USING ELECTROPHORETIC TECHNIQUES IN VARIETAL IDENTIFICATION, BIOSYSTEMATIC ANALYSIS, PHYLOGENETIC RELATIONS AND GENETIC RESOURCES MANAGEMENT

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SUMMARY: Personal experience gained with Lotus, Amaranthus, Medicago, and Vicia species; and flax and cotton cultivars led to conclusion that polyacrylamide gel techniques are a valuable tool to identify species and cultivars. This identification is very important for plant breeders, certification authorities and also in genetic resources management. Polyacrylamide gel techniques allow us to: 1) identify variation among the taxa of each species; 2) screen the purity of the ever expanding number of cultivars; 3) verify whether or not two or more morphologically identical accession in the collection were also electrophoretically identical; and 4) exploit the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield.

Key Words: Electrophoresis, phylogenesis, genetic resources.

INTRODUCTION

Early, distinctness, uniformity and stability (DUS) of any cultivar have relied on morphological methods, which are subjective and which may be influenced by environmental conditions (6). However the morphological makers were not quite enough to expose the genetic diversity between the morphological overlap cultivars and the morphological identical accessions (4). The need, therefore, for new tool was disparate. The advent of the electrophoretical as an analytical tool provide an indirect methods for genome probing by exposing structural variations in enzymes or other protein genome (3, 5). Electrophoretic makers appear to be due to neutral genes which are not linked to any loci that affect the cultivar and value (5). They are also independent of cultivar morphology and physiology, and offer significant advantages over morphological methods of variety and/or species identification in that they are rapid, relatively cheap, eliminate the need to grow plants to maturity and are largely unaffected by the growth environment. The biochemical methods have some disadvantages e.g. that they are profoundly influenced by tissue specificity and developmental stage. This disadvantage can be overcome by using the electrophoretic markers of a conservative proteins e.g. seed storage proteins. Recently, the number of available markers in plants has increased dramatically with the use of molecular biology techniques, with these techniques, it is now possible to identify variation at the DNA level which may not be expressed as differenced in visible or protein phenotype. However, using these techniques need enough capital outlay which is not available for most of the scientists in the developing countries.

In our laboratory we use the electrophoretic markers of the seed storage proteins to: 1) identify between cultivars, 2) to check species identification, 3) to assist biosystematic analysis, 4) to study phylogenetic relationships of the species, and 5) to generate pertinent information to complement evaluation and passport data and thereby increase the knowledge of the genetic diversity of the materials in the germplasm collections.

Polyacrylamide Gel Methodology

Sample preparation

Seed meals were routinely prepared by grinding ungerminated seed sample (in a Jank and Kunkel water-cooled mill).

In our study, total protein extracts were prepared by extracting appropriate protein of the seed meals with 0.2 M Tris /HCl, pH 6.8 distilled water, and 0.125M Tris/borate, pH 8.9. All these extracts were carried for 24 h at 4°C and then centrifuged at 10.000 rpm for 20 min. The supernatant was used for electrophoresis.

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Electrophoresis

The method for discontinuous PAGE techniques were based on that of Laemmli (8). For cultivar identification, we apply different gel techniques. One will notice that every technique is differently suitable for differentiation.

Staining

The staining for proteins was done with Commassie Blue R250. A mixture of acetic acid: methanol: water (3:17:20) was used for destaining.

Advantages and drawbacks of the electrophoretic approach in biosystematic analysis cultivar identification, species relationships, and germplasm management

Electrophoretic markers like other genetic markers have a number of advantages and some drawbacks. However, to asses the genetic diversity between the cultivar of a given species, a combined method of morphological, electrophoretic, cytological, and RFLPS (Restriction Fragment Length Polymorphism) are recommended. In our study, especially on the accession of *Lotus, Amaranthus*, and *Medicago*, we used morphological, cytological, and electrophoretic markers to study species relationships, to study some taxonomical problems and to complete passport data of recorded samples. In this report we are concerned with the electrophoretic markers which have some advantages over other genetic markers. We can conclude these advantages in that 1) The universal distribution of the proteins so that there is no theoretical limits to using the electrophoretic markers, 2) the electrophoretic markers are less effective to the environmental fluctuations, 3) they are too proximity to the primary genetic information (third hand copy of DNA), 4) the discriminatory power of electrophoresis is usually very high and distinction between genotype can often be achieved with less effort and with the analysis of fewer individuals than the morphologically based system, 5) the operating costs are relatively low.

