Pertanika J. Trop. Agric. Sci. 21(2): 113 - 122 (1998)

ISSN: 1511-3701

Variability in Eggplant (*Solanum melongena* L.) and Its Nearest Wild Species as Revealed by Polyacrylamide Gel Electrophoresis of Seed Protein

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Keywords: ***Solanum melongena*** **L., SDS-PAGE, seed protein, variability**

ABSTRAK

Satu kajian telah dilakukan untuk menentukan dan membezakan genotip-genotip terong (Solanum melongena L.) dan spesis-spesis liar terdekatnya dengan elektroforesis gel poliakrilamid natrium dodesilsulfat (SDS-PAGE) keatas protin biji boleh larut. Lima puloh empat aksesi kumpulan-kumpulan tanaman dan spesis liar terong telah digunakan untuk analisis. Hasil dari corak penjaluran elektroforetik menunjukan bahawa terdapat satu variasi yang besar diantara dan didalam kumpulan-kumpulan terong dari segi bilangan, saiz, kedudukan, keamatan warna dan hadir atau tak hadir jalur-jalur protin didalam profil, yang mana boleh digunakan untuk pencaman dan pencirian kultivar-kultivar terong. Kultivar terong jenis buah panjang telah menunjukkan banyak jalur-jalur khusus-sepunya yang memisahkanya dari kumpulan-kumpulan lain. Manakala, satu kevariabelan besar profil protin biji terdapat didalam kultivar-kultivar jenis buah bulat dan primitif dan kumpulan-kumpulan rumpai dan liar terong, termasuk yang paling liar dari S. incanum, yang menunjukan bahawa kumpulan-kumpulan ini mengandungi pelbagai genotip yang berbeza.

ABSTRACT

A study was undertaken to determine and distinguish the genotypes of eggplant (Solanum melongena L.) and its nearest wild species by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of soluble seed proteins. Fifty-four accessions of cultivated and wild groups of eggplant were subjected to the analysis. The results of electrophoretic banding patterns show that there was a great deal of variation within and between groups of eggplant in terms of numbers, sizes, positions, staining intensities and presence or absence of protein bands in the profile, which can be used for characterization and identification of eggplant cultivars. Elongated fruit eggplant cultivars exhibited nine common group-specific bands which distinguish them from the other groups. Meanwhile, a great variability in seed protein profiles was detected in the round fruit type and primitive cultivars, weedy and wild groups of eggplant including the truly wild S. incanum, which indicated that these groups consist of diverse genotypes.

INTRODUCTION

Solanum melongena L. (eggplant) has a wide range of morphological diversity ranging from wild and weedy to semi- or fully-cultivated forms. Many characters are overlapping from one group to another group, which makes it difficult to distinguish between and within groups (Lester and Hasan 1991; Karihaloo and Rai 1995). Horticulturally, it is important to be able to recognize and distinguish the different genotypes and, in particular, different cultivars of this species because the cultivars often differ in their fruit quality and other important agronomic characters which are obscure and difficult to detect.

Morphological and physiological characters have traditionally been used for the identification

of eggplant cultivars (Brezhnev 1958). Many of these characters, particularly leaf and stem pigmentation and fruit size, shape and colour, which are often important in the definition of an eggplant cultivar, can be discerned only in adult plants. Thus, it is necessary to grow out plants, which is very laborious and time-consuming. Furthermore, the phenotypic characters are strongly influenced by climatic factors and soil conditions, and their use may lead to unreliable or erroneous determinations.

These problems can be avoided by using seed storage protein electrophoresis because protein banding patterns produced through this technique are generally unaffected by external factors and the genotype of each cultivar can be rapidly determined. The seed storage proteins are also known to be highly polymorphic with respect to their size and charge in almost all species investigated (Cooke 1984). Hence, they provide sufficient variability in protein composition to detect differences between genotypes. Furthermore, the seed protein profiles are known for their stability, uniformity and additive nature (Smith 1976). For these reasons, SDS-PAGE of seed proteins is used as a method for cultivar identification in many cultivated species (Sathaiah and Reddy 1985; Gupta and Robbelen 1986; Gardiner and Forde 1988; Huaman and Stegemann 1989; Rao *et al* 1990; Stegemann *et. al* 1992; Yupsanis *et. al* 1992; Wang *et. al* 1994). However, this method has not been widely used for the identification of cultivars and varieties of many vegetables, particularly the eggplant.

