Assessment of Diversity in Himalayan Buckwheats: Variations in SDS-PAGE Profile of Soluble Proteins Extracted from Single Seeds

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ABSTRACT

Buckwheat (*Fagopyrum* spp.) is an ancient crop, which has long been grown in East Asia and the Himalayan region. It is the most important crop of mountain region at about 1800 m elevation both for grains and greens. Unlike common cereals, which are deficient in lysine, buckwheat has excellent protein quality in terms of essential amino acid composition. SDS-PAGE profiles of total soluble proteins extracted from single seeds of different accessions of buckwheat from the Indian Himalayas were studied to distinguish between the accessions of buckwheat. Comparisons based on Jaccard's coefficient and UPGMA clustering revealed interrelationships broadly in conformity with conventional treatments. On the basis of variations in the seed protein profiles accessions of buckwheat segregated into three broad groups. Cluster 1 included accessions of *F. esculentum* except VL-7. Even though VL-7 has been identified as *F. esculentum* on the basis of its morphological features, it showed only 37.3% similarity in SDS-PAGE profile with *F. esculentum*. On the other hand, IC-13145, which has been identified as *F. himalianum* showed >90% similarity with *F. esculentum*. Our results indicate that *F. himalianum* belongs to the *esculentum* group rather than as a different species. A moderate level of variability was detected between accessions of *F. esculentum*. Cluster 2 included all accessions of *F. tataricum*. *F. cymosum* emerged as separate group distinct from both *esculentum* as well as *tataricum*. Our results indicate that SDS-PAGE electrophoresis of proteins extracted from single seeds can be used to analyze species relationship between accessions of buckwheat growing in different regions of Indian Himalayas.

Key words: *Buckwheat accessions, diversity, SDS-PAGE, soluble proteins.*

Introduction

Buckwheat is a minor crop in the world but is an indispensable food in the temperate and hill regions of East Asia, Europe and the Asia pacific region. It is a major staple food crop in the Indo-Himalayan region (Jiang and Xing, 1992). Buckwheat is a multi-purpose crop used for food, feed, medicine and manure. The tender shoots are used as a leafy vegetable while the flower and green leaves are used for the extraction of rutin (Marshall and Pomeranz, 1982). Buckwheat flowers are a good source of honey.

The genus *Fagopyrum* consists of about 19 species, some of which have been recently discovered (Ohnishi, 1998; Ohsako and Ohnishi, 1998). Of the two cultivated species, *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn, *F. tataricum* is mainly cultivated in the Himalayas. Based on molecular data, the genus *Fagopyrum* has been divided into two phylogenetic groups viz., the *cymosum* group comprising of two cultivated species *F. esculentum* and *F. tataricum* and two wild species *F. cymosum* and *F. homotropicum* and the *urophyllum* group comprising *F. urophyllum* and other wild species (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a, b; Ohsako and Ohnishi, 2000).

Assessment of genetic variation in a species is important for initiation of effective breeding programmes because it provides the basis for tailoring desirable genotypes. RAPD and SDS PAGE profiles have been successfully used for analysis of diversity in many crops including buckwheat (Javornik and Kump, 1993; Tsuji and Ohnishi, 1998, Ohnishi and Asano, 1999), cowpea (Mignouna *et al.*, 1998), soybean (Thompson *et al.*, 1998), *Brassica juncea* (Rabbani *et al.*, 1998), bean (Duarte *et al.*, 1999), *Vicia sativa* (Potokina *et al.*, 2000). Even though much work has been done on the analysis of phylogenetic relationships between different species of the genus *Fagopyrum* using isozyme profiling and RFLP variations in cpDNA (Ohnishi 1998, Ohsako and Ohnishi 1998, Ohsako and Ohinishi 2000), not much information is available on inter- as well as intra-specific variations in molecular fingerprints in this genus. The selection of an appropriate molecular tool for screening of accessions in gene bank collections and elucidation of inter and intra-specific variations have always been an important consideration. Seed protein electrophoresis has found wide application in resolving systematic relationships and in characterising

cultivated varieties in crop plants, especially cereals and legumes (Ladizinsky and Hymowitz, 1979; Chen *et al.*, 1987; Singh *et al.*, 1991; Yupsanis *et al.*, 1992; Przybylska *et al.*, 1998) crop origin and evolutionary studies (Rogal and Javornik, 1996).

