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Polymorphism of Subunits Among Four Types of Seed Proteins on Common and Tartary Buckwheat

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Abstract

Polymorphisms of subunits among four types of seed proteins including albumin, globulin, prolamin, and glutelin on 20 common and tartary buckwheat accessions were studied by means of SDS-PAGE. It is clear that albumin and globulin in buckwheat seeds have similar subunit spectrums to each other and all have the highest number of bands in all four types of seed proteins. They are much different from prolamin and glutelin. There are certain differences of the spectrum of four types of seed proteins among accessions. Among them, the differences between common and tartary buckwheat are the biggest. The total intraspecific polymorphism of four types of seed protein samples on common buckwheat or tartary buckwheat produced up to 87 or 72 markers useful in genetic mapping, about four or six times more than their corresponding full seed protein samples.

Keywords: seed protein subunits, buckwheat, albumin, globulin, prolamin, glutelin

1. Introduction

Buckwheat belongs to genus *Fagopyrum* Mill (Lin, 1994; Chen, 1999) and contains about 23 species (Chen, 2012). Among them, there are two cultivated species, that is, common buckwheat (*F.esculentum* Moench) and tartary buckwheat (*F. tataricum* (L.) Gaertn.). The two cultivated buckwheat species are different from each other on genetic properties, origin, and optimum growth environment (Chen, 1999, 2001; Chen et al., 2004) and seed protein constituents.

Buckwheat seed proteins are of high content and of high quality (Guo et al., 2007). They, as full-value protein and a resource of high quality protein, have amino acid composition including all amino acids necessary to human. The seed proteins and their constituents as direct products of genotype are all controlled by genes, reflecting the differences among varieties and among species and being used for identification of varieties and species, for gene mapping as genetic markers (Hu & Wang, 1991; Yan et al., 1999), and for phylogeny and genetic polymorphism (Hu et al., 1984; Ma et al., 1997; Ladizinsky & Hymowitz, 1979a, 1979b). There are many related researches in rice (Li et al., 1996), corn (Su et al., 2002), cotton (Zhang et al., 1998), pea (Xia & Xing, 2003), and cushaw (Huang et al., 2005) crops.

In 1907, OSBORNE (see Osborne et al., 1986) classified the seed protein into four categories by their solubility in different solvent: albumin dissolved in water, globulins dissolved in salt solution, prolamin dissolved in alcohol solution, and the remaining glutelin dissolved only in acid or alkali solution.

There are some reports on contents of the four types of seed proteins. Li and Ding (2000) reported that there are 40%-70% of albumin and globulin, 0.7%-2.0% of prolamin, and 23%-59% of glutelin and other protein leftover in commercial buckwheat flour. JAVORNIK et al. (1981) reported the similar proportion of 18% of albumin, 43 % of globulin, 1% of prolamin, and 38% of glutelin and other protein leftover. Pomeranz (1983) reported that there are 80% of albumin and globulin in common buckwheat seed proteins. Wei et al. (1995) reported that common buckwheat seed proteins contain 31.8%-42.3% of albumin, 25.4%-26.1% of glutelin, and 1.7%-2.3% of prolamin. Guo and Yao (2006) reported that albumin was the predominant protein fraction (43.8%) followed by glutelin (14.6%), prolamin (10.5%), and globulin (7.82%) in tartary buckwheat flour.

Zeller et al. (2004) reported the spectrum of protein subunits of the whole seed on common buckwheat. Li et al.

(2008) reported the spectrum of protein subunits of the whole seed on 10 buckwheat species. But there are few reports in genetic variation among the four types (albumin, globulin, glutelin, and prolamin) of seed protein on common and tartary buckwheat.

This study will focus on the genetic variations of four types of seed proteins on cultivated buckwheat, in order to provide some ways to genetic mapping, proteomics, and evolution research of common and tartary buckwheat.

2. Materials and Methodes

Random twenty accessions of common buckwheat and tartary buckwheat were provided by IPGB (the Institute of Plant Genetics and Breeding, Guizhou Normal University) and used in this study (Table 1).

