of gel electrophoresis of seed proteins for identification and classification of species and infra-specific taxa, and the ease with which accessions of both inbreeding and outbreeding populations can be characterised using a small sample of seed of any viability, make it an ideal tool for genetic resource mangement. Examples are given of applications of this technique for correcting misidentifications, surveying variation in and assisting classification of stored germplasm, identifying duplicates and redundancies, and checking for contamination or genetic shift during seed multiplication or regeneration. It is suggested that a seed protein profile could become a standard germplasm descriptor for appropriate seed-propagated species.

## KEYWORDS

Seed bank, germplasm descriptor.

## INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) of seed proteins is now a recognised technique for identifying and characterising cultivars of crop and forage plants (Cooke 1984; Stegemann 1984; Cooke *et al.* 1984; Ferguson and Grabe 1984; Gardiner *et al.* 1986). Seed protein banding patterns have also been shown to have considerable value in taxonomic and evolutionary studies in a wide range of families (Ladizinsky and Hymowitz 1979; Vaughan 1983).

The types of seed protein involved and the value of the results vary both with the genus and with the extraction and electrophoretic techniques employed. The number and patterns of band displayed are frequently large and complex, and undoubtedly represent the expression of a considerable number of genes. Mastenbroek *et al.* (1981) point out that both SDS-PAGE (sodium dodecylsulphate-PAGE) and isoelectric focussing under denaturing conditions allow the examination of single gene products (protein sub-units) rather than intact proteins, as is the case with PAGE under non-denaturing conditions. Most work has been done with storage proteins, but enzymatic proteins have also been utilised, and it has been plausibly suggested (Φard and de Wet 1983) that the latter are likely to be more species-specific and less variable within and between populations because they are under greater selective constraint. Thus they may be potentially more useful for taxonomic studies but less useful for distinguishing cultivars and accessions of species.

Because of the value of SDS-PAGE of seed protein for identification and classification, and the fact that it can be carried out on a small sample of live or dead seed without the necessity for germinating seedlings or characterising individual genotypes, it has many potential applications in genetic resource management. This paper presents examples from research carried out by Plant Physiology Division DSIR on behalf of Grasslands Division and the N.Z. Forage Germplasm Centre, DSIR, Palmerston North.

## METHODS

Details of the SDS-PAGE techniques used at Palmerston North have been described by Gardiner *et al.* (1986). Total seed proteins are extracted, but it is assumed that storage proteins dominate the profiles produced. Excellent results have been so far obtained with several grass general including *Lolium, Festuca, Dactylis* and *Bromus.*

Most of the species investigated are outbreeding, and individual genotypes from the same population show considerable variation in the banding patterns of their seed proteins (Ferguson and Grabe 1984; Gardiner *et al.* 1986). However a bulk sample of at least fifty (usually

considerably more) seeds produces a repeatable and characteristic seed protein profile which quantitatively represents the abundance of different protein bands in the population. The number and variability of the bands is such that large numbers of different cultivars can be recognised, particularly in *Lolium* and *Festuca*.

## RESULTS AND DISCUSSION

The following are brief examples of the type of application where SDS-PAGE of seed proteins has been found useful.

**Checking identifications**

Accurate identification of stored germplasm is essential for its effective use. Many accessions received by seed banks, either from the field or from other institutions, are incorrectly named, and may be used in trials and breeding programmes or as a basis for published research before this is discovered. Either as a routine measure, or where there is reason to believe a mistake has been made, seed protein PAGE can establish the true identity of a line in a few days without the need to raise plants. For example, during a study of the genus *Lolium*, seed protein profiles were prepared for two accessions received as *L. rigidum*, four as *L. temulentum* and three as *L. remotum* (Fig. 1). One line of *L. rigidum* (lane 2) is totally different from the other and clearly resembles *L. temulentum*. This identification has subsequently been confirmed. Also evident in Fig. 1 is a clear polymorphism within *L. temulentum*, which deserves further investigation, and the close taxonomic relationship of *L. temulentum* and *L. remotum*.