To date, seed protein SDS-PAGE of *S. melongena* had been conducted as part of a taxonomic study of the genus *Solanum* (Edmonds and Glidewell 1977; Pearce and Lester 1979) but there was no serious attempt to determine differences between cultivars of eggplant on the basis of protein profiles. The objectives of this study were to determine seed protein banding patterns for 54 accessions of eggplants and to ascertain the level of protein variation present in the species as well as to determine the possibility of using SDS-PAGE of seed proteins for cultivar identification and characterization in eggplants.

MATERIAL AND METHODS

Seed Materials

The seeds of 54 accessions of eggplant complex representing a range of geographical origins and morphological varieties were used (Table 1). They were grouped as i) most advanced cultivars with elongated large fruit (fruit length three times width), ii) advanced cultivars with round fruit (fruit either round, obovate or pear-shape) with diameter greater than 5 cm, iii) primitive cultivars with small fruit (less than 5 cm diameter), iv) prickly plant of weedy (*S. cumingii* Dun.) or wild (*S. insanum* L.) groups and v) the truly wild relative of *S. incanum* L. group (Lester and Hasan 1990).

Preparation of Seed Flour

Dry seeds (0.5 g) were dehulled using a micro Feinmuhle-Culatti sample mill, followed by separation through a Hearson's blower to obtain clean seeds. The seeds were ground in a micro disembrator for 10 - 15 min to produce very fine seed flour, which was then defatted in five changes of diethyl ether and allowed to dry on filter paper.

Protein Extraction

Soluble proteins were extracted by soaking 0.05 g of defatted protein flour in 0.5 ml H_2O in a centrifuge vial. The suspension was centrifuged and 100 µl of protein solution (supernatant) was incubated with 100 µl Tris-HCl buffer (pH = 6.8) consisting of 20% glycerol and 5 mM mercaptoethanol. 0.1 ml of bromophenol blue (W/V) was added into the samples as a tracking dye in order to mark the moving protein front.

Gel Preparation

The crude protein was fractionated by the method of SDS-discontinuous polyacrylamide slab gel electrophoresis (SDS-PAGE) in a vertical format in a Studier-type slab gel apparatus. The separations were performed on 2.5% stacking gel and 12.5% separation gel consisting of 10% sodium dodecyl sulphate (SDS) and Tris-HCl at pH of 6.8 and 8.8 respectively. Fifteen wells were made in the stacking gel by inserting a comb into the gel before polymerization. Ammonium persulphate (10%) was used to initiate the polymerization and N,N,N'N' tetramethylethylenediamine (TEMED) was used as a catalyst (Hames and Rickwood 1986).

Electrophoresis

The 20-µl sample solution of each accession was loaded in the well. Sample solutions of two

TABLE 1
Accessions of Solanum melongena and its nearest wild species used in the study arranged according to their cultivar groups and electrophoresis run of different gels

