The Content Variation of 7S, 11S Globulins and Their Subunits of Seed Storage Protein of 706 Chinese Soybean Germplasm*

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Abstract The contents of 7S and 11S globulins and their respective subunits, and the 11S/7S ratios were tightly associated with the nutrition and processing properties of soybean protein. For improving the nutritional qualities and the functional properties of soybean seed proteins, obtaining germplasm with different contents of 7S and 11S globulins and their respective subunits and different ratios of the 11S/7S is a desirable objective. 706 accessions from Chinese germplasm were investigated by Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS – PAGE). The results showed that 7S globulin was divided as three subunits (α′, α, β), and 11S globulin was divided into three bands which were represented as A_3, $A_1aA_1bA_2A_4$ (Acid subunits) and $B_1aB_1bB_2B_3B_4$ (Basic subunits), respectively. There were great genetic diversity of the relative contents of 11S, 7S globulins and their subunits in 706 Chinese soybean germplasm. Remarkably negative correlation between the relative contents of 7S and 11S globulins ($r = -1.00$, $P < 0.01$) existed. The average and variation range of the relative content of 7S, 11S fraction of 603 Chinese landraces and 103 cultivars were 40.00%, 20.58% ~ 56.65%, 60.00%, 43.35% ~ 79.42% and 38.21%, 30.33% ~ 52.67%, 61.79%, 47.33% ~ 69.67%, respectively. The average and variation range of the 11S/7S ratios of 603 Chinese landraces and 103 cultivars were 1.54, 0.77 ~ 3.86 and 1.65, 0.90 ~ 2.30, respectively. 63 accessions screened from 706 germplasm showed polymorphism of band type for the subunit relative contents.

Key word *Glycine max* (L.) Merrill; Germplasm; SDS – PAGE; 11S, 7S globulin and their subunits; Relative content

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Soybean is one of the major protein sources for human and livestock. Soybean protein has the physiological and healthful functions, such as anti-cancer, preventing heart and vein diseases, and the properties related to food hobby and processing (Fukushima et al., 1991; Liu et al., 2000; Jiang et al., 2000). The major components of soybean seed proteins are 11S globulin (glycinin) and 7S globulin (conglycinin), which account for about 70% of the total seed protein and are tightly cor

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relative to the nutrition and properties of protein such as gel-making ability, thermal stability, and emulsifying capacity (Fukushima et al., 1991; Kitamura et al., 1995; Yagasaki et al., 2000). Compared with the 7S globulin, the 11S globulin is relatively rich in sulfur-containing amino acids, which are restrictive amino acids of soybean protein nutrition. Furthermore, the 11S globulin shows superior functional gel and film properties to those of the 7S globulin, and the 7S globulin shows superior functional emulsifying capacity to the 11S globulin. The 11S/7S protein ratio has been reported to correlate positively with many functions and properties of soybean protein, and used as a simple index to evaluate the relation between the contents of protein compositions and the functions and properties (Kitamura et al., 1995). For improving the nutritional qualities and the functional properties of soybean seed proteins, obtaining germplasm with different contents of 7S and 11S fraction and their respective subunits, and different ratios of the 11S globulin/7S globulin is a desirable objective (Ogawa et al., 1989; Tsukada et al., 1986; Fukushima et al., 1991; Kitamura et al., 1995; Hajika et al., 1998; Yagasaki et al., 2000).

Kitamura et al. (1981) found the average 11S/7S ratios to be 1.2 in 1700 germplasm, and obtained 2 accessions with high ratios. Xu Bao (1990) analyzed the 11S/7S ratios of 213 wild soybean accessions and found the ratio variation range to be 0.36~4.40. Wang Lixia et al. (2004) found the average 11S/7S ratios to be 1.885 in 1757 germplasm. The content variation of 11S and 7S globulins and their respective subunits, and their ratios in Chinese germplasm are still known little. Therefore, to evaluate properly the content variation of 11S, 7S globulin and 11S/7S ratios of Chinese germplasm, and to excavate the elite of the protein subunit mutants, are necessary for the purposes of the genetics and breeding research (Wang et al., 2004). In this paper, we present the relative content variation of 11S and 7S globulins and their ratios of 706 Chinese germplasm, and the content mutant types of protein subunits screened out.