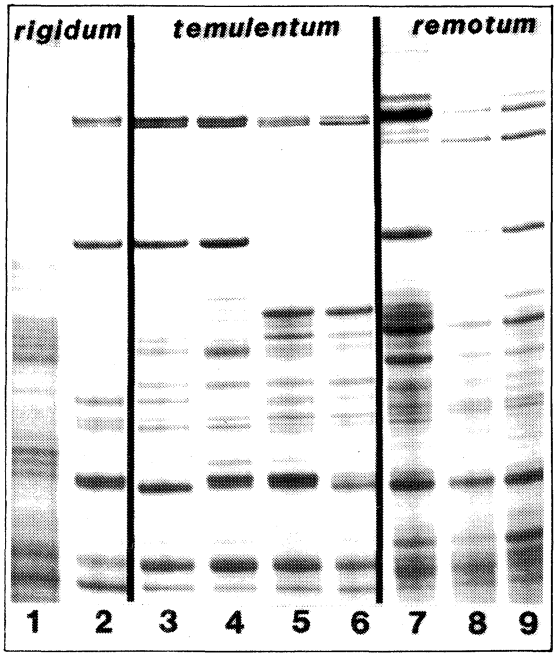



**Figure 1. Seed protein banding patterns for 9 accessions of *Lolium*, received as: 1-2 *L. rigidum*, 3-6 *L. temulentum*, 7-9 *L. remotum*.**

Mistakes can also arise during seed handling. Ellett perennial ryegrass was included as a control in a field trial of new selections. When it clearly appeared atypical in its early growth, the seed protein profile of the sample drawn for sowing was compared with that of the stored bulk, another line of Ellett, and other cultivars (Fig. 2). The sown sample (lane 4) is quite different from both lines of Ellett (lanes 2 and 3) and appears identical with R.v.P. Vigor (lane 6). Subsequent field observations confirmed this identification.

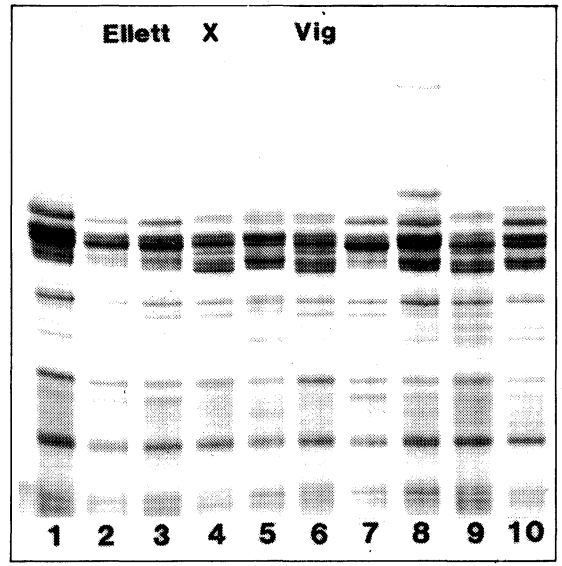



**Figure 2. Seed protein banding patterns for 10 cultivars and selections of perennial ryegrass (*Lolium perenne*): 1 Nui, 2-3 Ellett, 4 seed sown as Ellett (X), 5 Ruanui, 6 R.v.P. Vigor, 7 Ballantrae selection, 8 Droughtmaster, 9 late-flowering selection, 10 dryland selection.**

**Surveying variation in stored germplasm**

A future priority of seed bank management will be to assess and classify the variability present in collections and identify duplicate accessions and redundancies, so as to reduce the number of accessions that have to be maintained and facilitate efficient use of large collections (Frankel 1986). Such information will also assist with planning future genetic conservation strategies (Jain 1979). PAGE of seed proteins is an ideal method for outlining patterns of genetic variation. Fig. 3 shows a number of accessions from an extensive collection of *Bromus willdenowii* (*B. catharticus* auct.) made throughout New Zealand over 20 years ago. Very little variation is evident either within or