Cultivar/group name; Run/gel no.	Electrophoresis lane no.	Accession no. (UPM/B...)	Country of origin
Elongated large fruit;			
Run 1:	1	047	Malaysia
	2	200	Philippines
	3	203	"
	4	080	Indonesia
	5	206	Philippines
	6	204	"
	7	082	Malaysia
	8	077	Thailand
	9	161	Malaysia
	10	068	Thailand
	11	014	Malaysia
	12	026	"
	13	007	"
	14	090	"
	15	066	"
Round or obovate large fruit;			
Run 2:	1	017	Malaysia
	2	131	"
	3	028	"
	4	079	Italy
	5	137	Malaysia
	6*	203	Philippines
	7*	026	Malaysia
	8	202	Phillipines
	9	067	Malaysia
	10	071	France
	11	163	France
	12	184	"
	13	198	Phillippines
	14	181	Malaysia
	15	188	Phillippines
Round or obovate large fruit;			
Run 3:	1	214	Phillippines
	2	176	Italy
	3	081	France
	4	073	Spain
	5	178	Italy
	6	083	France
	7	082	Malaysia
	8	161	"
	9	154	Italy
	10	149	Greece
	11	157	Italy
	12	190	"
	13	151	Taiwan
	14	075	"
	15	089	Malaysia

Continued Table 1

Cultivar/group name; Run/gel no.	Electrophoresis lane no.	Accession no. (UPM/B...)	Country of origin
Elongated large fruit;			
Run 4:	8*	082	Malaysia
	9*	161	"
Primitive cultivar;			
Run 4:	1	005	"
	2	023	"
	6	027	"
	7	029	"
Wild/weedy form;			
Run 4:	10	111	"
	11	121	India
	12	311	Thailand
	13	313	"
	14	326	Malaysia
	15	347	Indonesia
Solanum incanum L.			
Run 4:	3	307	Saudi Arabia
	4	295	Kenya
	5	301	India

* = lane with elongated fruit cultivar used as the reference samples

accessions of elongated fruit cultivars chosen as reference proteins were loaded in two wells of standard samples of each gel for comparison and reproducibility checking of the system. The electrophoresis was carried out at about 5^0C with a constant current of 30 mA for 5 h. The gels were stained and fixed for 1 h in a solution containing 0.25% brilliant blue R (W/V), 40% methanol and 7% acetic acid, and then gently agitated in destaining solution containing 30% methanol and 10% acetic acid. The resultant protein profiles in the gel were then recorded photographically and stored in 7% acetic acid in the dark. Duplicate electrophoresis runs were performed for each sample.

Analysis of Electrophoregrams

Each electrophoresis run was analysed separately. Comparisons of the protein banding patterns were made between accessions within each gel. The protein bands were examined and assessed visually and identified according to their number, position, size, staining intensity and the presence or absence of the bands in the profile. Bands which were very clear, thick and densely stained were considered as major bands whereas those that were very thin and weakly stained were considered as minor bands. An identification letter was given to each of the major bands.

RESULTS

The electrophoretic banding pattern of SDS-PAGE of seed proteins of eggplant (*S. melongena* L.) and its nearest wild taxa representing diverse morphological groups and geographical origins are depicted in Plates 1-4 and *Fig. 1.* The banding patterns revealed variations in the numbers, positions, width, staining intensities and the presence or absence of bands among different accessions and groups such that each accession or group exhibited a unique banding pattern. The zymograms showed that eggplants have five common major protein bands (a, f, g, h and i). The banding patterns can be divided into 3 regions: region I with one broad strongly staining protein band (band a) moving at the same rate in all accessions; region II with 6 - 11 protein bands (bands b, c, d, e, f, g, and ai, ci, di, ei and fi) of which two bands (f and g) are fast moving at the anodal end and region III consisting of two very broad strongly stained bands (h and i) present in all accessions. Differences in the protein profiles are described below.