1 Materials and Methods

1.1 Materials

706 Chinese germplasm were included in this study. The 603 accessions of them came from different ecotypic region of China, and 103 were the cultivars released or great extended recently in China. All accessions were planted in June, 2002, with two replications at Jiangpu Experimental Station of Nanjing Agricultural University, Nanjing, China. Each of accessions was grown in three row plot 1.5 m long with 0.4 m between rows. The soil was the clay loam with 7.1pH, which contained 0.67% organic matter, 0.11% total nitrogen, 0.052‰ rapidly available phosphorus and 0.72‰ rapidly available Potassium. The plots were harvested and the seeds were used as materials for protein SDS PAGE in Lab.

1.2 Protein sample preparation

The defatted soy flour was obtained by pulverizing the peeled soybean seeds of experimental materials, degreased with aether (w/v = 1:3) overnight. 0.25g of soybean meal were homogenized with 5 ml of 0.05 M tris hydrochloric acid (Tris-HCl) (pH8.0) containing 0.01 M 2-mercaptoethanol, extracted for 1 hr under room temperature, and centrifuged for 15 min (5000rpm). Total globulins were precipitated by adjusting pH of the supernatant to 4.5 and centrifuging for 10 min (5000rpm), and then freeze-dried and stored at 4℃ until analysis. The total globulin fraction was dissolved in 1.0 ml of 0.1 M tris hydrochloric acid solution (Tris-HCl) (pH8.0) containing 0.2% sodium acetate buffer (SDS), 0.01 M 2-mercaptoethanol, 30% sucrose, and 5 M urea. 10μl of the solution was applied to the gel.

1.3 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The protein was fractioned by SDS polyacrylamide gel electrophoresis using a condensed gel concentration of 5% and a separation gel concen-

tration of 9.5%. Electrophoresis was carried out at 12 mA for 1 hr, then 25 mA for 4 ~ 5 hr, with 0.025M tris hydrochloric acid solution (Tris-HCl) as the electrode buffer.

The gel was stained for half an hour in 0.2% Coomassie brilliant blue G250 in water methanol acetic acid (50: 43: 7) and destained with water – methanol – acetic acid (17: 2: 1) overnight.

The electrophoretic patterns were analyzed by ImageMaster VDS of Pharmacia Biotech with ImageMaster 1D Elite V4.00 soft. The relative protein quantity of each subunit (protein band) of 11S and 7S fraction was calculated from their respective percent area on the densitograms against the total subunits area of 11S and 7S globulin, and then the 11S/7S protein ratios were estimated.

2 Results and Discussion

2.1 SDS – PAGE patterns and scanning profiles

Fig. 1 showed the SDS-PAGE patterns and scanning profiles of the 11S and 7S fractions of soybean major storage protein. The protein bands were basically similar among all 706 Chinese soybean germplasm. The subunits of 11S and 7S globulins were well separated by SDS – PAGE. The 7S protein fraction (β – conglycinin) was separated into three band types (α′, α, β subunits). The 11S protein (glycinin) was separated into three band types (an acidic A_3 polypeptide, a group of acidic polypeptides ($A_{1a}A_{1b}A_2A_4$) and a group of basic polypeptides ($B_{1a}B_{1b}B_2B_3B_4$)). The A_3 polypeptide and the polypeptide group of $A_{1a}A_{1b}A_2A_4$ formed the acidic subunits. The polypeptide group of $B_{1a}B_{1b}B_2B_3B_4$ formed the basic subunits. These results were basically consistent to earlier reports (Xu Bao et al., 1990; Ogawa & Tayama et al., 1989; Hajiki et al., 1996).