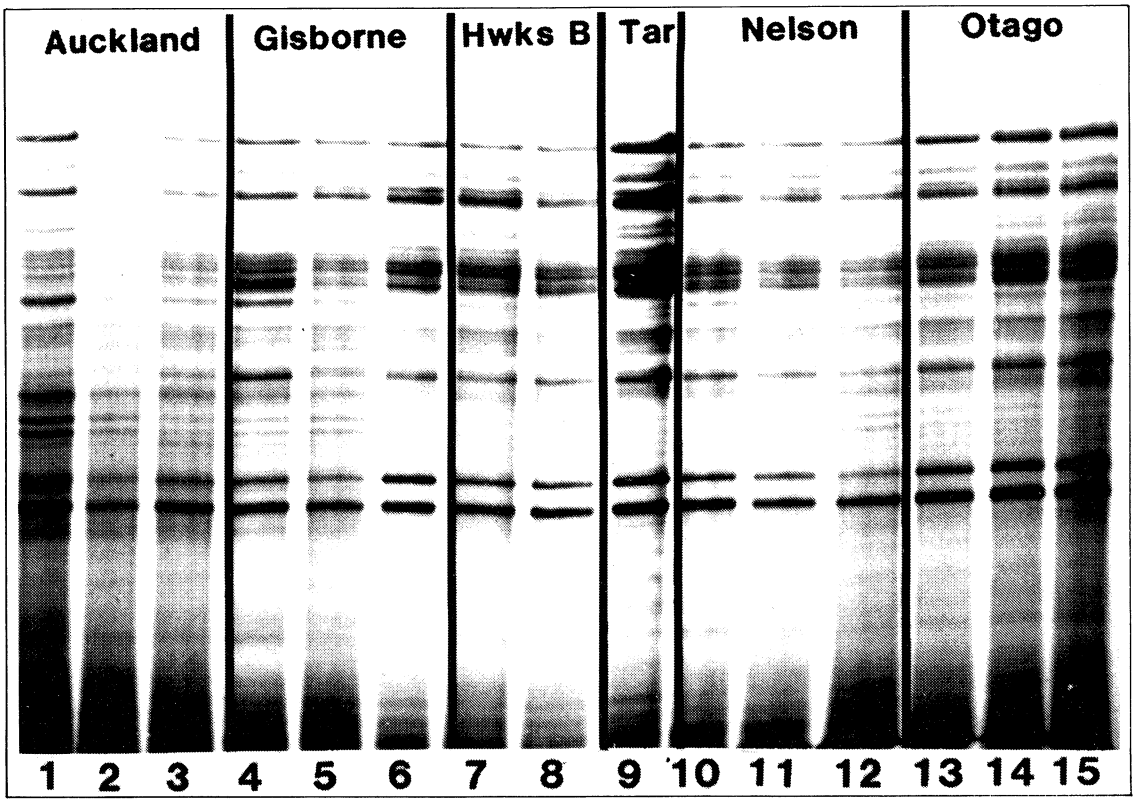



**Figure 3. Seed protein banding patterns for 15 N.Z. collections of prairie grass (*Bromus willdenowii*) from 6 districts: 1-3 Auckland, 4-6 Gisborne, 7-8 Hawke's Bay, 9 Taranaki, 10-12 Nelson, 13-15 Otago.**

between localities, indicating little need to regenerate the collection in entirety, and also suggesting that most New Zealand populations of this largely inbreeding species derive from one source. By contrast, old regional populations of perennial ryegrass (not shown) can all be differentiated by SDS-PAGE.

That the genetic relationship of populations is reflected in their seed protein banding pattern is shown by comparing profiles of some turf ryegrass cultivars (Fig. 4), where the known relationship of Barry and Arno to Manhattan is evidenced by a number of common bands. The profile of Yorktown, which includes both Manhattan and Pennfine in its ancestry (Lofts Pedigreed Seed Inc. 1980), combines the banding patterns of both these parents. It may be hypothesized that Derby, derived from plants collected from old turf stands (Pepin 1982), bears a close genetic relationship to the older cultivar Pennfine, which it resembles in being early flowering.