Despite all these advantages, there are some drawbacks. The main drawbacks are 1) the need for international system for band nomenclature, especially for the seed proteins of the plants which are not fully characterized, 2) electrophoretic markers are profoundly influenced by tissue specificity, 3) electrophoretic techniques require technically complement and trained laboratory staff, particularly for gel evaluation.

Examples of using polyacrylamide techniques in cultivar and species identification, and phylogenetic relationship of the species

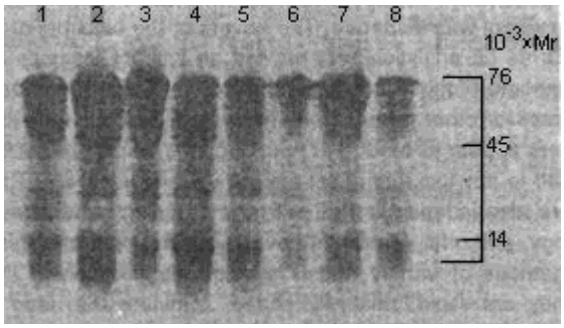


Figure 1: SDS-PAGE of the total seed proteins of flax cultivars extracted with 0.125 M Tris/borate buffer, pH 8.9. 1. Viking, 2. Fany, 3. Ariana, 4. Giza5, 5. Giza6, 6. Lidgate, 7. Atlanta, 8. Antarese (10).

1. Cultivar identification

The way in which electrophoresis is utilized for varietal identification varies from species to species. However, two principle approaches can be recognized.

A. The direct, multi-locus approach. This involve the electrophoretic examination of protein display as substantial degree of molecular polymorphism. The analysis of seed storage protein is a good example of this approach.

B. The indirect, single locus approach. This involves the utilization of protein which, despite polymorphism, are derived from a single locus. Such proteins will generally be enzymes and are known as isozymes (or allozymes).

In our laboratory, we use the former approach. Since each electrophoretic technique exposed some of the physical traits of the proteins, therefore, we use the different electrophoretic techniques (PAGE, SDS-PAGE, Porosity-PAGE, IEF and mapping) in cultivar identification. It was also reported by Stegmann (13) and Sammour

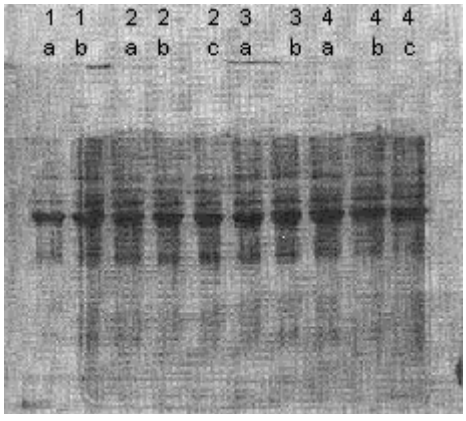


Figure 2: SDS-PAGE of the total seed proteins of Vicia cultivars extracted with 0.2 M Tris/HCl buffer, pH 6.8. 1. Giza3, 2. Rome, 3. Giza402, 4. Giza2, R.Sammour (Unpublished data)

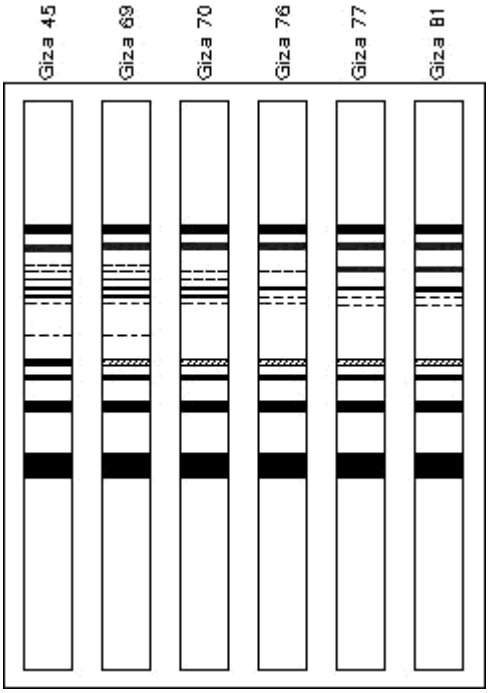


Figure 3: Total seed proteins extracts of cotton cultivars extracted with 0.2 M Tris/HCl, pH 6.8 and analyzed on SDS-PAGE under reducing conditions. R. Sammour (Unpublished data)

(10) that using buffer ion that interfere with carbohydrates, like the borate ion, governs part of the migration and one can obtain a typical shift of the pattern by changing the buffer.