The present work was undertaken to analyse the inter- and intra- specific genetic diversity in accessions of buckwheat growing in different regions of Indian Himalayas using single seed protein SDS-PAGE analysis.

Materials and Methods

Accessions of buckwheat used for the present investigation were procured from the National Bureau of Plant Genetic Resources, New Delhi and Vivekananda Laboratory of Hill Agriculture, ICAR, Almora, India and from hilly regions of Meghalaya. The accessions are listed in Table 1. Single seed of each accession were used for SDS-PAGE of proteins. The hull portion of each seed was removed and the grout was deflatted by washing with cold acetone for 4–6 hours. Acetone was removed by filtration and the samples were air dried. The deflated meal was powdered in liquid nitrogen and homogenized in a pre-chilled mortar and pestle in 50 mM Tris-Cl buffer (pH 6.8) containing 100mM NaCl, 10Mm EDTA, 100 mM Glycine, 10% SDS and 1mM PMSF. The homogenate was kept for 45 minutes at 4°C and centrifuged at 10,000 rpm for 15 min. The extracted proteins were recovered as clear supernatant. Protein concentration in each sample was determined according to Bradford (1976).

SDS-Polyacrylamide gel electrophoresis

SDS-PAGE of seed protein was carried out on 12% polyacrylamide slab gel following the method of Laemmli (1970). Samples containing 50 µg protein was loaded on 1.5 mm thick 12% acrylamaide gels. Electrophoresis was carried out at a constant voltage of 100V for 6 hours in cold room. The gels were stained for 3–4 hours in 0.25% (w/v) Coomassie Brilliant Blue R-250 prepared in glacial acetic acid (4:5:1). Distaining was carried out in methanol: water: glacial acetic acid. Followed by 2nd distaining in methanol: water: glacial acetic acid (4:5.3:0.7). Protein bands were visualized in a transilluminator under white light. The molecular mass of the dissociated peptides

Table 1. List of *Fagopyrum* accessions/cultivars used for the present study

No.	Accession	Name Species	Source
1.	IC-188669	*F. esculentum*	NBPGR *
2.	IC-18751	*F. esculentum*	NBPGR
3.	IC-13376	*F. esculentum*	NBPGR
4.	IC-13145	*F. himalianum*	NBPGR
5.	IC-13141	*F. esculentum*	NBPGR
6.	IC-13417	*F. esculentum*	NBPGR
7.	EC-323729	*F. esculentum*	NBPGR
8.	Local	*F. esculentum*	VPKAS **
9.	Kamroo local	*F. esculentum*	VPKAS
10.	OC-2	*F. esculentum*	VPKAS
11.	VL-7	*F. esculentum*	VPKAS
12.	SanglaB-1	*F. tataricum*	VPKAS
13.	SanglaB-2	*F. tataricum*	VPKAS
14.	SanglaB-3	*F. tataricum*	VPKAS
15.	Sangla B-5	*F. tataricum*	VPKAS
16.	Sangla B-6	*F. tataricum*	VPKAS
17.	Sangla B-7	*F. tataricum*	VPKAS
18.	KBB-3	*F. tataricum*	VPKAS
19.	Himpriya	*F. tataricum*	VPKAS
20.	Kuppa local	*F. tataricum*	VPKAS
21.	Shimla B-1	*F. tataricum*	VPKAS
22.	*F. cymosum*	*F. cymosum*	NBPGR

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was determined by co-electrophoresis of the standard molecular markers.