Table 1. Buckwheat accessions and their name, symbol, and origin

Species	Accessions	Symbol	Name	Native to
Common buckwheat	ES2004062003	E88	Sobano	Germany
	ES2004092501	E91	Wild Tian	Yunnan
	ES2004102902	E69	QianxiTian	Qianxi, Guizhou
	ES2004102901	E24	WugangTian	Wugang, Hunnan
	ES2004081101	E62	BijieTian	Bijie, Guizhou
	ES2004082001	E85	CzechTian	Czech
	ES2004010102	E48	WeiningTian 1	Weining, Guizhou
	ES2005061606	E20	YuQiao 4	Yulin, Shaanxi
	ES2004092801	E77	DaozhenTian	Daozhen, Guizhou
	ES2005061603	E16	LiuQiao 1	Liupanshui, Guizhou
Tartary buckwheat	TA2004081101	T11	JiujiangKu	Jiujiang, Jiangxi
	TA1999111501	T7	Kuqiao 1	Guizhou
	TA2001100501	T43	YanheKu	Yanhe, Guizhou
	TA2004102902	T8	WugangKu	Wugang, Hunan
	TA2004041502	T22	QianheiQiao 1	Weining, Guizhou
	TA2004102901	T25	QianxiKu	Qianxi, Guizhou
	TA2003100102	T37	ShuichengKu	Shuicheng, Guizhou
	TA2001112201	T14	Kuciqiao	Weining, Guizhou
	TA2001112202	T45	LaoyaKu	Weining, Guizhou
	TA2004060102	T29	WeiningKu 3	Weining, Guizhou

2.1 Preparation of Seed Protein Samples

The preparation for total protein samples of the whole seed followed Li et al. (2008). Their extracting solution was made up of 50 μl of 55% isopropyl alcohol, 20 μl of 1 mol/L Tris-HCl (pH 8.0), and 30 μl of double-distilled water. The above extracting solutions mixed with 100 μl of solution C (including 10 μl 10% SDS, 20 μl glycerin, and 10 μl double-distilled water) were prepared for SDS-PAGE.

The preparation of samples of albumin, globulin, prolamin, and glutelin in seed total protein followed the above preparation procedures of the total protein samples of the whole seed except different extracted solutions which are ddH_2O, 3% NaCl solution, 20% ethanol solution, and 0.1 mol/L acetic acid solution, respectively. These samples for albumin, globulin, prolamin, and glutelin are prepared independently from seed powders.

2.2 SDS-PAGE Electrophoresis

The main procedures for SDS-PAGE see LI et al. (2008). Three layers of gel, that is, a layer of concentration gel and two layers of separation gel, were used for SDS-PAGE analysis of buckwheat seed protein. The main parameters of the two layers of separation gels were as follows: T (total content of Acr and Bis in gel) = 15.6%

and 11.3% respectively, C (crossing ratio, that is a rate of Bis / (Acr+Bis)) = 2.6%, and pH 8.8 and 6.8 respectively; with SDS added to each layer. The SDS-PAGE was run at 4 ^{o}C and 20 mA for 12 h.

2.3 Dyeing

After electrophoresis, the gel was dyed for about 24 hours in a solution prepared by dissolving 0.125g Coomassie Brilliant Bright Blue R250 in 500 ml of fixative (454 ml 50% methyl alcohol and 46 ml glacial acetic acid) and then destained twice in a solution containing 7.5% glacial acetic acid and 4.6% methyl alcohol until the gel was clear, and then it was photographed for analysis.

2.4 Analysis of Data

The molecular weight markers of protein subunits used in this experiment were 116.0, 66.2, 45.0, 35.0, 25.0, 18.4, and 14.4 kDa, which were made by Fermentasg. A standard curve was obtained using these markers, and the molecular weight of seed protein subunits estimated.

3. Results and Analysis

The spectrums and idiograms of four types of seed protein and the seed total protein on 10 common buckwheat accessions and 10 tartary buckwheat accessions were showed in Figures 1-3.

