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CHARACTERIZATION OF MUSTARD [*BRASSICA JUNCEA* (L.) CZERN. & COSS.] GERMPLASM BY SDS-PAGE OF TOTAL SEED PROTEINS

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Abstract

A comparative study of total seed storage protein was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to characterize oilseed mustard [*Brassica juncea* (L.) Czern. & Coss.] germplasm from Pakistan. Oilseed collections from Pakistan as well as oilseed cultivars from diverse origin were not differentiated from each other nor were vegetable cultivars found to be distinct from one another on the basis of their seed protein patterns. Eight types of protein were recognized based on the banding patterns of 52 accessions. Relative inter-type relationships of protein patterns were estimated using Jaccard's similarity index and a dendrogram showing the hierarchical clustering was constructed by unweighted pair-group method with arithmetic averages (UPGMA). The clustering of eight protein types generally agreed with our previous classification and the limited data already available on inter-accession relationships in oilseed mustard based on morphological traits and RAPD analysis. The differences between the profiles of accessions 'PAK-85835', 'PAK-85839' and 'PAK-85910' supported the idea that they were separate species from *B. juncea*. The results showed that the technique of SDS-PAGE applied to seed proteins was not feasible to distinguish the closely related oilseed collections and cultivars from each other as they were characterized by the same banding pattern and formed a common gene-pool. However, seed proteins were useful to discriminate *B. juncea* and *B. campestris*. It was also possible to distinguish the oilseed mustard from the vegetable form. Future studies should involve a greater number of local accessions of oilseed mustard from other parts of Pakistan, and those of vegetable and condiment forms to further elucidate the situation.

Introduction

Mustard [*Brassica juncea* (L.) Czern. & Coss.] displays a great polymorphism and is a source of different types of vegetables, condiment and oilseeds. Vegetable mustard is grown predominantly in China and Japan. On Canadian prairies, however, the mustard is a major source of condiment, while in Indian subcontinent, oilseed mustard is more important than the vegetable and condiment types (Chen *et al.*, 1997). The term mustard is believed to be derived from the early European practice of mixing the sweet 'must' of old wine with the crushed seeds to form a hot paste 'hot must' or 'mustum ardens' - hence the modern term mustard (Hemingway, 1976).

Recently, a considerable interest has been focused on the use of biochemical methods for plant variety discrimination and identification. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz, 1979; Sammour, 1989; Khan, 1992; Das & Mukherjee, 1995). They have also provided a promising tool to distinguish cultivars of a particular crop species (Cooke, 1984; Ferguson & Grabe, 1986; Gardiner & Forde, 1988; Gadgil *et al.*, 1989; Koranyi, 1989; Moller & Spoor, 1993; Jha & Ohri, 1996). However, few studies indicated that cultivar identification was not possible with the SDS-PAGE, as the electrophoretic patterns of the proteins were similar among the cultivars (Ladizinsky & Adler, 1975; Raymond *et al.*, 1991; Ahmad & Slinkard, 1992; de Vries, 1996).

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The genus *Brassica* contains a number of species of great economic importance which have been the subject of many taxonomic investigations based on external morphology, anatomy and cytology (Morinaga, 1934; U, 1935). Electrophoresis of crude protein extracts has been successfully used as an additional tool to establish these relationships. Comparative electrophoretic studies of proteins and enzymes have been carried out in order to study the taxonomic and genetic relationships among (Vaughan & Denford, 1968; Yadava *et al.*, 1979) and within brassica species (Vaughan & Gordon, 1973; Gupta & Robbelen, 1986). In mustard, however, very few studies have been conducted using seed protein electrophoresis (Uchimiya & Wildman, 1978; Yadava *et al.*, 1979). Moreover, these authors examined only inter-specific and revealed the taxonomic relationships among various brassica species including *B. juncea*. But, relatively little attention has been paid to distinguish the cultivars of mustard on the basis of their seed storage protein patterns. In the present study, an attempt has been made to investigate the feasibility of using SDS-PAGE of total seed storage proteins to characterize a collection of mustard at intra-specific level.

Materials and Methods

Plant material consisted of a total of 52 genotypes including 41 accessions collected from Pakistan; six oilseed cultivars from Pakistan, India, China and Australia; and four vegetable cultivars of mustard from Japan. One cultivar of *B. campestris* from Japan was also included as an out-group for comparison (Rabbani *et al.*, 1998a, 1998b).