Data analysis

The protein gels were observed carefully and the bands in each lane were scored as presence (1) or absence (0) of bands of comparable size. Faintly stained bands were not included in the analysis. Based on the scores similarity matrix was generated using Jaccard's coefficient of similarity. Cluster analysis was performed on the similarity matrix by the UPGMA method. All computations were performed using the software NTSYS-PC version 2.1 (Rohlf, 2000).

Results and Discussion

Total seed storage proteins were extracted individually from each accession and were separated using SDS-PAGE. The SDS-PAGE profile of seed proteins of different accessions of *F. esculentum*,

F. himalianum, *F. tataricum* and *F. cymosum* is presented in Fig. 1. The number of polypeptide bands observed in each accession ranged from a minimum of 14 in the accession OC-2 of *F. esculentum* to a maximum of 28 in the accessions of *F. tataricum*. The size of resolved polypeptides ranged between 110–14 kDa. Distinct qualitative variations were observed in the SDS-PAGE profile of seed proteins between *F. esculentum*, *F. tataricum* and *F. cymosum*. The SDS-PAGE profile of seed proteins of *F. himalianum*, on the other hand showed similarity with that of *F. esculentum*. Significant intraspecific variations were detected in the protein profile of different accessions of *F. esculentum*. Most of these variations were observed either in the protein bands ranging in size between 90–100kDa and 26–54 kDa (Fig. 1a–h). Interestingly most of the protein bands in the 26–54 kDa category belong to the legumin type family of seed proteins (Rout and Chrungoo 1996, Bharali 2002). Accessions of both *F. tataricum* (Fig. 1i) and *F. cymosum* (Fig. 1j), on the other hand, did not show any significant intraspecific variation in the SDS-PAGE profile of seed proteins. These results are consistent with the observation of Nishiyama *et al.* (1991) and Svetek (1994). One of the notable features of the SDS-PAGE profile was the presence of a distinct 110 kDa band in IC-13147 and EC-323729. Similarly a 79 kDa and a 38 kDa protein band were found exclusively in *F. cymosum* only.

The dendrogram generated on the basis of SDS-PAGE profiles of proteins isolated from single seeds of the different accessions of buckwheat showed the clustering of the accessions into 3 broad groups (Fig. 2). Cluster 1 included all accessions of *F. esculentum* except VL-7. Even though VL-7 has been identified as *F. esculentum* on the basis of morphological features, it showed only 37.3% similarity in SDS-PAGE profile with the *F. esculentum*. On the other hand IC-13145, this has been identified as *F. himalianum* belongs to the *esculentum* group rather than as a different species. Significantly the *esculentum* group showed further subgrouping into four clusters.

This could be ascribed to a high degree of seed protein polymorphism in *F. esculentum*. Cluster 2 includes all the accessions of *F. tataricum*. The accessions of *F. tataricum* did not exhibit any significant variation in SDS-PAGE profile of seed proteins. *F. cymosum* emerged as a separate group distinct from both *esculentum* and *tataricum*.

