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Genetic Diversity of Storage Proteins in Cultivated Buckwheat

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Abstract: Prolamin and albumin variations of the storage proteins in 76 cultivated buckwheat accessions (55 accessions of *Fagopyrum tataricum*, 21 accessions of *F. esculentum*) from 7 countries were characterized by A-PAGE and SDS-PAGE, respectively, for the purpose of evaluating the genetic diversity of cultivated buckwheat at the level of proteins. A total of 18 prolamin bands were detected, among which 88.89% bands were polymorphic. The number of albumin bands based on SDS-PAGE observed in accessions ranged from 4 to 10. Most intense bands were in the range of molecular weights from 29 to 97.2 kDa. The average of genetic similarity coefficient based on prolamin bands was 0.784 (in *F. tataricum* and *F. esculentum* were 0.892 and 0.681, respectively), while on prolamin and albumin bands was 0.742 (in *F. tataricum* and *F. esculentum* were 0.864 and 0.633, respectively). Accessions of *F. tataricum* and *F. esculentum* showed significant interspecific variation in the A-PAGE and SDS-PAGE profile of the storage proteins. The cluster analysis indicated that all the accessions could be divided into 3 groups and 3 subgroups. The genetic variations among cultivated buckwheat accessions were associated with their geographic origins in some degree.

Key words: Tartary buckwheat (*Fagopyrum tataricum*), common buckwheat (*Fagopyrum esculentum*), genetic diversity, prolamin, albumin

INTRODUCTION

Buckwheat is a minor crop in the world but is an indispensable food in the temperate and hill regions of East Asia and Europe. It is a major staple food crop in the Indo-Himalayan region. Buckwheat is a multipurpose 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, 1998a, b). Among these species, only two types of buckwheat are used as food around the world: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Buckwheat is known as a valuable source of protein and its amino acid composition is nutritionally superior to that of cereal grains (Pomeranz and Robbins, 1972; Ikeda, 2002). Although, the digestibility of buckwheat protein is relatively low (Tomotake *et al.*, 2006), it has many physiological activities which are potential for human health, such as hypocholesterolemic activity in rats,

suppression in body fat, constipation, mammary carcinogenesis and colon carcinogenesis and in the formation of cholesterol gallstones in hamsters (Kayashita *et al.*, 1996; Kayashita *et al.*, 1997; Liu *et al.*, 2001; Tomotake *et al.*, 2000, 2001).

Assessment of genetic variation in a species is important for initiation of effective breeding programs because it provides the basis for tailoring desirable genotypes. SDS-PAGE profiles have been successfully used for analysis on diversity in buckwheat (Ohnishi, 2000; Morita *et al.*, 2006; Tang, 2007). Even though much work has been done on the analysis of genetic variations between different species of the genus *Fagopyrum* using isozyme profiling (Huh and Huh, 2000), RAPD (Murai and Ohnishi, 1996; Kump and Javomik, 2002), AFLP (Tsuji and Ohnishi, 2001; Iwata *et al.*, 2005), not much information is available on inter- as well as intra-specific variations in storage protein variations in cultivated buckwheat. While the storage proteins have the advantage of reflecting numerous variations as they belong to very polymorphic multigenic families (Doll and Brown, 1979). In addition, storage proteins are also reliable bio-chemical makers for the evaluation of genetic diversity (Branlard *et al.*, 1989).

In this study, the genetic variations of storage protein in 76 cultivated buckwheat accessions, which were collected from various areas, were investigated by A-PAGE and SDS-PAGE methods, respectively. The objective of this study was to evaluate the genetic diversity of cultivated buckwheat accessions at the level of proteins with reference to the improvement of buckwheat.

MATERIALS AND METHODS

Plant materials: Seventy-six cultivated buckwheat accessions from 7 different countries were analyzed (Table 1), including fifty-five accessions of *F. tataricum*, twenty-one accessions of *F. esculentum*. Among them, ‘PI’ accessions were kindly provided by Dr. Croston (American National Plant Germplasm System), the others were collected from Sichuan, Yuannan and Tibet Province of China in 2007.

A-PAGE: Prolamin protein were extracted from single seeds with a solution of 25% (V/V) α-ethanol and 0.05% (W/V) methyl green and fractionated by a standard acid-polyacrylamide-gel electrophoresis (A-PAGE) at pH 3.1 (Draper, 1987). PI 481635 and Chayutianqiao was used as reference in A-PAGE analysis for their bands were more and clear distinct relatively than compared with the others.