**Assisting taxonomic classification**

Observation of variation in and apparent relationships between banding patterns of seed proteins can assist taxonomy and biosystematics. *Bromus* sect. *Ceratochloa* contains many useful forage species, but also some unsolved taxonomic and nomenclatural problems. Fig. 5 indicates that:

- South American hexaploid species (lanes 1-9, 11) can be differentiated from North American octoploids such as *B. sitchensis* and *B. aleutensis*. The latter two accessions shown here (and also the misidentified "*B. unioloides*" cv. Una) appear to be identical, as their morphology suggests.
- *B. willdenowii* (*B. catharticus* auct.) cv. Grasslands Matua, based on South American material, is clearly distinct from the Australian Priebe and the United States Lamont, (lanes 1 and 2), which have indistinguishable banding patters, despite their supposedly different origins (Barnard 1969; Hanson 1972).
- Three South American species naturalised in New Zealand and provisionally identified as *B. stamineus*, *coloratus* and *fonkii*, (lanes 4, 6 and 8) exhibit related banding patterns. The profile of the New Zealand *B. stamineus* (lane 4) differs considerably from an

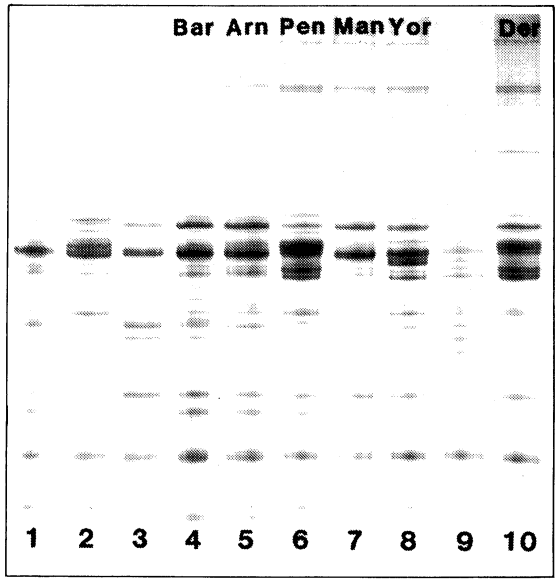
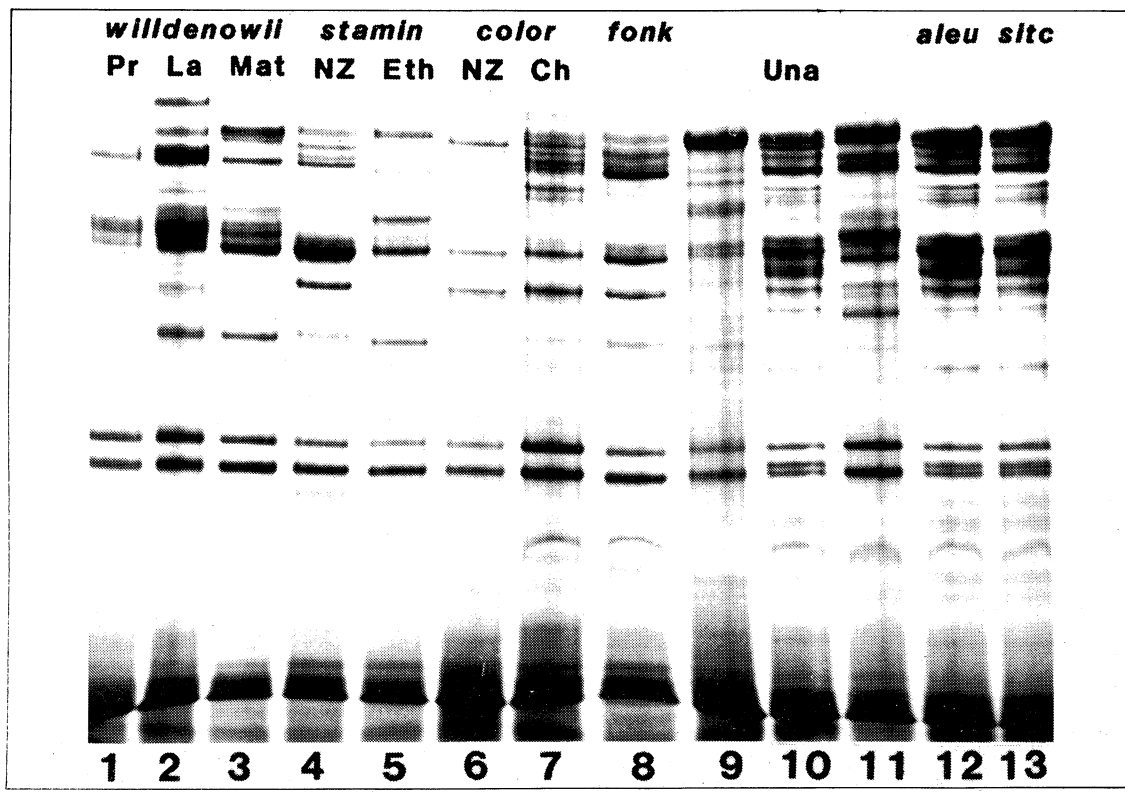