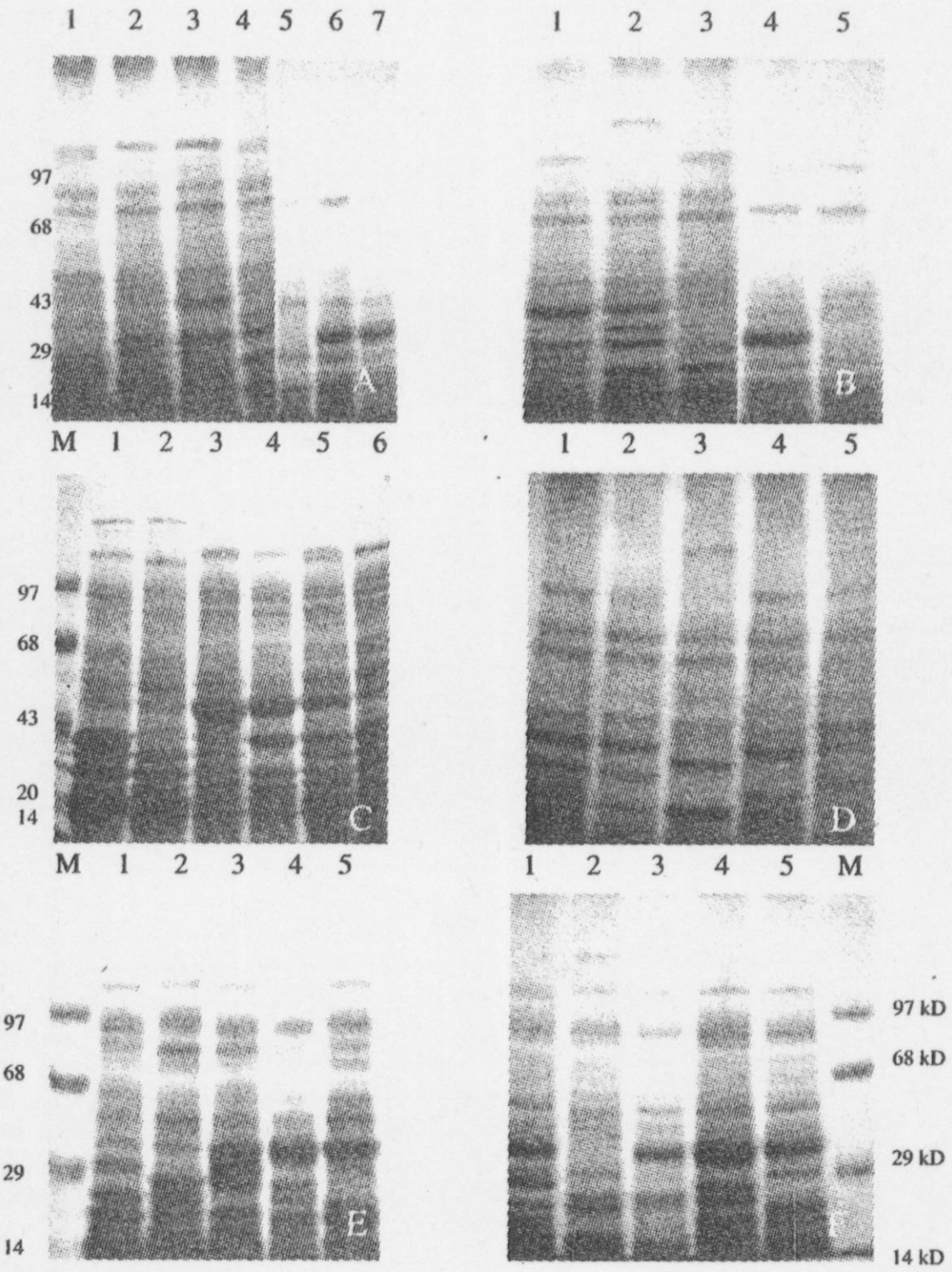

Fig. 1. SDS-PAGE profile of seed proteins from single seeds of *Fagopyrum* spp. — A: Lane 1-IC-188669-1, 2-IC-188669-2, 3-IC-188669-3, 4-IC-188669-4, 5-IC-188669-5, 6-IC-188669-6, 7-IC-188669-7 B: Lane 1-IC-18751-1, 2-IC-18751-2, 3-IC-18751-3, 4-IC-18751-4, 5-IC-18751-5 C: Lane 1-IC-13376-1, 2-IC-13376-2, 3-IC-13376-3, 4-IC-13376-4, 5 -IC-13376-5 D: Lane 1-IC-13141-1, 2-IC-13141-2, 3-IC-13141-3, 4-IC-13141-4, 5-IC-13141-5, 6-IC-13141-6 E: Lane 1-IC-13417-1, 2-IC-13417-2, 3-IC-13417-3, 4-IC-13417-4, 5-IC-13417-5 F: Lane 1-EC-323729-1, 2-EC-323729-2, 3-EC-323729-3, 4-EC-323729-4, 5.EC-323729-5.