SDS-PAGE: Glutein proteins were extracted from grains of different accessions of buckwheat with 50 mmol L⁻¹ Tris-Cl buffer (pH 6.8) containing 100 mmol L⁻¹ NaCl, 10 mmol L⁻¹ EDTA, 100 mmol L⁻¹ Glycine, 10% SDS and 1 mmol L⁻¹ phenyl methyl sulphonyl fluoride (PMSF). The

crude homogenate was kept for 45 min at 4°C and centrifuged at 10,000 rpm for 15 min. The extracted proteins were recovered as clear supernatant. SDS-PAGE of the extracted proteins was carried out on 12% isolation gel and 3.7% concentration gel under reducing conditions following the method of Laemmili (1970). For standards we used wide range protein markers (M.W. 6500~200,000, which were synthesized by Shanghai Sangon Biotech Ltd, China). Electrophoresis was carried out at a constant current of 20 mA for 3-4 h. The gels were stained for 3 h in 0.25% (w/v) coomassie brilliant blue R-250 followed by destaining in methanol:water:glacial acetic acid (4:5.3:0.7). Protein bands were visualized in a transilluminator under white light.

Statistic analysis: Evaluation of variation in the storage proteins was performed by calculating the individual band frequency for each accession. Polymorphism was scored for the presence (1) or absence (0) of bands. The data matrix was then used to calculate Genetic Similarity (GS) values according to Nei and Li’s (1979) method:

$$GS = 2N_{ij}/(N_i+N_j)$$

where, N_{ij} is the number of prolamin and albunn bands in common between accessions i and j and N_i and N_j are the total number of prolamin and albunn bands observed for accessions i and j, respectively. Based on the GS matrix, a dendrogram showing the genetic relationships between accessions was constructed using the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) through the computer software NTSYS-pc version 2.10 (Rohlf, 2000).

Table 1: Accessions of cultivated buckwheat used in the study

Species	Origin	Accession/name	No.		
Tartary buckwheat (<i>F. tataricum</i>)	Bhutan	PI 481645, PI 481646, PI 481647, PI 481648, PI 481649, PI 481650,	29		
		PI 481651, PI 481652, PI 481653, PI 481654, PI 481655, PI 481656,			
		PI 481658, PI 481660, PI 481661, PI 481644, PI 481662, PI 481663,			
		PI 481664, PI 481665, PI 481666, PI 481667, PI 481668, PI 481669,			
		PI 481671, PI 481672, PI 481673, PI 481674, PI 481675			
		China		Yingheikuqiao, Zaoqiao, Xiyuanliqiao, Yuanliqiao, Yuanziqiao, Wenchuankuqiao, Kandingkuqiao, Huilikuqiao 1, Huilikuqiao 2 Chuanqiao 1, Chuanqiao 2, Xiqiao1, Jiujiangkuqiao, Kuciqiao, Songmingkuqiao, Mianningkuqiao, Miruikuqiao,	17
		Mexico		PI 451723	1
		Nepal		PI 427235, PI 427237, PI 427238, PI 427239	4
		The United States		PI 199769, PI 476852, PI 503879	3
		Common buckwheat (<i>F. esculentum</i>)		Bhutan	PI 481628, PI 481629, PI 481630, PI 481632, PI 481635, PI 481637,
PI 481638, PI 481639, PI 481642, PI 481643					
China	Chayutianqiao, Songmingtianqiao, Wenchuantianqiao		3		
Nepal	PI 427236		1		
The Soviet Union	PI 280831, PI 280832, PI 633689		3		
The United States	PI 600909, G 24776		2		
Zimbabwe	PI 482595, PI 482596, PI 482597		3		

RESULTS

Prolamin variations: A total of 18 prolamin bands were detected in 76 accessions of cultivated buckwheat (Fig. 1), among which 88.89% were polymorphic. Each accession had 6~12 bands with the mean of 9.46. Thirty-two prolamin patterns were identified. The prolamin patterns in 44 accessions of *F. tataricum* (such as PI 481645, PI 481675, Kangdingkuqiao and Huilikuqiao, etc.) were identical. Thus, these accessions could not be distinguished by A-PAGE.