In all of the cultivar studied, as in other out breeding species the distinctness of a cultivar is a statistical feature of the cultivar as a whole and not the property of single individuals. When morphological or agronomic characters are considered distinctness is estimated by growing large numbers of plants and characterizing populations using the mean values of characters. A similar approach can be employed for electrophoretic analysis by extracting a bulk sample of seeds rather than individuals. Water, Tris/borate extracts of flax, cotton, and Vicia seed storage proteins were analyzed on different electrophoretic techniques to take benefit of the parameters molecular size and molecular shape and charge. For flax, for example, analysis of the seed meals extracted with Tris/HCl on porosity or PH-gradient, SDS-PAGE shows (10, 13); that the cultivar identification is almost impossible, whereas the analysis of the seed storage proteins extracted with Tris/borate is the better suited method for identification (Figure 1). SDS-PAGE under reducing conditions were used very successfully for Vicia and cotton cultivars iden-

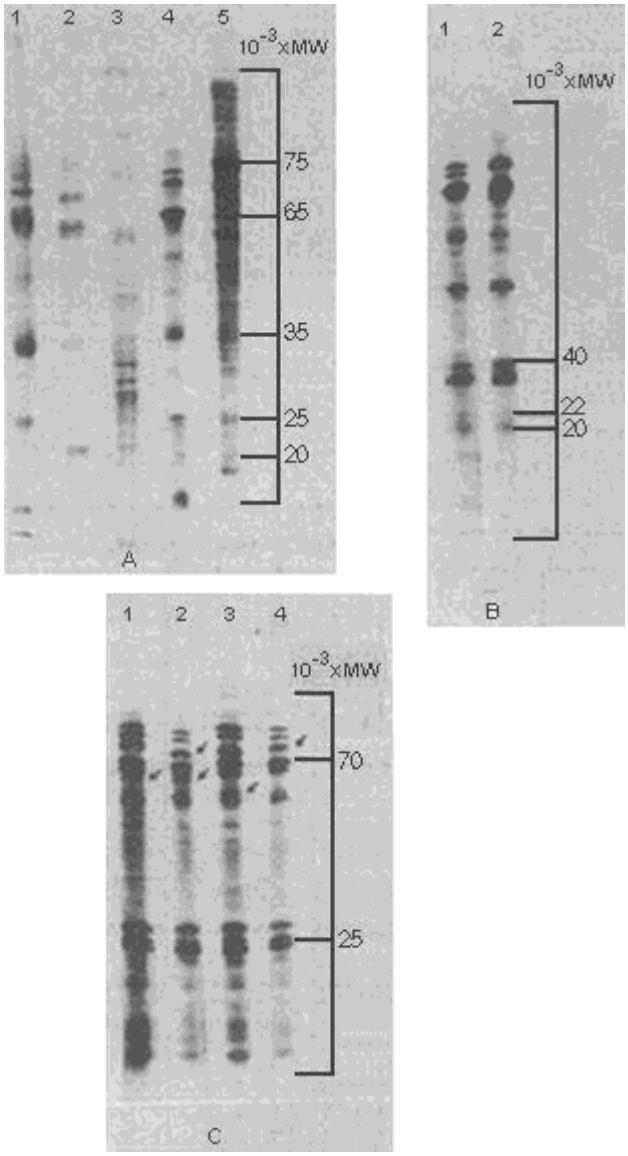


Figure 4: SDS-PAGE of the total seed proteins of the taxa of Lotus spp. collected from Egyptian flora A. 1. L. creticus, 2. L. polyphillus, 3. L. peregrinus, 4. L. halophilus, 5. L. corniculatus (7).

tification (Figures 2 and 3). It must be borne in mind that applying the mapping technique (IEF in first dimension and SDS-PAGE in second dimension) prove to be suitable in all cases (13). However, difficulties are encountered in PAGIF when the sample contain complex acids in high concentrations, like oxalic acid or phytic acid (10,13). Dialysis against arginine in urea is recommended.