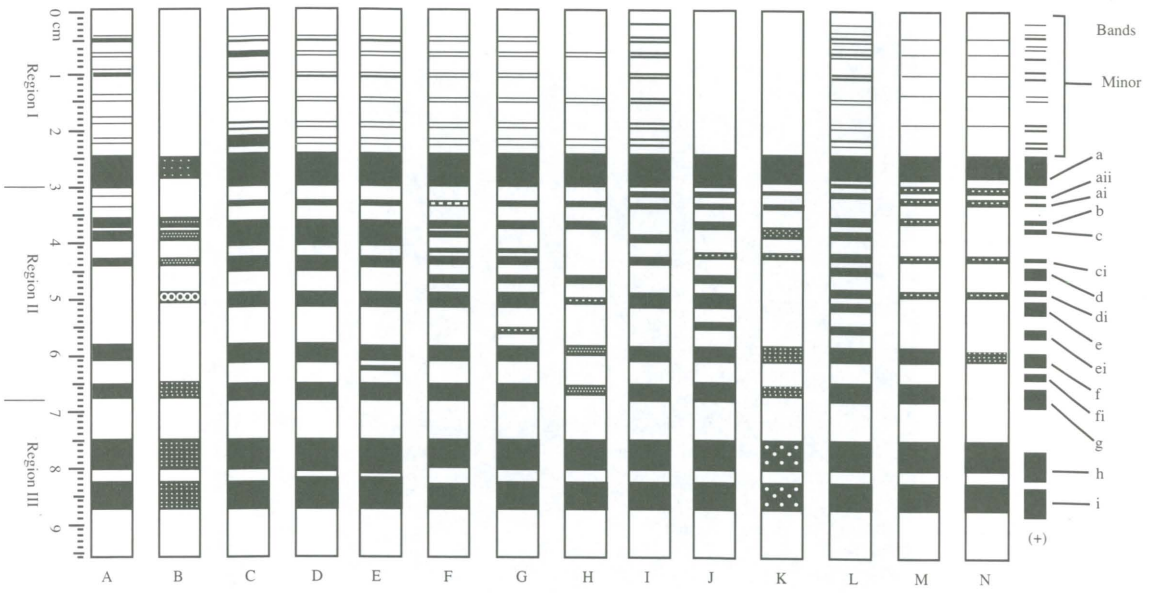

Fig 1. Diagramatic illustration of seed protein banding patterns found in Solanum melongena L. cultivar and its nearest wild species groups. A, elongated fruit cultivar; B – H, round or obovate fruit cultivar; I, primitive cultivar; J- L, wild or weedy (S. insanum) group; M and N, S. incanum group.

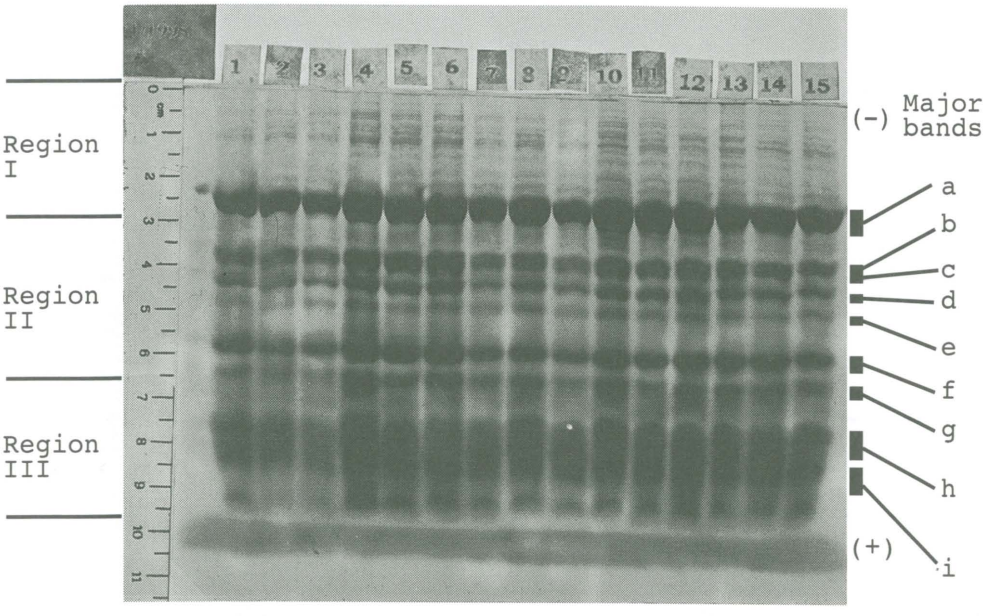

Plate 1. Seed protein banding patterns of elongated fruit cultivars of eggplant (Solanum melongena L.) grown in Southeast Asia. Each lane represents a different accession.