Based on the prolamin bands, the GS in cultivated buckwheat accessions ranged from 0.375 to 1.000 with the mean of 0.784, indicating that relatively higher genetic diversity existed in cultivated buckwheat. The GS value between PI 451723 and PI 633689 was the lowest. It was showed that the GS values ranged from 0.667 to 1.000 with an average of 0.892 in *F. tataricum*, the GS value between

Huilikuqiao 1 and PI 476852 was the lowest, while in *F. esculentum* was from 0.400 (between Wenchuantianqiao and PI 482595) to 0.947 (between PI 280831 and G 24776) with an average of 0.681. And the average GS value between *F. tataricum* and *F. esculentum* was 0.674. This indicated that distinct quantitative variations were observed between *F. tataricum* and *F. Esculentum* and the genetic variation within *F. esculentum* was more plentiful than in *F. tataricum*.

Albumn variations: A total of 15 albumn bands were detected in the tested materials, among which 93.33% were polymorphic (Fig. 2). Each accession had 4~10 bands with the mean of 7.76. Thirty-one albumn patterns were identified from 76 accessions. The albumn patterns in 45 accessions were identical.

Most intense bands were in the range of molecular weights from 29 to 97.2 kDa. Distinct quantitative

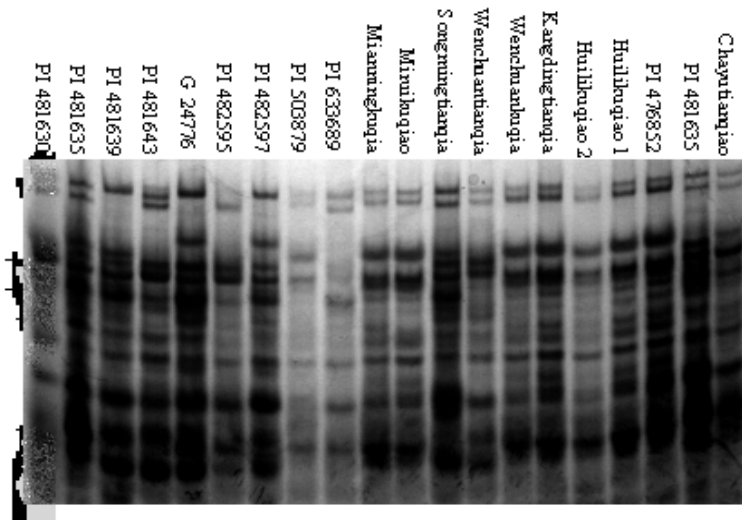


Fig. 1: Prolamin patterns of some cultivated buckwheat

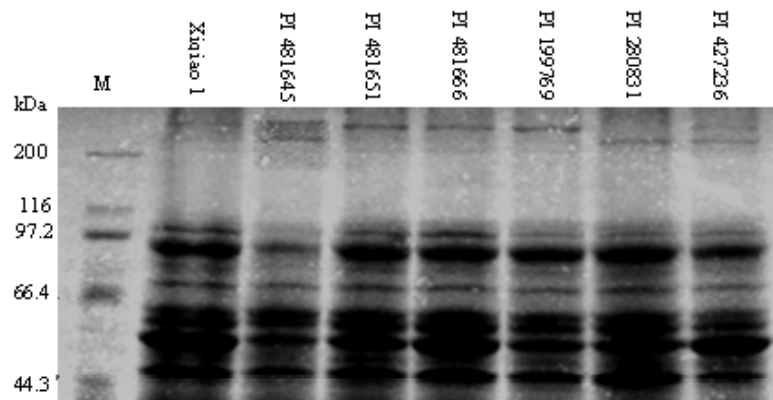


Fig. 2: SDS-PAGE patterns of albumn proteins in some accessions of cultivated buckwheat

variations were observed in the SDS-PAGE profile of storage proteins of *F. tataricum* and *F. esculentum*. Significant intraspecific variations were detected in the storage protein profile of different accessions of *F. esculentum*. This could be ascribed to a high degree of grain protein polymorphism in *F. esculentum*. Most of these variations were observed in the protein bands ranging in size between 29 and 66.4 kDa. 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). While accessions of *F. tataricum* and *F. esculentum* did not show any significant interspecific variations in the SDS-PAGE profile of storage proteins.

Prolamin and albumn variations: Based on the prolamin and albumn bands, the GS in cultivated buckwheat accessions ranged from 0.303 to 1.000 with the mean of 0.742. The GS value between PI 427238 and PI 482595 was the lowest. It was showed that the GS values ranged from 0.563 to 1.000 with an average of 0.864 in *F. tataricum*, the GS value between PI 503879 and PI 481669 was the lowest, while in *F. esculentum* were from 0.412 (between PI 280832 and Chayutianqiao) to 0.972 (between PI 280831 and G 24776) with an average of 0.633. Significant intraspecific variations were detected in the storage protein profile of different accessions of *F. esculentum*. Interspecific variations were existed between *F. tataricum* and *F. esculentum*.