2. Species identification

A. Lotus species

34 taxa of six species of lotus were morphologically,

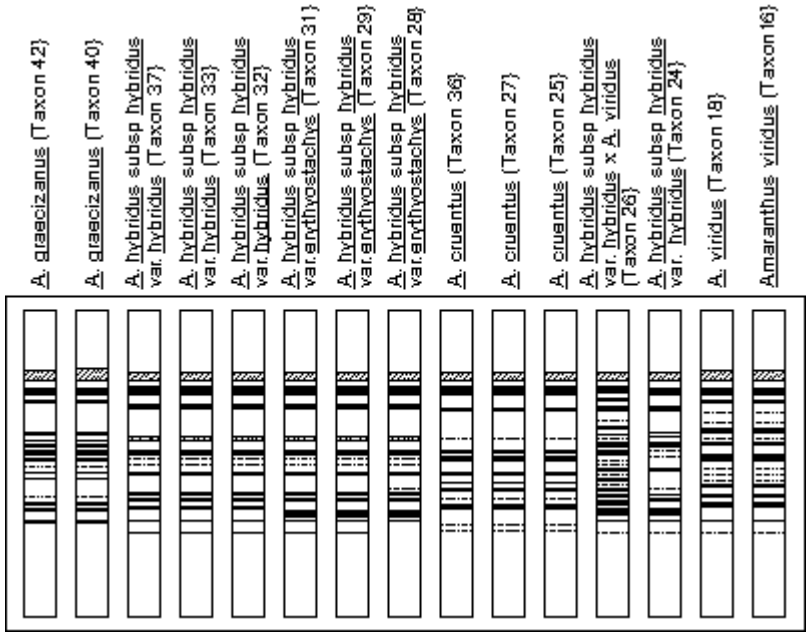


Figure 5: SDS-PAGE of the total seed proteins of *Amaranthus* spp. subspecies, and varieties extracted with 0.2 M Tris/HCI pH 6.8 (1).

cytologically and electrophoretically studied. The six species were better differentiated on SDS-PAGE (Figure 4a). Electrophoretic analysis proved the presence of two cultivars of *L. corniculatus* (Firgure 4b) and 4 cultivars of *L. halophilus* (Figure 4c). These cultivars were morphologically, cytologically and electrophoretically different (7). This study was very useful in solving the taxonomical problems within this genus in Egypt.

B. Amaranthus species

Soluble extracts of seed storage proteins of 44 taxa of *Amaranthus spp.* were analyzed on SDS-PAGE under reducing conditions (1). Figure 5 shows that *Amaranthus* taxa can be divided into two groups. Group one with basic chromosome number x=17 and group two with basic chromosome number x=16. It is very evident the relation between the chromosome number and the electrophoretic pattern. The taxon No. 26 of *A. hybridus* showed a banding pattern with a combination of feature from *A. hybridus* and *A. viridus*. This taxon indicated the possibility of hybridization between *A. hybridus* and *A. viridus*. This possibility was in consistent with the suggestion of Sauer (11) who postulated a free and intercrossing between both cultivars and weedy species of *Amaranthus*. The data also confirm the separation of *A. cruentus* from *A. hybridus* and *A. sylvestris* and *A. sylvestris* from *A. graecizans*.

C. Medicago species

Seed extracts of 96 taxa of 16 species of Medicago were analyzed on SDS-PAGE. Figure 6 depicted that each species has a characteristic profile of heavily staining invariable bands, and in addition bands which show variation between cultivars. The variable bands for cultivar is denoted with arrows. Electrophoretic similarity between *M. intertexta* and *M. arandesis* confirmed the classification of *M. intertexta* (2). The data also proposed the classification of *M. cillaris* as a variety under *M. intertexta*.

3. Phylogenetic relationships of Vicia species

Electrophoretic patterns of total seed proteins analyzed on polyacrylamide gel electrophoresis (Figure 7) was found to be diagnostic traits of the Vicia species (9). Index matrix of similarity coefficient (s) between pairs of different electrophoregrams of seed proteins subunit in 17 species of Vicia (Table 1) indicate the close similarity in the seed protein profiles of the cultivated and *Vicia narbonensis subsp. narbonensis* which was suggested to be the immediate progenitors of *V. faba*. study also indicated that *V. serratifolia* should be included as a subspecies of *V. narbonensis*.