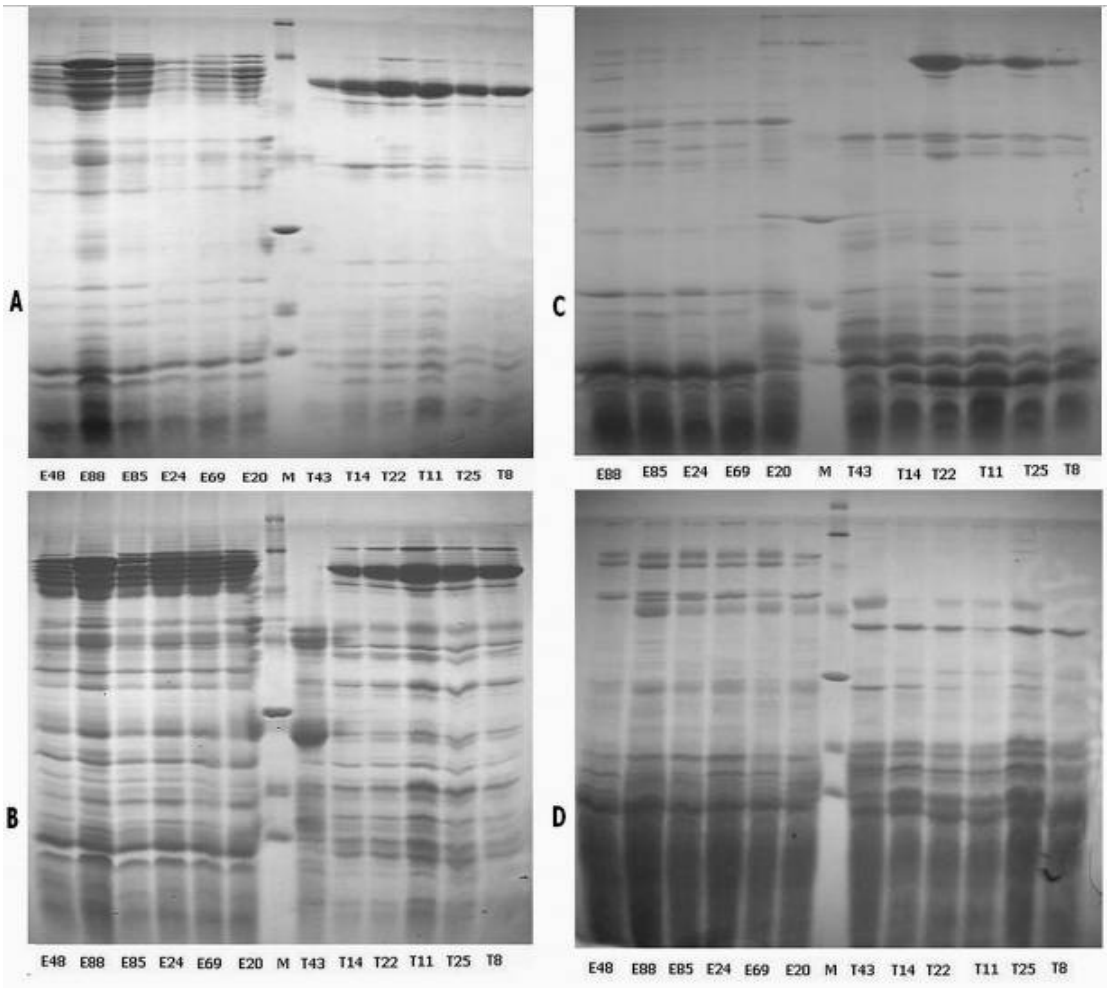

Figure1. The spectrums of albumin (A), globumin (B), prolamin (C), glutelin (D) subunits of some common buckwheat accessions and tartary buckwheat accessions