The loss of variability in *tartary* buckwheat could have occured during the process of domestication. Ohnishi (1998) has suggested that during the process of domestication *tartary* buckwheat acquired four variants through mutations and each of the variants got fixed in the local populations during diffusion of buckwheat cultivation into the Indian Himalayas.

Accessions of *F. esculentum* showed greater closeness with accessions of *F. tataricum* than with *F. cymosum*. Similar observation has also been made by Yasui and Ohnishi (1998a, b) who have

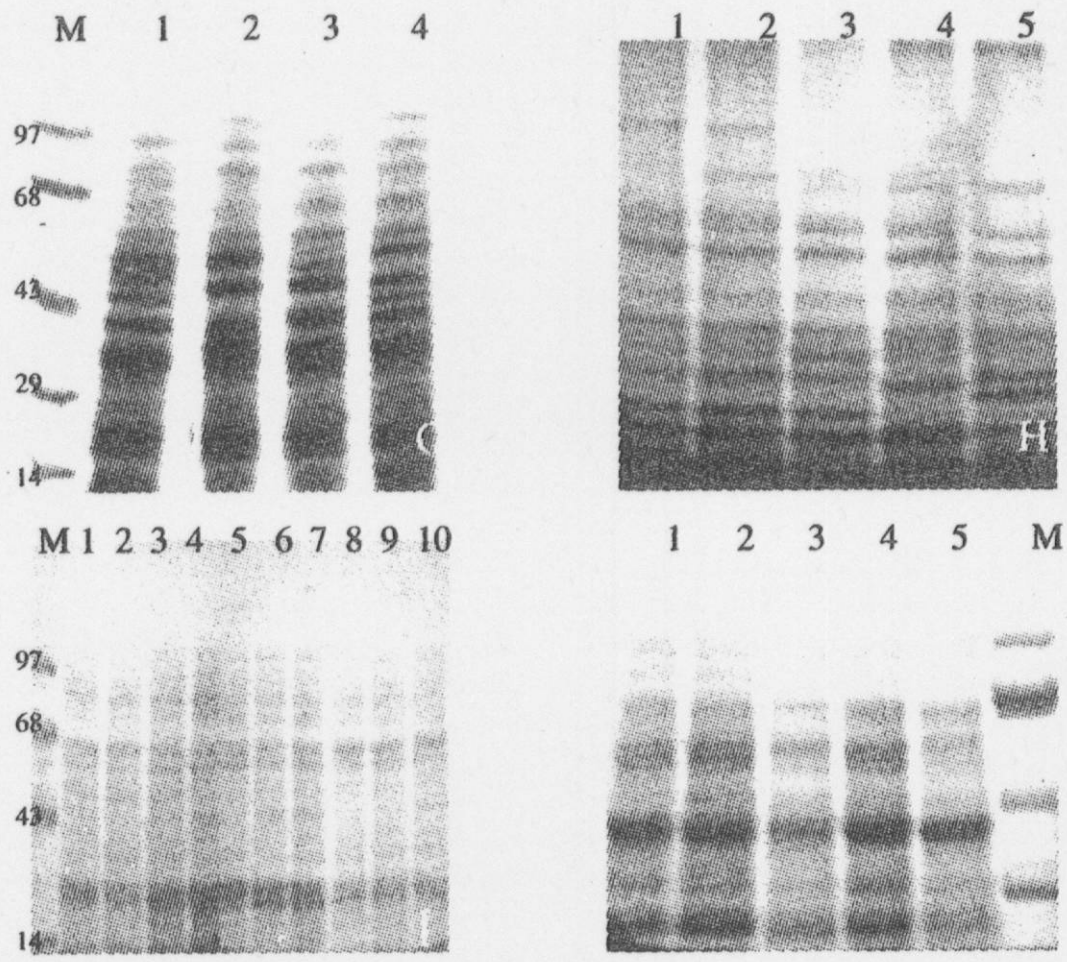

Fig. 1. SDS-PAGE profile of seed proteins from single seeds of *Fagopyrum* spp. — G: Lane 1-OC-2, 2-Kamroo local, 3- Local, 4-VL-7 H: Lane 1-IC-13145-1, 2-.IC-13145-2, 3-IC-13145-3, 4-IC-13145-4, 5-IC-13145-5 I: Lane 1.KBB-3, 2-Himpriya, 3-Kuppa local, 4-Shimla B-1, 5-Sangla B-1, 6-Sangla B-2, 7-Sangla B-3, 8-Sangla B-5, 9-Sangla B-6, 10-Sangla B-7 J: Lane 1–5. *F. cymosum.* (Marker in kD).