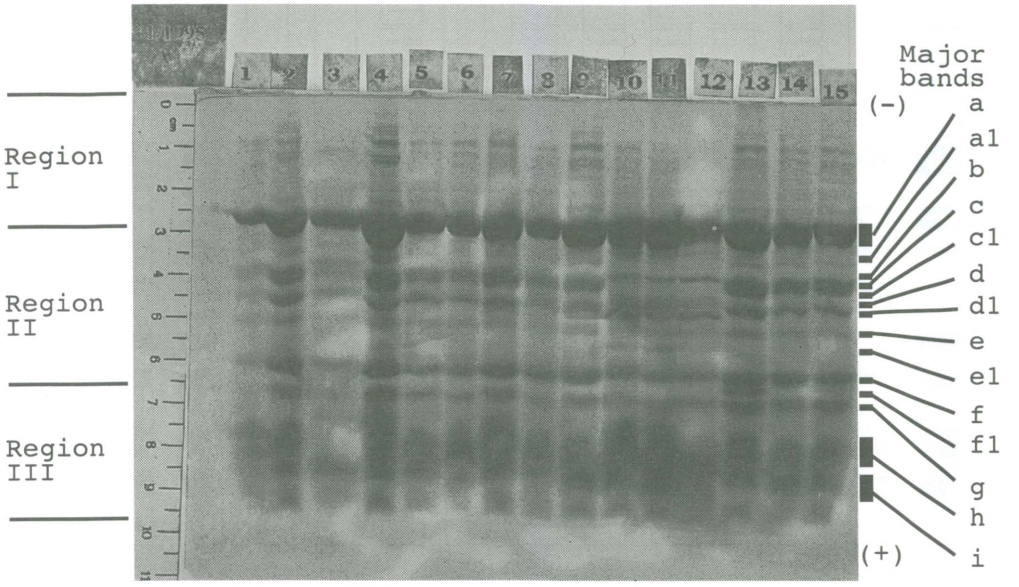

Plate 2. Seed protein banding patterns of typical round fruit cultivars of eggplant (Solanum melongena L.). Each lane represents a different accession. 1 - 3, 5, 8, 9, 13 - 15 = Southeast Asian accessions; 4, 10-12 = Meditterranean accessions; 6 and 7 = accessions of elongated fruit cultivar as references.