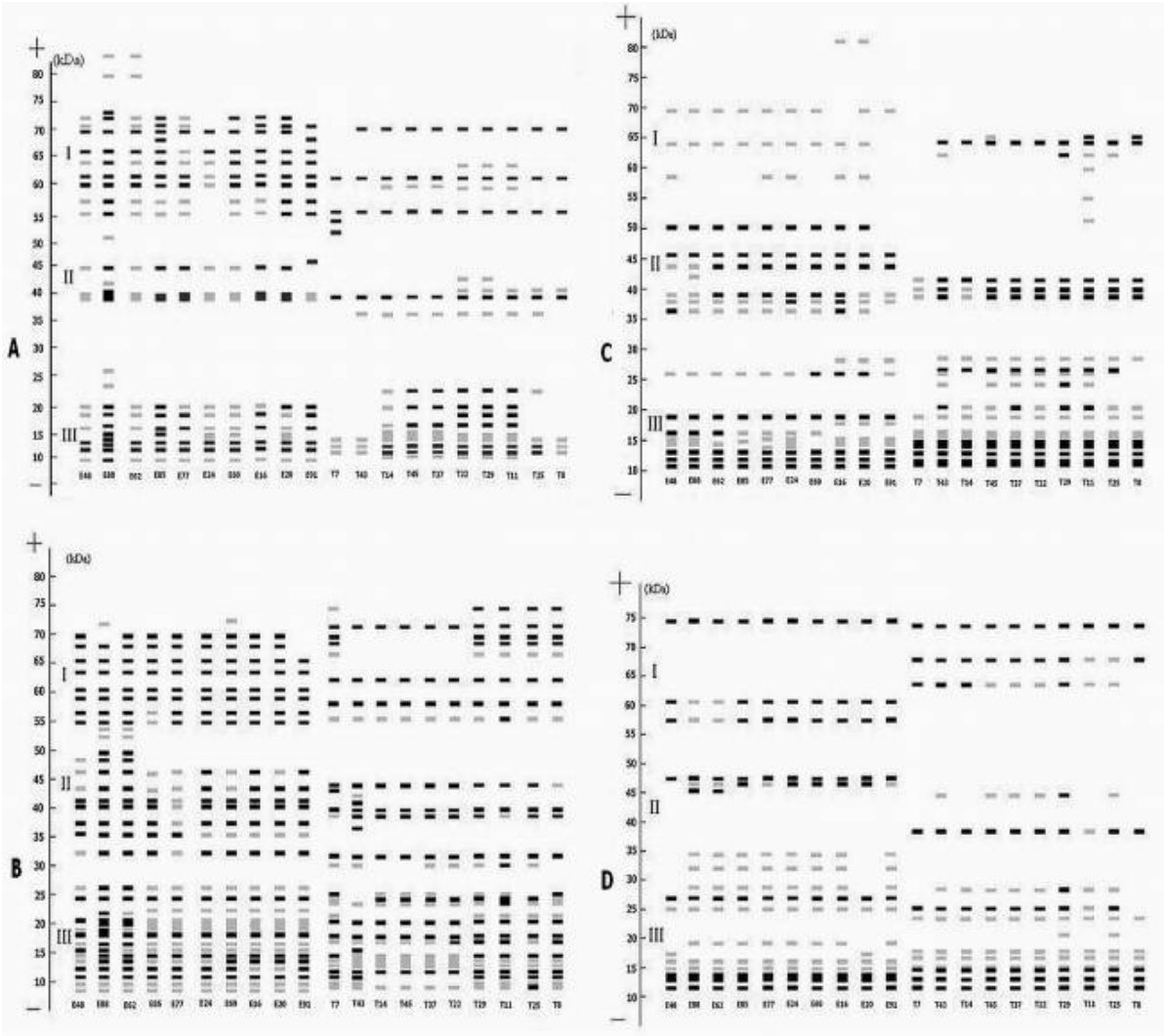

Figure 2. The idiograms of albumin (A), globumin (B), prolamin (C), glutelin (D) subunits of 10 common buckwheat accessions and 10 tartary buckwheat accessions

Notes: Dark rectangles denote stable bands among different seeds of the accession and light rectangles denote bands that vary among different seeds of the accession.