Total seed protein was extracted from 20-25 mg of seed meal with a sample buffer containing 0.5M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Fifteen µl of extract solution were applied into the sample wells with the micro-syringe. Electrophoresis was carried out in the modified discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) using 30% acrylamide resolving gel (1M Tris-HCl buffer, pH 8.8 and 0.27% SDS) and 30% stacking gel (0.25M Tris-HCl, pH 6.8 and 0.2% SDS). The electrode buffer was Tris-glycine (9 g of Tris HCl and 43.2 g glycine in 3 liter water at a pH 8.9). After electrophoresis, staining of the gels was done in 0.2% Coomassie Brilliant Blue R-250 solution containing 10% acetic acid and 40% methanol for about one hour. Gels were then destained by washing with a solution containing 5% acetic acid and 20% methanol until the color of background disappeared and electrophoresis bands were clearly visible.

Depending upon the presence (1) and absence (0) of the bands, Jaccard's similarity index (S) was calculated for all possible pairs of protein types by the following formula (Sneath & Sokal, 1973):

Similarity (S) = W/(A + B - W)

where W = number of bands of common mobility; A = number of bands in protein type 'A' and B = number of bands in protein type 'B'. The similarity matrix thus generated was converted to a dissimilarity matrix (Dissimilarity = 1 - similarity) and used to construct a dendrogram by the unweighted pair-group method with arithmetic averages (Sneath & Sokal, 1973). All the analyses were carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993).

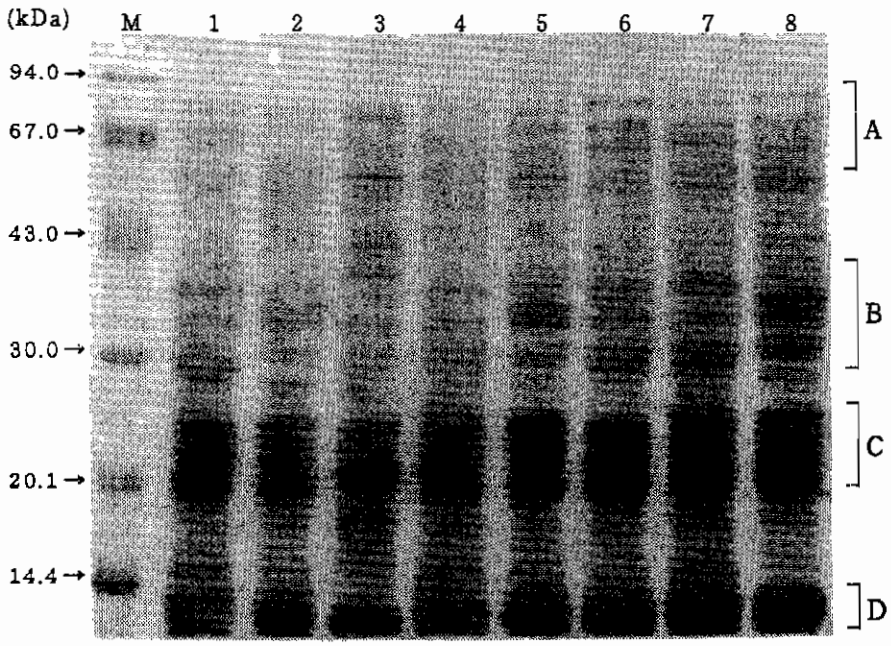

Fig. 1. SDS-PAGE variation in the eight phenotypes of total seed protein electrophoregrams in mustard; M molecular weight marker.

Results

Among 52 accessions tested, very close relationship was found between the oilseed collections as well as cultivars. Except 'PAK-85835', 'PAK-85839', 'PAK-85870' and 'PAK-85910', the electrophoretic seed protein profiles of all the remaining accessions/cultivars of oilseed mustard were alike, even though cultivars represented a wide range of geographic origin. The seed protein patterns were also uniform among the vegetable cultivars of mustard from Japan except 'Unzenkobu-takana'. Based on the combination of various banding patterns, eight types of total protein electrophoregrams were recognized among all the accessions/cultivars screened (Fig. 1). Forty-three oilseed collections and cultivars contained protein type 1, three vegetable cultivars consisted of protein type 6, whereas each one of the remaining six protein types was represented by single accessions (Table 1). Protein type comparisons were carried out on bulk seed samples. The differences occurred in the degree of separation in the highest and middle protein sub-unit regions. Polypeptide banding patterns could be divided into four regions, A to D (Fig. 1). Region A (79.5 to 55.5 kDa) contained relatively weakly stained protein bands. Region B (38.0 to 28.5 kDa) had upto 8 protein bands, most of them dark stained in various protein type accessions. Both regions A and B exhibited a considerable range of variation between eight protein types as bands with different mobilities and intensities were observed. Region C ranged from 24.5 to 19.5 kDa and showed broad bands, mostly dark stained in all protein types. Region D included the lowest weight protein subunits in 13 kDa molecular weight at the cathode end. Banding pattern in region D was almost uniform between all protein types.