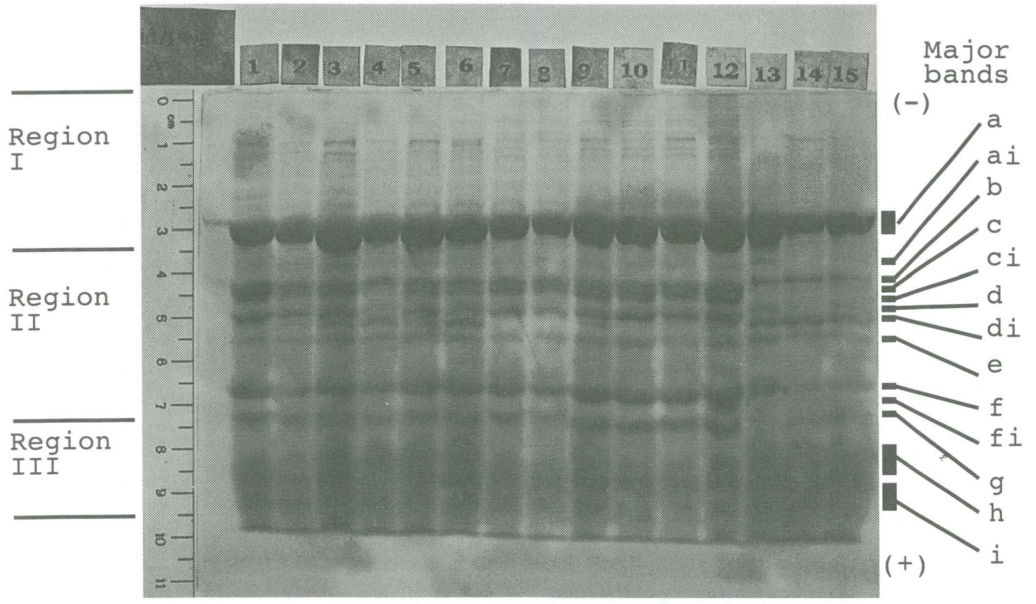

Plate 3. Seed protein banding patterns of round fruit cultivars (pear-shaped or obovate fruit types) of eggplant (Solanum melongena L.). Each lane represents a different accession. 1, 13 - 15 = Southeast Asian accessions; 2 - 6, 9 - 12 = Meditterranean accessions; 7 and 8 = accessions of elongated fruit cultivar as references.

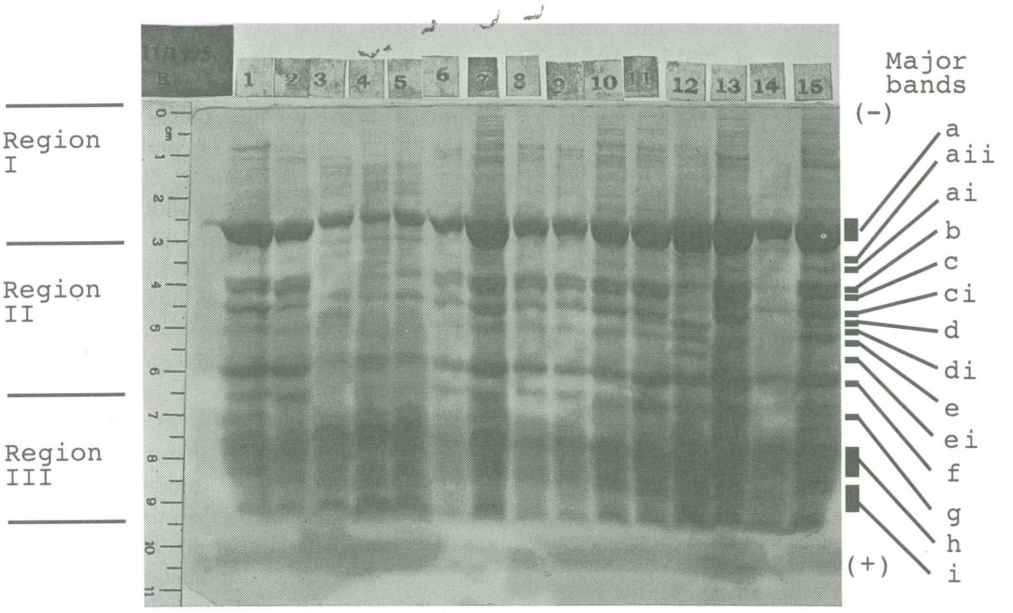

Plate 4. Seed protein banding patterns of a range of eggplant (Solanum melongena L.) groups found in Southeast Asia. Each lane represents a different accession. 1, 2, 6 and 7 = primitive cultivar; 10 - 15 = wild or weedy forms; 3 - 5 = S.incanum L.; 8 and 9 = accessions of elongated fruit cultivar as references.

Variation Within and Between Advanced Cultivars.

For a commercial elongated large fruit cultivar, 9 major protein bands (a, b, c, d, e, f, g, h and i) were recognized in the profiles of each accession (Plate 1 and *Fig. 1(A)*). The pattern of these protein bands was highly constant and uniform among accessions. This result agrees well with the earlier findings that diverse accessions of cultivated eggplant still possess essentially the same major seed protein profiles (Pearce and Lester 1979). Although there are some differences between accessions within this cultivar due to variations in the staining intensities of minor bands in region I of the profiles, such differences were unable to separate the accessions into distinct groups.