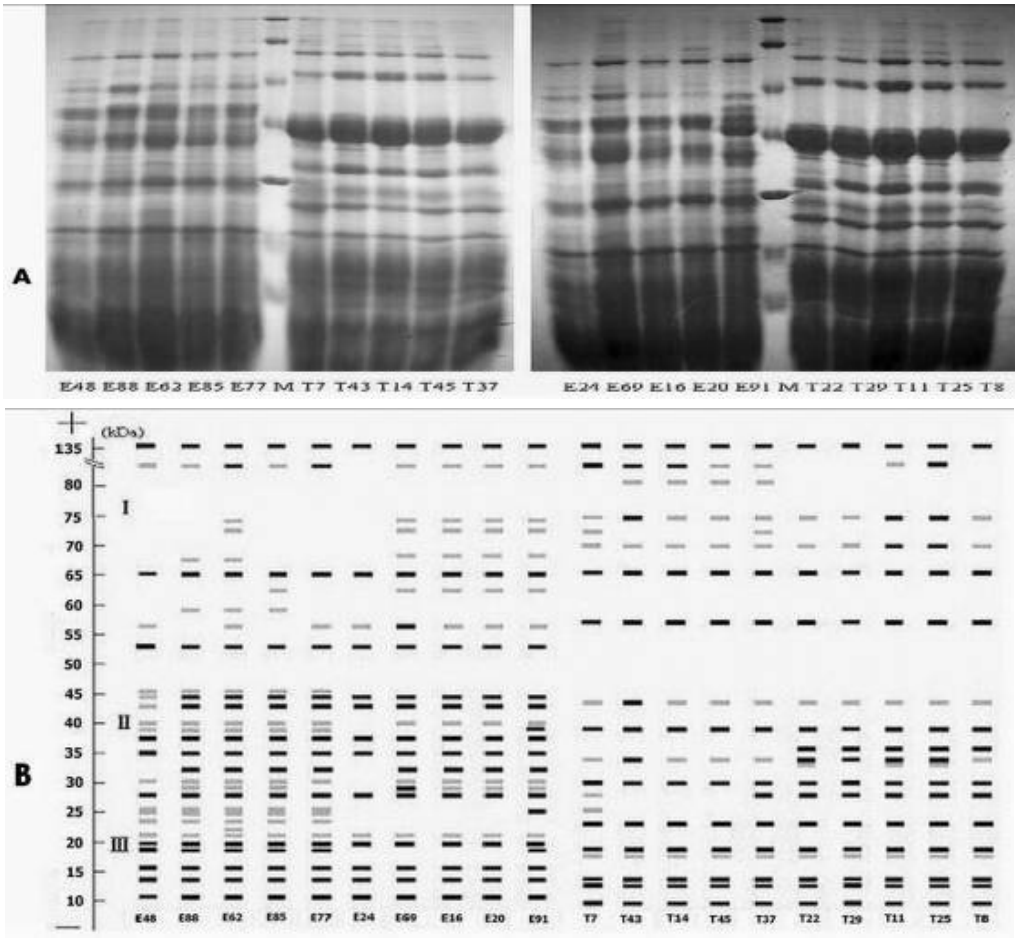

Figure 3. The spectrum (A) and idiogram (B) of seed protein subunits of 10 common buckwheat accessions and 10 tartary buckwheat accessions

Notes: Dark rectangles denote stable bands among different seeds of the accession and light rectangles denote bands that vary among different seeds of the accession.

3.1 Albumin

Figures 1A and 2A showed that, common buckwheat has richer bands of albumins with molecular weight range of 9-85 kDa than tartary buckwheat. Among them, the bands in range of 55-75 kDa in common buckwheat are very dark and different from those in tartary buckwheat. There are obvious variations among accessions of common buckwheat, especially E88 having the highest number of protein subunit bands (up to 25 bands) and E24 the lowest (only 14 bands). E88 and E62 have the high molecular weight band PS83.5. All ten common buckwheat accessions shared twelve bands, that is, PS66.2, PS64.0, PS61.8, PS59.4, PS39.5, PS38.2, PS20.1, PS18.4, PS16.6, PS13.5, PS11.9, and PS9.3. Except these shared bands, there are 18 bands varied among different accessions of common buckwheat.

The albumin subunits of tartary buckwheat accessions had totally 21 bands, fewer than common buckwheat. Among them, the band in range of 63.7-58.4 kDa is a wide black band, maybe consisted of many subunits. There are also obvious intraspecific variations among accessions. Among them, the accessions T22, T29, and T11 have the highest number of subunits up to 18 bands and T7, T8, and T43 the lowest with only 8 bands. Among total 21 bands, there are six bands (PS61.0, PS55.5, PS38.9, PS13.5, PS12.8, and PS11.9) shared by all ten tartary buckwheat accessions and 15 bands varied among accessions.

According to the difference of intraspecific shared bands of albumins, common buckwheat and tartary buckwheat can be identified.

3.2 Globulin

Figures 1B and 2B showed that there are 36 bands of globulin subunits with molecular weight range of 8-75 kDa

in ten common buckwheat accessions, similar to their albumin subunit range. Among them, most of bands are shared by most common buckwheat accessions. E62 have the highest number of bands (up to 33) and E91 have only 14 bands.