It was showed that the average GS value of *F. tataricum* accessions within one country or between two countries were all higher than that among all the accessions (0.742), while opposite to *F. esculentum*. The average GS value within the accessions of *F. tataricum* from the United States was the lowest, while in Nepal was the highest. The average GS between the United States and other countries were all lower relatively, which inferred that the genetic relationships among the United States were far, relatively. The result was similar to prolamin variations (Table 2).

The result was showed that the average GS value of *F. esculentum* accessions within one country or between two countries were all lower than that among all the accessions (Table 3). The average GS value within Bhutan and China were quite similar (0.736, 0.706, respectively), within the Soviet Union was the lowest. The average GS value between the Untied States and the Soviet Union was 0.664, which was the highest between two countries, the lowest was between China and Zimbabwe. It inferred that the genetic variations among the *F. esculentum* accessions from different countries were higher, the genetic variations within the Soviet Union accessions was relatively higher than other countries accessions of *F. esculentum*.

Table 2: Average value of GS within and between countries based on prolamin and albumn bands of *F. tataricum*

GS	Bhutan	China	Nepal	The United States
Bhutan	0.918			
China	0.883	0.878		
Nepal	0.931	0.890	0.936	
the United States	0.852	0.856	0.870	0.803

Table 3: Average value of GS within and between countries based on prolamin and albumn bands of *F. esculentum*

GS	Bhutan	China	The Soviet Union	The United States	Zimbabwe
Bhutan	0.736				
China	0.583	0.706			
The Soviet Union	0.650	0.583	0.530		
The United States	0.621	0.640	0.664	0.643	
Zimbabwe	0.643	0.513	0.543	0.530	0.598

Based on the prolamin and albumn bands, 76 cultivated buckwheat accessions could be divided into three major groups, too. Of which group 2 included 11 accessions from Bhutan, the Soviet Union and Zimbabwe and just one accession from Zimbabwe (i.e., PI 452597) consist of group (Fig. 3). Group 1 could be further divided into three subgroups. All accessions of *F. tataricum* and six *F. esculentum* (such as G 24776, Chayutianqiao, Songmingtianqiao, etc.) were clustered into subgroup Ia. Two accessions of *F. esculentum* (PI 633689 and PI 600909) which from the Soviet Union and the United States were clustered into subgroup Ib. Subgroup Ic were constituted by PI 481628 from Bhutan. Most of accessions come from the same country were clustered into one group. These results suggested that most of the accessions with close geographic origins had the tendency to cluster together indicating that the their geographic origins variations among cultivated buckwheat were associated with their geographic origins.

DISCUSSION

It has been proposed that prolamin pattern could provide valuable and exact information for the identification and utilization of germplasm. In this study, relatively higher genetic diversity of prolamin was found in cultivated buckwheat, especially in *F. esculentum*. Branlard *et al.* (1989) concluded that the number of diverse patterns was not generally very high for the wheats of a given country. Some results showed that prolamin patterns could reflect the systematic relationships among the type species of four genera in Triticeae. In this study, it was found that the prolamin variations among cultivated buckwheat were associated with their geographic origin. It was likely explained by genetic chemotaxis resulting from the geographical environment or incidental selection of other traits. The