**Figure 4. Seed protein banding patterns for 10 cultivars of turf ryegrass (*Lolium perenne*) 1 Sprinter, 2 PG84F, 3 Barclay, 4 Barry, 5 Arno, 6 Pennfine, 7 Manhattan, 8 Yorktown II, 9 Repell, 10 Derby.**

Ethiopian accession of this species (lane 5), but the species identified as *B. coloratus* (lane 6) agrees reasonably well with a Chilean accession of the same name (lane 7).

As already shown in Fig. 1, SDS-PAGE can also assist with the taxonomy of *Lolium*, which contains many poorly understood species (Terrell 1968). Fig. 6 shows seed protein profiles of accessions of *L. rigidum*, *gaudinii* and *parabolicae*. From this it appears that Australian Wimmera ryegrass differs considerably from European *L. rigidum* and shows some resemblances to *L. multiflorum*, which may be an introgressant (Barnard 1972). *L. gaudinii* is

**Figure 5: Seed protein banding patterns for 13 accessions of *Bromus* sect. *Ceratochloa*: 1 *B. willdenowii* Priebe, 2 *B. willdenowii* Lamont, 3 *B. willdenowii* Grasslands Matua, 4 *B. stamineus* N.Z., 5 *B. stamineus* Ethiopia, 6 *B. coloratus* N.Z., 7 *B. coloratus* Chile, 8 *B. fonkii*, 9 *B. brevis*, 10 "*B. unioloides*" Una, 11 *B. unioloides*, 12 *B. aleutensis*, 13 *B. sitchensis*.**



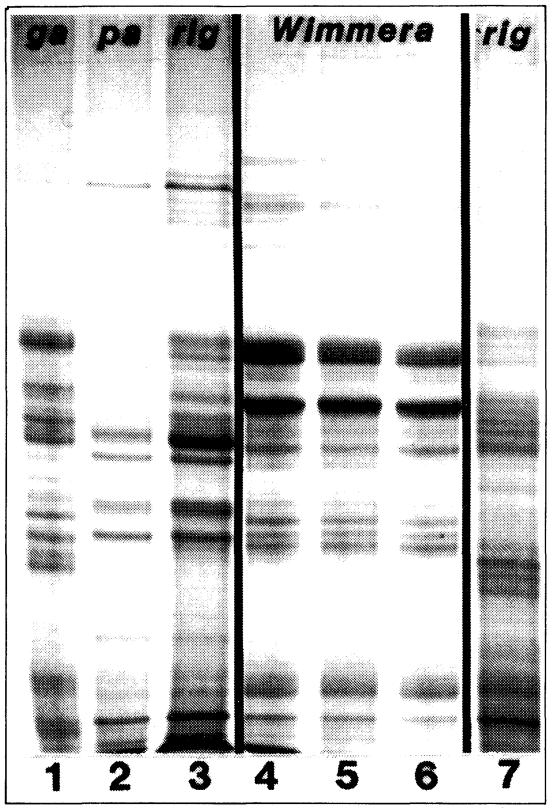



**Figure 6. Seed protein banding patterns for 7 accessions of the *Lolium rigidum* complex: 1 *L. gaudinii*, 2 *L. parabolicae*, 3 *L. rigidum*, 4-6 Wimmera ryegrass (Australia), 7 *L. rigidum*.**