Ten tartary buckwheat accessions have 32 bands of globulins. Among them, most are shared by all tartary buckwheat accessions and only ten varied among accessions. Among all ten tartary buckwheat accessions, T7 has the highest number of bands (26 bands) and T14 and T45 have the lowest number of bands (18 bands).

There are obvious differences between common buckwheat and tartary buckwheat in band number and spectrum of globulins.

3.3. Prolamin

Figures 1C and 2C showed that ten common buckwheat accessions totally have 22 bands of prolamin in range of 10-70 kDa. Among them, there are 10 bands with variation and six black shared bands (PS45.8, PS44.1, PS37.3, PS38.8, PS26.3, and PS18.8) within the species. E20 have the highest number of bands (up to 18) and E91 have the lowest (only 13 bands).

Tartary buckwheat accessions also have 22 bands of prolamin. Among ten tartary buckwheat accessions, T11 has the highest number of bands (19 bands) and T7 has the lowest (only 8 bands).

There are the special bands PS50.5, PS45.8, PS44.1, PS37.3, and PS36.1 in common buckwheat, different from those (PS41.8 and PS40.2) in tartary buckwheat, which can be used in identification of common buckwheat and tartary buckwheat.

3.4 Glutelin

Figures 1D and 2D showed that there are 17 glutelin bands in ten common buckwheat accessions in range of 10-75kDa. Among them, ten bands are shared by accessions and 7 bands are polymorphic. Most accessions (60%) have the same spectrum of glutelin subunits. Among all accessions of common buckwheat, E88 and E62 have the highest number of bands (17 bands) and E48 the lowest (11 bands). All common buckwheat accessions shared ten bands and have 7 bands varied among accessions.

There are total 15 bands of glutelins in ten tartary buckwheat accessions. T25 has all 15 bands and T8 has the fewest bands (10 bands). 40% of tartary buckwheat accessions shared the same spectrum.

Common buckwheat accessions have special bands PS74.6, PS60.8, PS57.3, PS47.3, and PS26.7, and tartary buckwheat accessions the special bands PS73.8, PS67.6, PS63.8, PS38.2, and PS23.7. Glutelins have more obvious differences of subunit spectrum between common buckwheat and tartary buckwheat than albumin, globulin, and prolamins.

3.5 Total Protein Subunits

Figures 3A and 3B showed that total protein subunits on 10 common buckwheat accessions have 31 bands mainly distributed in the range of 10-75 kDa. Among them, there is a high molecular weight band of 135.7 kDa. All 10 common buckwheat accessions shared subunits PS135.7, PS65.8, PS52.6, PS44.5, PS42.8, PS37.1, PS35.3, PS27.0, and PS19.4.

The total protein subunit spectrums on ten tartary buckwheat accessions have totally 22 bands and shared PS135.7, PS65.8, PS56.6, PS38.9, PS29.9, PS22.6, and PS18.7, with similar range to common buckwheat.

3.6 Comparison of Spectrums of Four Types of Seed Protein With Total Protein Spectrum

Comparisons of subunit spectrum between total proteins and their four different types of seed protein on common buckwheat and tartary buckwheat were listed in Table 2. It is clear that total protein samples of common buckwheat and tartary buckwheat all have richer bands than their four types of seed proteins respectively but do not cover all of their four types of protein bands and that the preparation of the protein samples in different solvents can produce a lot of new bands, indicating the extracting solvent for total protein can not extract all protein in buckwheat seeds. Total seed protein samples of common buckwheat and tartary buckwheat have some special bands different from those of albumin, globulin, prolamin, and glutelin, indicating that the four solvents can not also extract all proteins in buckwheat seeds.