Table 1. Protein type composition based on SDS-PAGE.

Protein type	No. of accesions	Accessions/cultivars falling in protein type
Type-1	43	1, 3, 4, 6 ~ 22, 24 ~ 36, 38 ~ 47*
Type-2	1	PAK-85835 (2)
Type-3	1	PAK-85839 (5)
Type-4	1	PAK-85870 (23)
Type-5	1	PAK-85910 (37)
Type-6	3	Negarashina (48), Hakarashina (49), Kikarashina (50)
Type-7	1	Unzenkobu-takana (51)
Type-8	1	Sendai-bashouna (52)

*Numbers indicate the accessions/cultivars codes as given in Rabbani *et al.* (1998b).

In total, 29 polypeptide bands were distinguished among eight types of protein identified. The accession in type 3 showed the maximum number of bands (18), while the minimum number of bands (12) were present in type 4 protein. Fifteen bands were observed in each of types 1 and 8, while types 2, 5 and 7 showed 14 bands each. Distinction of particular types was due to characteristic polypeptide bands observed in some of the eight protein types. For example, a polypeptide band having molecular weight of 79.5 kDa was specific for 1, 4 and 7 types protein, while a band with 76.5 kDa molecular weight was absent in these protein types and was specific for protein types 6 and 8. Similarly, types 5 and 8 had a characteristic band pattern at position 33.7 kDa, but this band pattern had not been found in any of the other types. Likewise, type 3 had prominent bands at position 72, 38 and 17.5 kDa, whereas other protein types lacked these characteristic band patterns. Distinction of type 5 was also due to the presence of a characteristic polypeptide band with approximately 74 kDa. Polypeptides having an approximately molecular weight of 28.5, 24.8, 19.5 and 13.0 kDa were present in all the types (Fig. 1).

Based on banding patterns, Jaccard's similarity indices were calculated between all possible pairs of eight protein types. The similarity estimates among eight types ranged from 23 to 93% (Table 2). Among the 8 types identified, type 1 and 7 accessions showed the highest similarity with each other, while the lowest affinity was observed between type 4 and 8. Interestingly, 'Unzenkobu-takana' (type 7 protein) had more closeness of 93% and 86% with oilseed accessions from Pakistan in type 1 and 4, respectively, than with other vegetable cultivars (type 6) from Japan (80%). In general, protein types 1, 4, 6 and 7 exhibited closer affinity with one another, which varied between 80 and 93%. Similarly, types 2, 5 and 8 proteins showed a higher level of similarity with each other (61 to 75%), while protein type 3 accession was the least similar to any of the other protein types. The dendrogram based on dissimilarity matrix using UPGMA, showed a division into three major groups: A, B and C (Fig. 2). Group A comprised accessions in protein types 1, 4, 6 and 7; single accession 'PAK-85839' of type 3 formed group B, while types 2, 5 and 8 accessions constituted group C. Cluster analysis placed all of the oilseed collections/ cultivars and Japanese vegetable cultivars close to each other, showing high level of genetic similarity among them. However, considerable polymorphism was present between mustard type-proteins and the accessions from other four types that clustered into separate groups. Accession 'PAK-85839' was the most dissimilar entry, which exhibited the lowest level of similarity with all other protein types and clustered independently. Likewise, 'PAK-85835' and 'PAK-85910' (protein types 2 and 5) grouped with 'Sendai-bashouna' (protein type 8), showing relatively a high level of similarity with *B. campestris* than with any of the mustard protein types.

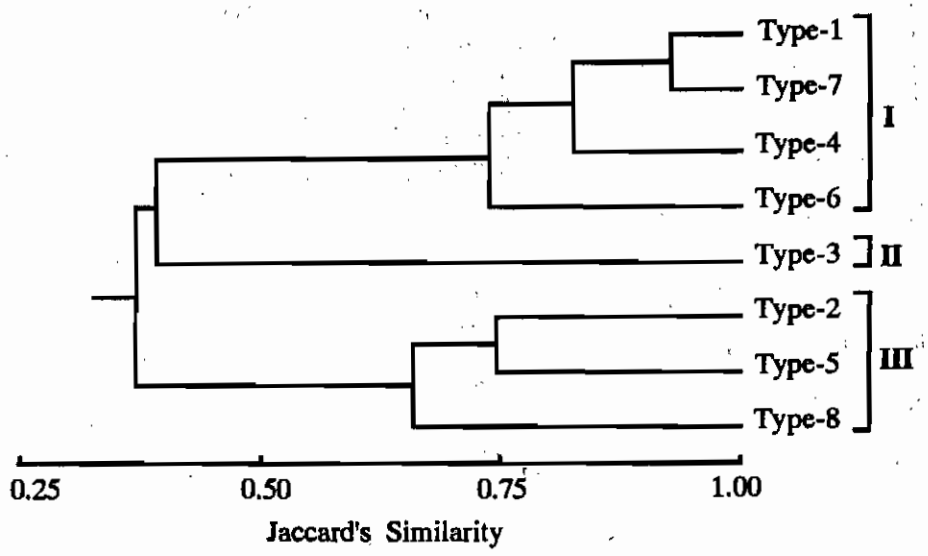

Fig. 2. UPGMA dendrogram showing the relationships among eight types of mustard accessions revealed by total seed protein electrophoresis.