Plates 2 and 3 illustrate the characteristic SDS-PAGE protein banding pattern in the round or obovate fruit cultivar group. A wide range of variation in seed protein profiles was found between the accessions of this group. A few accessions, as indicated in lanes 5, 8, 14 and 15 (Plate 2) and lanes 10 and 11 (Plate 3), showed a similar protein banding pattern with accessions of an elongated fruit cultivar in lanes 6 and 7 (Plate 2) and in lanes 7 and 8 (Plate 3) (*Fig. 1(A)*).

There are, however, 20 accessions of the round fruit cultivar which showed differences in protein profiles from those of the elongated cultivar. Two accessions (lanes 1 and 3 of Plate 2) produced very weak staining bands, which may be because the seeds were poorly filled as a result of the hybrid origin of the original source (*Fig. 1(B)*). Six accessions (lanes 2, 4, 9 and 13 of Plate 2 and lanes 1 and 12 of Plate 3) also displayed a different profile (*Fig. 1(D)*) from those of an elongated cultivar by producing a wider band at positions ai, b, c and d in region II. Among these accessions, one (lane 4 of Plate 2) showed densely stained bands with clear minor bands in region I of the profile (*Fig. 1(C)*), and two (lane 13 of Plate 2 and lane 12 of Plate 3) which exhibited a distinctive protein banding pattern by producing an extra band (fi) in region II (*Fig. 1(E)*).

Further, distinct protein profiles of round fruit cultivar were observed from accessions in lanes 2 - 6 and 9 of Plate 3. They were clearly differentiated from the other accessions by the presence of bands ci and di in region II of the profile (*Fig. 1(F)*).

Furthermore, the accessions in lanes 10 - 12 of Plate 2 and lanes 13 - 15 of Plate 3 gave an extremely distinct protein banding pattern. They

were easily distinguished from the other accessions by the absence of band c in the profile (*Fig. 1(G)*). However, accessions in lanes 14 and 15 of Plate 3 could be further distinguished from the other accessions by the absence of bands ci, d and ei in the profile (*Fig. 1 (H)*).

Variation Within and Between Primitive Cultivars and Wild Groups.

The photoelectrophoregram of seed protein profiles for different accessions of primitive cultivar, weedy and wild groups of eggplant is shown in Plate 4. For comparison, control lines of elongated cultivar were in lanes 8 and 9. Three accessions (lanes 1, 2 and 6) of primitive cultivar showed only slight differences in seed protein profile from those of the elongated cultivar. One accession (lane 7) of primitive cultivar and three accessions (lanes 10, 11 and 13) of the weedy group shared a similar protein banding pattern (*Fig. 1(I)*) but showed some differences from those of the elongated cultivar by producing densely stained bands and the presence of bands ai and aii in the profiles.

One accession (lane 14) of weedy group (Plate 4)) showed a clear difference in protein profile from those of the elongated cultivar by the absence of bands c, d and e in the profile (*Fig. 1(K)*). One accession in lane 12 of the same group also showed the differences by the absence of bands c and d and the presence of bands ci, di and ei in the profile (*Fig. 1(J)*). Another accession of the weedy group (lane 15) also exhibited a specific protein banding pattern distinct from the other groups by producing bands in all position with thick and densely stained bands, particularly in region I of the profile (*Fig. 1(L)*).

The truly wild *S. incanum* (lanes 3 - 5) was clearly differentiated from the other groups by the absence of bands c, d and e and the presence of bands aii, ai, ci and di in the profile (*Fig. 1(M)*). The accession in lane 3 is even more distinct from the other accessions by the absence of bands b and g in the profile (*Fig. 1(N)*).

DISCUSSION

The overall differential banding patterns of seed proteins revealed both qualitative and quantitative variations between and within the groups of eggplant. There are 14 protein banding patterns (*Fig. 1*) characterizing the different types of eggplant used in this investigation. Major bands which contributed to the variability of protein banding patterns were mainly found in region II of the profile. These made it possible to divide genotypes of eggplant into different cultivars or groups (Table 2). These differences appear reproducible, as similar protein profiles were obtained when further samples of the same accessions were analysed in different gels.