Table 2. Comparison of seed protein subunits among full seed proteins and their four different constitutional types

Species	Protein types	albumin	globulin	prolamine	glutelin	∑	Full protein
Common buckwheat	Total number of bands	30	36	22	17	105	31
	Range of band number among accessions	14-25	14-33	13-18	11-17	\	14-29
	Constant band	2	7	5	5	19	10
	Variable band	29	29	17	12	87	21
	MW range (kDa)	9-83.5	10-75	10-83	10-75		10-135
Tartary buckwheat	Total number of bands	21	32	22	15	90	22
	Range of band number among accessions	8-18	18-26	8-19	10-15	\	17-19
	Constant band	3	8	3	4	18	10
	Variable band	18	24	19	11	72	12
	MW range (kDa)	9-71	10-75	10-65	10-75	\	10-135
Number of interspecific polymorphism bands		18	33	16	16	83	20

Note: MW stands for molecular weight.

Table 2 also showed that there are 105 and 90 total seed protein subunit bands from four different types of seed proteins on common buckwheat and tartary buckwheat, respectively, 3.4 and 4.1 times as many as those from total protein samples of the whole seed, respectively. There are 89 and 72 total variable seed protein subunit bands from four different constitutional types of seed protein of common buckwheat and tartary buckwheat, respectively, 4.1 and 6.0 times as many as those from total seed protein samples, respectively. There are 83 total interspecific variable seed protein subunit bands from four different types of seed protein between common buckwheat and tartary buckwheat, 4.2 times as many as those from total seed protein samples. These results indicated that SDS-PAGE of four different types of seed protein can produce much more total bands and variable bands than total seed protein samples.

4. Discussion

4.1 Identification of Buckwheat Species and Varieties by Means of Four Types of Seed Protein

Su et al. (2002) reported that the four types of corn seed protein by means of SDS-PAGE can be used for variety identification. Jia et al. (1998) showed that water-solution proteins are the main part in cotton seed protein. Huang et al. (2005) discovered that cushaw (*Cucurbita*) have water-soluble seed protein much different from their salt-soluble seed protein, which can be used as protein fingerprint in identification of species and variety.

Researches on seed protein subunits of buckwheat focused mainly on common buckwheat and tartary buckwheat by means of total protein samples of seeds (Tamami et al., 1995; Zeller et al., 2004). There are few reports about four types of buckwheat seed protein. This study first focused on variation among accessions of common buckwheat and tartary buckhwheat based on four types of buckwheat seed protein and discovered that there are many intraspecies and interspecies variations of anyone of the four types.

Li et al. (2008) showed that because of heterostyle and insect-media cross-pollination of common buckwheat there are fewer variations among common buckwheat accessions than those among tartary buckwheat accessions. The results in this study discovered greater variations of four types of buckwheat seed protein, four or six times as many as their total seed protein samples, which can be provide rich seed protein finger markers for genetic mapping on common buckwheat. And this study also showed that there are some different special bands of four types of seed protein on common buckwheat from tartary buckwheat, indicating an effective way to identification of buckwheat species.

This study also showed that the total seed protein samples of common buckwheat and tartary buckwheat do not include all bands in spectrum of albumin, globulin, prolamin, and glutelin, indicating incomplete extraction of total seed protein. The way to extract all protein of buckwheat seeds needs further research.

4.2 Composition of Four Types of Buckwheat Seed Protein

Bejosano and Corke (1999) reported four types of buckwheat seed protein and discovered that in common buckwheat seeds globulins and glutelins are composed of several ranges of seed protein subunits, albumins mainly comprise lower molecular weight protein subunits, and prolamins consist of middle- and lower-molecular weight protein subunits. Tamami et al. (1995) reported the main protein of common buckwheat seed powder and showed that prolamine are composed of the molecular range (30-40 kDa) of protein subunits and glutelins show single molecular weight range (80-90kDa). Wei (1995) reported that globulins of tartary buckwheat are composed of subunits with molecular weight 43.0kDa and 27.0kDa. Kayashita et al. (1995) discovered two globulin subunits with 67.9kDa and 44.3kDa in tartary buckwheat. The results of common and tartary buckwheat in this study showed that albumin and globulin are similar to each other, mainly composed of high molecular weight range (55-70 kDa) and covered a wide range (8.0-75.0 kDa), and glutelins comprise several molecular ranges (57.3-75 kDa, 25.0-35.0 kDa, 11.8-19.3 kDa), prolamins mainly consist of high (60-70 kDa) and low molecular weight ranges (10.9-18.8 kDa), which are much richer than those from the above reports. Their reasons resulting in the differences among reports may be the differences on accession number, genetic ground, and research methods.

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