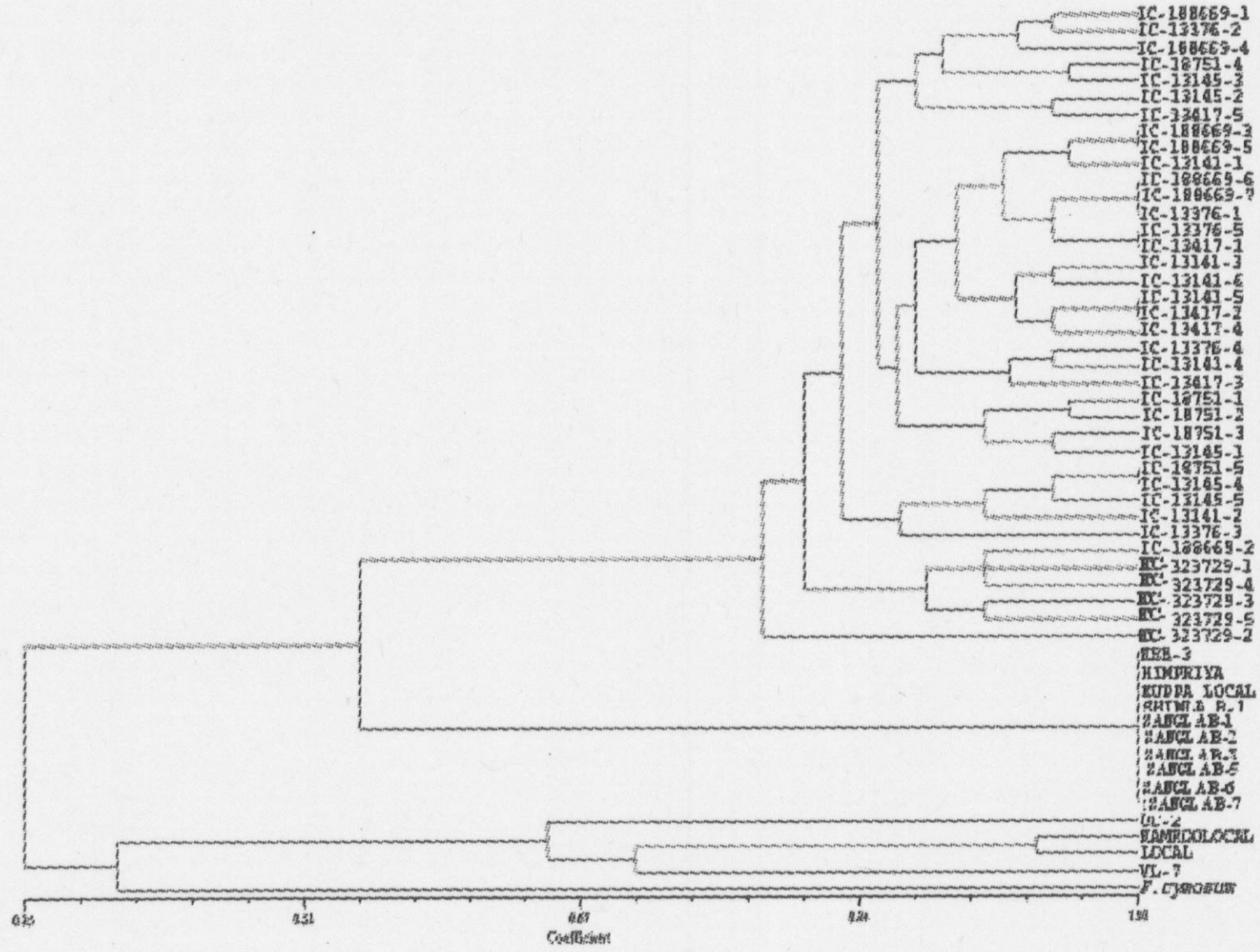

Fig. 2. UPGMA dendrogram generated from SDS-PAGE profile of proteins isolated from single seeds showing clustering pattern among the accessions of *Fagopyrum*.

compared the nucleotide sequence of *rbcl* and *accD* region of cpDNA in the genus. These results are in agreement with the molecular systematic studies of Ohnishi and Matsuoka (1996), Ohsako and Ohnishi (1998) and Ohsako and Ohnishi (2000) on *Fagopyrum*.

Our results agree well with the recognized taxonomic divisions within the genus *Fagopyrum*. Our results, however, indicate that the taxonomic position of IC-13145 needs further investigation. This study has demonstrated the usefulness of single seed SDS-PAGE profile technique in elucidation of biodiversity in the genus *Fagopyrum*.

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References

Bharali, S. 2002. *Isolation, cloning and molecular analysis of the legumin gene of common buckwheat* (*Fagopyrum esculentum*). Ph.D. thesis, North-Eastern Hill University, Shillong, India.

Bradford, M.M. 1976. A rapid sensitive method for quantification of microgram quantities of protein utilizing the principal of dye binding. Analytical Biochemistry **72**:248–254.

Chen L.F.O., M.C. Cheng and S.C.G. Chen. 1987. Similarity and diversity of seed proteins in rice varieties. *Botanical Bulletin of Academia Sinica* **28**:169–183.