CONCLUSION

Although electrophoretic analysis of seed storage proteins in now widely recognized as a technique for cultivar identification in the breeding species, PAGE of seed pro-

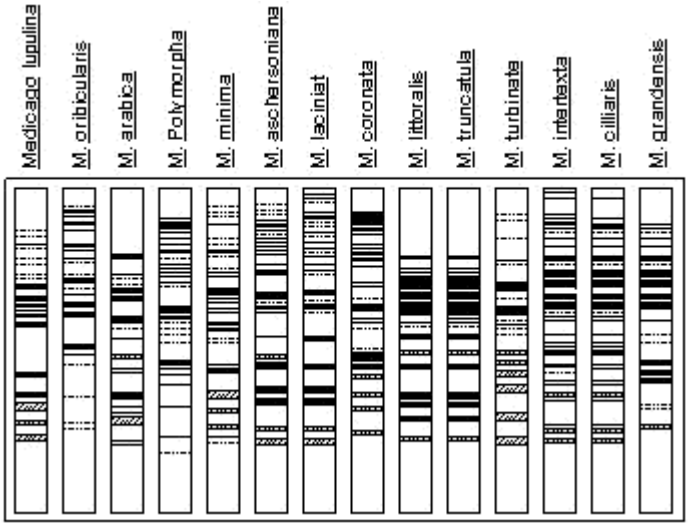


Figure 6: SDS-PAGE of the total seed proteins of Medicago spp. extracted with 0.2 M Tris/HCl pH 6.8 buffer.

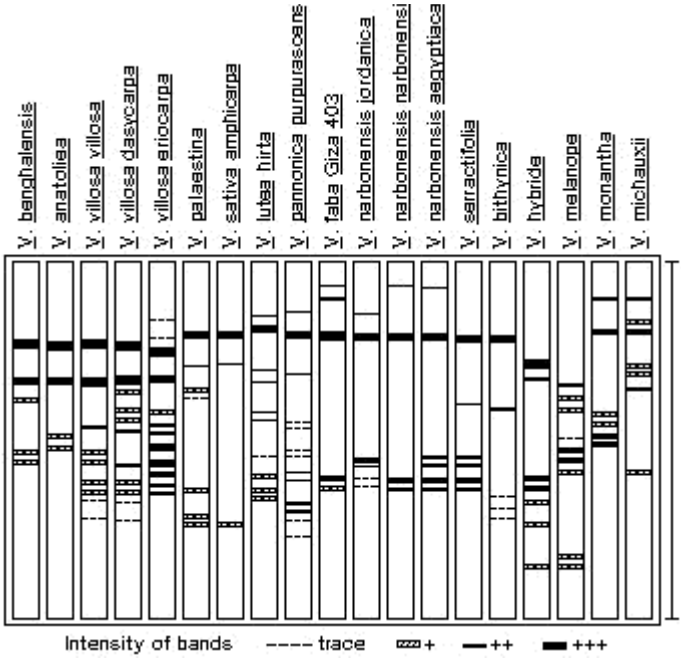
teins has been used on a limited scale for the identification of cultivar of other species (3,4). As shown in the present study, this technique might be used on large scale to include the largely out breeding species. However, in cultivar identification, it has been recommended to use more than one polyacrylamide gel technique to expose the minor genetic variation between the cultivars of a specific crop. This technique has been successfully used a number of time to check pedigrees and also to test for genetic shift after seed multiplication and re-isolation have proved an ideal for checking species identifications and identify duplicate accessions. They can also assist biosystematic analysis, as for example in the study of *Lotus* spp (7). Another group where the technique is being used to assist classification is *Amaranthus* spp (1).

SDS-PAGE of proteins from bulked seed samples thus join isoenzyme electrophoresis as another new tool for varietal identification.

Table 1: Matrix of similarity coefficients (CS) between pairs of different electrophoregrams of seed protein subunits in 17 species of Vicia.