It is evident that the elongated fruit cultivars had the most constant and uniform pattern of major protein bands that gave characteristic group-specific seed protein banding patterns, thus enabling them to be distinguished easily from those of the other groups (*Fig. 1(A)* and Table 2). Although elongated advanced cultivars have many of the same bands, there are, however, small variations between accessions. The elongated fruit type is the most common eggplant cultivar grown for commercial purposes. Thus, they may have a common parent in their pedigrees which could explain the similarity of their electrophoregrams. It is common for the agricultural cultivar seed protein banding pattern to be very stable during commercial seed production, but the pattern may alter slightly when an accession is multiplied, particularly if it is regenerated from a very small sample size (Gardiner and Forde 1987).

The other groups of eggplant did not show a strong common protein banding pattern, but displayed a great deal of variation at the groups level (*Fig. 1(B-N)*). Great variability in seed protein profiles was detected in the round fruit cultivar indicating that the cultivar actually consists of different genotypes. This high variability is not surprising because round fruit cultivar is an outbred heterogeneous population whose accessions were mostly obtained from small isolated populations in diverse agro-ecosystems.

Primitive cultivar also showed variable seed protein banding patterns between different accessions, and the protein banding pattern of the weedy group, including the truly wild *S. incanum*, was even more variable. The substantial differences in the protein profiles within these populations are mainly due to the outbreeding nature of these taxa (Karihaloo and Rai 1995).

The majority of the accessions used in this investigation could be readily distinguished by their soluble seed protein banding patterns. Although in a few cases the weedy accessions showed similar major protein compositions to

TABLE 2
Groups or cultivars of eggplant accessions arranged according to their electrophoresis seed protein banding patterns obtained from this study

Protein banding pattern	Cultivars or groups				
	Elongated fruit	Round fruit	primitive	Wild/weedy	*S. incanum*
A	007, 014, 026, 047, 066, 068, 077, 080, 082, 090, 161, 200, 203, 204, 206,	137, 149, 157 181, 188, 202	005, 023, 027	–	–
B	–	017, 028	–	–	–
C	–	079	–	–	–
D	–	067, 131, 124	–	–	–
E	–	190, 198	–	–	–
F	–	073, 081, 083 154, 176, 178	–	–	–
G	–	067, 071, 151, 163, 184	–	–	–
H	–	075, 089	–	–	–
I	–	–	029	111, 121, 313	–
J	–	–	–	311	–
K	–	–	–	326	–
L	–	–	–	347	–
M	–	–	–	–	295, 301
N	–	–	–	–	307

the accessions of the cultivated group, there are strong differences in the patterns of the minor bands found in the profiles of those accessions. It could be postulated that the weedy group was derived from cultivated eggplant from which it has since been isolated as a feral taxon. Another possibility is that both cultivated and wild taxa developed from a common ancestor (Lester and Hasan 1991; Karihaloo and Rai 1995).

CONCLUSION

The results of this study demonstrate that the SDS-PAGE seed protein banding patterns are reproducible and differ from one accession to another. Therefore the profiles serve as genotype fingerprints for eggplant cultivars and can be used for identification, breeding or patent purposes. This method could be used to construct an atlas of the seed protein profiles of the eggplant group for reference. Furthermore, profiles of important groups could be filed as one crop descriptor. With a substantial variation in seed protein composition in eggplant, it is possible to use the SDS-PAGE technique to identify eggplant cultivars within a couple of hours without the necessity of growing out the plants. The SDS-PAGE pattern of bulk seed extracts could also form a much-needed adjunct to the present system of classifying the eggplant complex and at least partially resolve the various groups which complicate the species.

ACKNOWLEDGEMENT

We would like to thank MPKSN for supporting this study through IRPA grant no. 07-05-044-50339.

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(Received 13 August 1996)
(Accepted 24 March 1999)