Duarte, J.M., J.B. dos Santos and L.C. Melo. 1999. Genetic diversity among common bean cultivar from different races based on RAPD markers. *Genetic and molecular biology* **22**:419–426.

Javornik, B. and B. Kump. 1993. Random amplified polymorphic DNA (RAPD) markers in buckwheat. *Fagopyrum* **13**:35–39.

Jiang, J. and J. Xing. 1992. Dalianshan region in Sichuan province one of the habitats of tatary buckwheat. pp. 17–18, In: *Proceedings of 5th international symposium on Buckwheat*, 20–26 August 1992, Taiyuan, China. Agriculture Publishing House, Taiwan.

Ladizinsky G.W. and T. Hymowitz. 1979. Seed protein electro-phoresis in taxonomic and evolutionary studies. *Theoretical and Applied Genetics* **54**:680–685.

Marshall, H.G. and Y. Pomeranz. 1982. Buckwheat description, breeding, production and utilization, pp. 157–212 In: *Advances in cereal science and*

technology, Y. Pomeranz (ed.), American Association of Cereal Chemistry, St. Paul, MN.

Mignouna, H.D., N.Q. Ng, J. Ikea and G. Thotapilly. 1998. Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. *Journal of Genetics and Plant Breeding* **52**:151–159.

Nishiyama, K., S. Lachman and M. Miura. 1991. Electrophoretic property of buckwheat seed protein. Procceedings of ICOBB, pp. 215–222. Miyazaki.