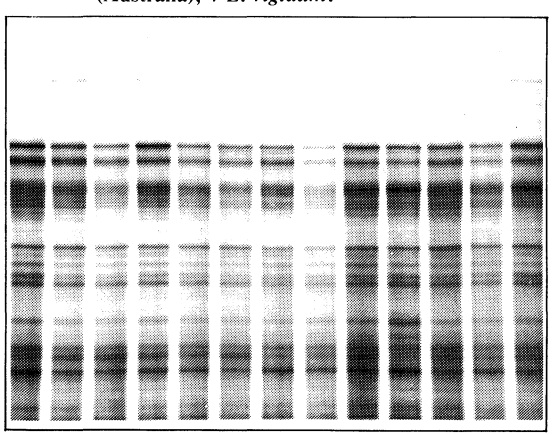


**Figure 7. Seed protein banding patterns for 13 lines of Grasslands Manawa ryegrass (*Lolium hybridum*) of varying year, site and generation.**

distinguishable from, but resembles *L. rigidum* (lane 7), however the *L. rigidum* accession in line 3 matches *L. parabolicae* (lane 2). Considerably more work is needed to determine whether seed protein banding patterns support established taxa or allow them to be subdivided.

**Monitoring genetic change**

Seed protein profiles of populations can be highly stable in different years, generations and sites (Fig. 7). SDS-PAGE is therefore well suited for detecting genetic change through contamination or selection during seed multiplication. Fig. 8 shows half-sib isolations made from seven inter-pollinated ryegrass plants on two dates eleven years apart, the plants having been vegetatively maintained in the interim. Allowing for differences in overall intensity between the two harvests, four of the pairs show a reasonable match but in the other three (plants 1, 2 and 4) the difference is so great as to suggest that the parents are no longer the same plants.

In another instance, seed from two adjacent but slightly different cocksfoot plots distinguishable by SDS-PAGE was harvested in one operation. Of the seven bags of seed dressed, four could be identified as resembling one plot and three the other, enabling them to be separated if desired.

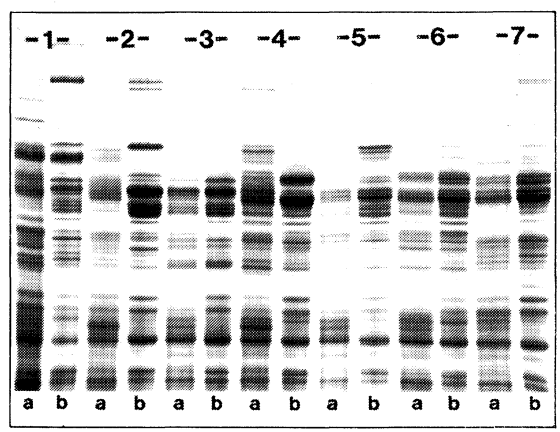



**Figure 8. Seed protein banding patterns of repeat isolations from 7 ryegrass plants, taken in 1966 and 1977 repsectively.**

## CONCLUSIONS

Because of the value of these applications, a PAGE facility is a recommended adjunct to a seed bank, and a seed protein profile may beome a routine descriptor for stored accessios of appropriate seed-propagated species, just as isozymes are already being used to characterise accessions in the world potato collection (International Potato Centre, 1984 p. 58). It may also become routine to use this technique to check for genetic change during regeneration of stored germplasm. In this connection it should be noted that even old inviable seed of valuable

material may be worth retaining for future checks on the genetic constitution of progeny lines, as evidenced by their seed protein banding patterns.

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## SYMPOSIUM DISCUSSION

Dr R. Burdon, Forest Research Institute

How does this technique of acrylamide gel electrophoresis work with vegetative material.

Gardner

I think the problems are likely to be more difficult with vegetative material because there will be a greater influence of environmental conditions, but it may be possible.

Dr W. Bushuk, University of Manitoba

Our experience was that it was much easier to differentiate cultivars using isozymes rather than storage protein. We obtained good patterns for any tissue, root tissue or stem tissue in inbreeding species.

You made a comment that you thought each of the bands was the product of a single gene. Have you confirmed this using 2-dimensional procedures?

Forde

No, I haven't confirmed this. It is something we could look into but for our purposes we were using it as a blind set of fingerprints and it seemed to be quite adequate.

Sir Otto Frankel, CSIRO

How does the enzyme pattern in which you find diversity or similarity relate to diversity on the plant?

Forde

We are working on cultivars of which we known the breeding history and we fell there is very good correspondence.