Table 2. Jaccard's similarity coefficients between eight protein types of mustard accessions based on observed seed protein banding patterns.

Protein type	Type-1	Type-2	Type-3	Type-4	Type-5	Type-6	Type-7	Type-8
Type-1	1.000							
Type-2	0.450	1.000						
Type-3	0.435	0.333	1.000					
Type-4	0.800	0.368	0.364	1.000				
Type-5	0.450	0.750	0.280	0.300	1.000			
Type-6	0.750	0.350	0.348	0.667	0.421	1.000		
Type-7	0.933	0.400	0.391	0.857	0.400	0.800	1.000	
Type-8	0.364	0.611	0.320	0.227	0.706	0.400	0.318	1.000

Discussion

Seed protein analysis by SDS-PAGE has proved to be an effective way of revealing the differences and relationships between taxa. The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky & Hymowitz, 1979). The present investigation revealed no variation in different accessions and cultivars of oilseed mustard with regards to their total seed protein profiles. The genetic affinities among the mustard germplasm from Pakistan determined by the seed protein profile study here generally corroborated the morphological and RAPD analysis (Rabbani *et al.*, 1998a, 1998b). Phenotypically, most of the oilseed accessions as well as cultivars also showed close association. This uniformity of seed protein profiles also agreed with the findings of Ladizinsky & Alder (1975) and Ahmad & Slinkard (1992) who examined

different cultivars of cultivated chickpea and concluded that seed protein was a very conservative trait in chickpea. Similarly, Raymond *et al.*, (1991) and de Vries (1996) also reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce, respectively. Likewise, Tomooka *et al.*, (1992) observed similar patterns in the local mung bean strains collected from south-east Asia using SDS-PAGE analysis. Ladizinsky & Hymowita (1979) also stated that taxonomic categories below the species level, despite morphological and ecological differences, still possess basically the same seed protein profiles.

Considerable variability was detected when the various accessions/cultivars were arranged into different types according to their protein banding patterns. The maximum extent of similarity was shown by the accessions in protein types 1 and 7. Accessions 'PAK-85839' and 'PAK-85910' were more closer to 'Sendai-bashouna' and therefore, most likely the *B. campestris*. Such conclusion was further supported by RAPD markers, in which 'PAK-85910' showed highest similarity (80%) with 'Sendai-bashouna' than with any of the other accessions (Rabbani *et al.*, 1998b). The overall clustering pattern of protein types supported our previous classification based on morphological traits and RAPD analysis. The molecular study based on RAPDs showed a close relationship between oilseed accessions themselves as well as with oilseed cultivars, while the pattern of 'PAK-85839' and 'PAK-85910' was found to be distantly different from all other accessions (Rabbani *et al.*, 1998b). 'PAK-85839' was different from all other collections from Pakistan on the basis of morphological grounds: dwarf plants with sect leaves, compact short inflorescence, longer siliques, yellow seeds. Phenotypically, 'PAK-85910' also had unique features as well. It displayed relatively longer hypocotyl, longer pods with heavier seeds, with upper leaves fully clasping the stem (Rabbani *et al.*, 1998a). Such morphological and RAPD differences between 'PAK-85839' and 'PAK-85910' and other accessions were also supported by seed protein patterns.

Previous research on brassica species using SDS-PAGE of seed storage proteins focused on species discrimination (Vaughan & Denford, 1968; Yadava *et al.*, 1979). The present work was initiated to investigate the potential of electrophoresis for intra-specific characterization of mustard on the basis of their total seed protein. The results clearly showed that it was impossible to discern closely related oilseed collections and cultivars used from each other, however, seed proteins were useful to distinguish different types of mustard from one another and from other brassica species. Therefore, it is concluded that protein profiles provided a sound basis for discussing biosystematic relationships among different species and diverse forms of same species. However, it was impossible to discriminate closely related collections and cultivars of same form or species. It is necessary to conduct further analysis of protein type diversity using a large number of local strains from other parts of the country and those of vegetable and condiment mustard of diverse origin to further elucidate the situation.

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