Ohnishi, T. and Y. Matsuoka. 1996. Search for wild ancestor of buckwheat. II Taxonomy of the *Fagopyrum* (Polygonaceae) species based on morphology, isozyme and chloroplast DNA variability. *Genes and Genetic System* **71**:383–390.

Ohnishi, O. 1998. Search for wild ancestor of buckwheat I. Description of new *Fagopyrum* (Polygonaceae) species and their distribution in China and the Himalayan hills. *Fagopyrum* **15**:18–28.

Ohnishi, O. and N. Asona. 1999. Genetic diversity of *Fagopyrum homotropicum*, a wild species related to common buckwheat. *Genetic Resources and Crop Evolution* **46**:389–398.

Ohsako, T. and O. Ohnishi. 2000. Intra and interspecific phylogeny of wild *Fagopyrum* (Polygonaceae) species based on nucleotide sequences of noncoding regions in chloroplast DNA. The *American Journal of Botany* **87**:573–582.

Ohsako, T and O. Ohnishi. 1998. New *Fagopyrum* species revealed by morphological and molecular analysis. *Genes and Genetic System* **73**:85–94.

Potokina, E., D.A. Vaughan, E.F. Eggi and N. Tomooka. 2000. Population diversity of the Vicia sativa agg. (Fabaceae) in the flora of former USSR deduced from RAPD and seed protein analysis. *Genetic resource and crop evolution* **47**:171–183.

Przybylska J., Z. Zimniak-Przybylska and P. Krajewski. 1998. Diversity of seed albumins in the grass pea (*Lathyrus sativus*): An electrophoretic study. *Genetic resource and crop evolution* **45**:423–431.

Rabbani, M.A., A. Iwabuchi, Y. Murakami, T. Suzuki and K. Takayanagi. 1998. Genetic diversity in mustard (*Brassica juncea* L.) germplasm from Pakisthan as determined by RAPDs. *Euphytica* **103**:235–242.

Rogal, S. and B. Javornik. 1996. Seed protein variation for the identification of common buckwheat (*Fagopyrum* Moench) cultivars. *Euphytica* **87**:111–117.

Rohlf, F.J. 2000. NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Version 2.1. Exeter Publishing. Setauket, NY.

Rout, M.K. and N.K. Chrungoo. 1996. Partial characterization of the lysine rich 13S globulin from buckwheat: Its antigenic homology with seed proteins of some other crops. *Biochemistry and Molecular Biology* **40**:587–595.

Singh S.P., R. Nodari and P. Gepts. 1991. Genetic diversity in cultivated common bean I. allozymes. *Crop Science* **31**:19–23.

Svetek, S. 1994. Electrophoretic analysis of buckwheat (*Fagopyrum esculentum* Moench). Procceedings of IPBA, Rogla, Slovenia. pp. 161–171.

Thompson, J.A., R.L. Nelson and L.O. Vodkin. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Science* **38**:1348–1355.

Tsuji, K. and O. Ohnishi. 1998. Phylogenetic relationships among cultivated landraces and natural populations of tartary buckwheat (*Fagopyrum tataricum*) revealed by RAPD analysis. Procceedings 7th Intl Symp Buckwheat, pp. VI-41–49.

Yupsanis T.M., M. Moustakes and Karakoli. 1992. Seed protein electrophoresis for varietals identification in rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science* **168**:95–99.

Yasui, Y. and O. Ohnishi. 1998a. Interspecific relation among *Fagopyrum* species revealed by nucleotide sequences of the rbcL and accD genes and their intergeneric region. *American Journal of Bot*any **85**:1134–1142.

Yasui, Y. and O. Ohnishi. 1998b. Phylogenetic relationship among *Fagopyrum* species revealed by nucleotide sequences of the ITS region of the nuclear rRNA gene. *Genes and Genetic System* **73**:201–210.