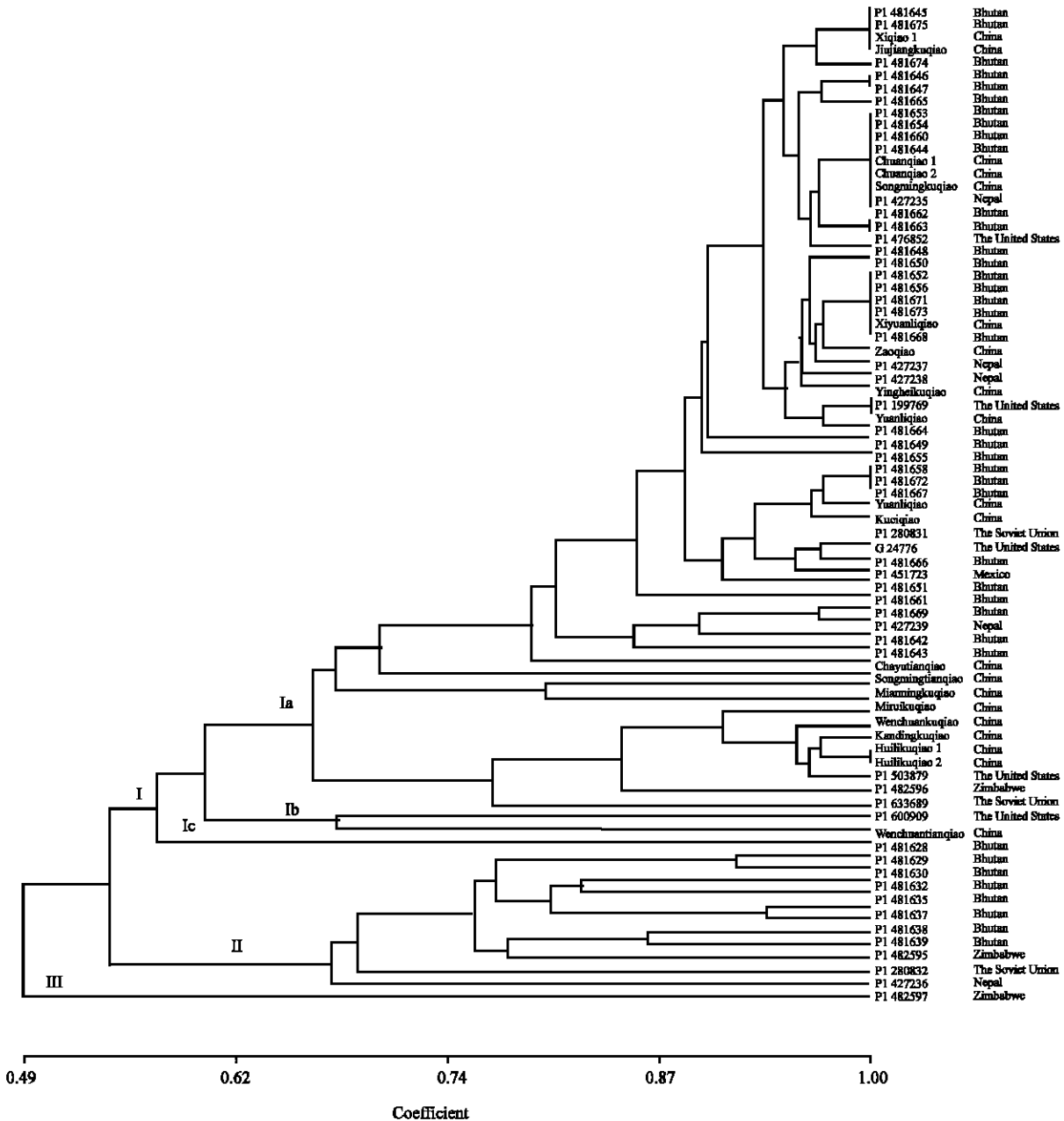


Fig. 3: Cluster analysis of 76 accessions from cultivated buckwheat based on prolamin and albumin bands

prolamin and albumin patterns in most of *F. tataricum* were identical and caused these accessions could not be distinguished by A-PAGE and SDS-PAGE. The reason might be that *F. tataricum* is self pollination crop and its self-cross rate is larger than 95%. Furthermore, the locus detected by A-PAGE and SDS-PAGE were few and it couldn't revealed many information. Moreover, the geographical distribution of some accessions was closer and germplasm resources' exchange could caused fewer genetic variations. For example, in Fig. 3, most of

accessions from China, Nepal and Bhutan were clustered together for these counties are bound on each other.

Particular accessions of *F. esculentum* were clustered with *F. tataricum*. Similar observations have also been made by Yasui and Ohnishi (1998a, b) who compared the nucleotide sequence of the *rbcL-accD* region of cpDNA in the genus. These results are in agreement with the molecular systematic studies of Ohnishi and Matsuoka (1996) and Ohsako and Ohnishi (1998, 2000) on *Fagopyrum*. Present results indicate that *F. esculentum*

exhibited very close relationships with *F. tataricum*. These observations are also brought out by a combined dendrogram generated on the basis variations A-PAGE and SDS-PAGE profiles. These observations are in conformity with earlier findings reported on species relationships in *Fagopyrum* using different marker approaches (Kishima *et al.*, 1995).

In this study, we detected most intense bands were in the range of molecular weights from 29 to 66.4 kDa. Two most intense bands were in the range of MWs from 50 to 60 kDa. These proteins have been shown not to be associated with allergy (Urisu *et al.*, 1995, Nair and Adachi, 1999). Similar observations have also been made by Licen *et al.* (2005) who indicated six intense bands were detected from buckwheat endosperm extracts.

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