	V. benghalensis	V. anatoliaa	V. villosa villosa	V. villosa eriocarpa	V. palaestina	V. sativa amphicarpa	V. lutea hirta	V. pannonica purpurascens	V. faba Giza 403	V. narbonensis Jordanica	V. narbonensis narbonensis	V. narbonensis aegyptiaca	V. serratifolia	V. bithynica	V. hybrida	V. melanops	V. michauxii
V. benghalensis	1																
V. anatoliea	0.41	1															
V. villosa villosa	0.27	0.28	1														
V. villosa eriocarpa	0.27	0.33	0.55	1													
V. palaestina	0.31	0.38	0.27	0.38	1												
V. sativa amphicarpa	0.51	0.52	0.44	0.50	0.50	1											
V. lutea hirta	0.20	0.23	0.22	0.33	0.41	0.09	1										
V. pannonica purpurascens	0.48	0.57	0.33	0.33	0.41	0.19	0.23	1									
V. faba Giza 403	0.43	0.44	0.48	0.49	0.45	0.50	0.46	0.28	1								
V. narbonensis Jordanica	0.51	0.38	0.27	0.38	0.41	0.48	0.30	0.38	0.60	1							
V. narbonensis narbonensis	0.44	0.38	0.33	0.44	0.50	0.38	0.46	0.47	0.70	0.63	1						
V. narbonensis aegyptiaca	0.44	0.52	0.44	0.44	0.33	0.45	0.46	0.47	8.66	0.77	0.86	1					
V. serratifolia	0.44	0.47	0.55	0.77	0.66	0.48	0.53	0.42	0.62	0.68	0.59	0.66	1				
V. bithynica	0.27	0.14	0.33	0.33	0.25	0.19	0.15	0.14	0.23	0.27	0.22	0.20	0.24	1			
V. hybrida	0.24	0.42	0.33	0.38	0.50	0.29	0.38	0.38	0.16	0.31	0.22	0.29	0.32	0.63	1		
V. melanops	0.31	0.42	0.50	0.44	0.41	0.29	0.38	0.33	0.36	0.36	0.22	0.41	0.52	0.45	0.58	1	
V. michauxii	0.17	0.90	0.22	0.05	0.41	0.03	0.23	0.33	0.13	0.09	0.17	0.08	0.22	0.04	0.12	0.27	1

Figure 7: Electrophoretic patterns of the total seed proteins of Vicia species analysed by 10% polyacrylamide gel electrophoresis (9).



REFERENCES

1. Abdallah SA: Biosystematic study on some species of the genus Amaranthus L. in Egypt. M Sc Thesis. Tanta University, Tanta, Egypt, 1990.

2. Classen D, Nozzolillo C, Small E : A phenolic-taxonomic study of Medicago (Leguminosae). Can J Bot 60:2477-2495, 1982.

3. Cooke RJ : The characterization and identification of crop cultivars by electrophoresis. Electrophoresis 5:59-72, 1984.

4. Gardiner SE, Forde MB : Identification of cultivars and species of pasture legumes by sodium dodecylsulphate polyacrylamide gel electrophoresis of seed protein. Plant Varieties and Seeds, 1:13-26, 1988.

5. Gilliland TJ : Electrophoresis of sexually and vegetatively propagated cultivars of allogamous species. Plant Varieties and Seeds, 2:15-25, 1989.

6. Goodrich WJ, Cooke RJ, Morgan AG : The application of electrophoresis to the characterization of cultivars of Vicia faba L. FABIS Newsletter, 13:8, 1985.

7. Haidar AS : Phenetic relationships of some selected species of genus Lotus L. M Sc thesis, Tanta University, 1987.

8. Laemmli UK : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685, 1970.

9. Sammour RH : Seed protein electrophoresis of Vicia faba L. and their immediate progenitors, a reappraisal. Plant Breeding 101:176-181, 1990.

10. Sammour RH : Flax seed proteins: Comparison by various PAGE-techniques in slabs. Journal of Agronomy and Crop Science 160:271-276, 1988.

11. Sauer JD : The grain amaranthus and their relatives: a revised taxonomic and geographic survey. Ann Mo Bot Gard 54: 103-137, 1982.

12. Stegmann H : Retrospect on 25 years of cultivar identification by protein patterns and prospects for future. In : Biochemical Tests for cultivar Identification, Draper SR & Cooke RJ (eds) (1984), pp 20-31. The International Seed Testing Association, Zurich, Switzerland, 1983.

13. Stegmann H : Discrimination among intraspecific taxa using electrophoretic data. In: Proteins and Nucleic Acids in Plant Systematics (eds U Jensen and DE Fairbrothers), (1983), pp 124-128, Springer-Verlag, Berlin, 1983.

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