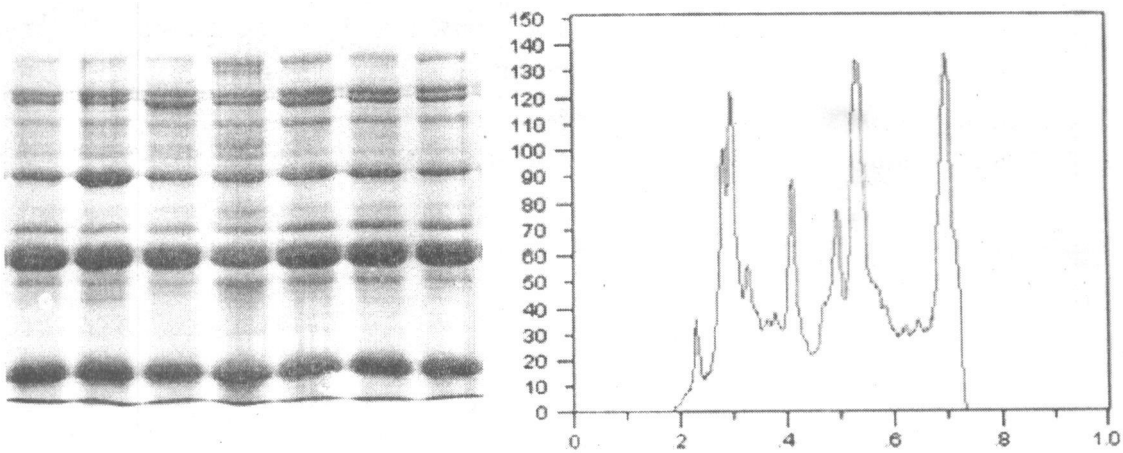

Fig. 1 SDS – PAGE pattern and scanning profile of 11S and 7S fractions of soybean seed storage protein
(Note: A and B indicated the polypeptide groups of $A_{1a}A_{1b}A_2A_4$ and $B_{1a}B_{1b}B_2B_3B_4$, respectively)

2.2 Quantification of 11S and 7S proteins and their subunits by densitometry

The 706 germplasm were divided into two groups for data analysis. One group (Group I) is the 103 cultivars released or extended recently. Another group (Group II) is the landraces (603 accessions). The results of statistical analysis showed that (Table 1).

2.2.1. There were great genetic variations of the relative contents of 11S, 7S globulins and their respective subunits in soybean germplasm, especially for that of Basic subunits (11.34% ~ 40.54% for the cultivar group; 10.23% ~ 46.59% for the landrace group). All of the frequency distributions of the relative content of 11S, 7S fraction and their subunits appeared to be normal distributions (Fig. 2: A, B).

2.2.2 The relative content variation ranges of the 7S, 11S globulins and their respective subunits of the landraces group (Group II) were basically greater than that of the cultivars group (Group I), except for that of $A_{1a}A_{1b}A_2A_4$ (A). This indicated that the genetic diversity of 7S, 11S globulins and their subunits of the landraces were not yet made

the best of in our soybean breeding.

2.2.3 The variation coefficient of 7S protein fraction was great than that of 11S protein fraction.

2.2.4 The variation ranges and coefficient of 11S/7S ratios of the landraces group (0.77~3.86, 21.36%) were great than that of the cultivars group (0.90~2.30, 18.06%). 18 accessions with low ratios (minor than 1) and 4 accessions with high ratios (more than 2.5) were identified from the 706 germplasm, especially for one with the ratio of 3.86. The average 11S/7S ratios of the groups of 603 Chinese landraces (Group Ⅱ) and 103 cultivars (Group I) was 1.54 and 1.65, respectively, and both great than that (1.12) reported by Kitamura (1981) in investigation of 1700 landraces and minor than that (1.85) reported by Wang LX et al. (2004) in investigation of 1757 landraces with the same method.

2.2.5 The frequency distributions of 11S/7S ratios of 706 germplasm appeared to be normal distributions (Fig. 2: C).

The investigating results indicated that it is feasible to excavate the content variation elite of soybean protein subunits from Chinese germplasm for the study and utilization of genetics and breeding.

Table 1 The relative content variation of the 11S and 7S, and 11S/7S ratios of soybean seed storage proteins

Group	Item	No. of accessions	Range of variation	Mean±Sd	Coefficient of variation
	7S fraction	103	30.33%–52.67%	38.21%±4.59%	12.01%
	α′	103	5.19%–15.96%	9.37%±1.84%	19.68%
	α	103	11.08%–25.24%	17.10%±3.07%	17.96%
	β	103	7.72%–17.54%	11.74%±2.04%	17.38%
I	11S fraction	103	47.33%–69.67%	61.79%±4.59%	7.43%
	A_3	103	7.03%–13.84%	9.48%±1.59%	16.76%
	$A_{1a}A_{1b}A_2A_4$(A)	103	15.93%–33.20%	23.88%±3.41%	14.26%
	Basic subunit(B)	103	11.34%–40.54%	28.43%±5.91%	20.80%
	11S/7S ratio	103	0.90–2.30	1.65±0.30	18.06%
	7S fraction	603	20.58%–56.65%	40.00%±5.00%	12.50%
	α′	603	4.27%–16.59%	10.53%±1.93%	18.32%
	α	603	5.90%–27.01%	16.87%±3.08%	18.28%
	β	603	6.99%–22.77%	12.60%±2.29%	18.16%
Ⅱ	11S fraction	603	43.35%–79.42%	60.00%±5.00%	8.33%
	A_3	603	0–16.99%	9.48%±1.90%	20.00%
	$A_{1a}A_{1b}A_2A_4$(A)	603	10.95%–32.11%	22.54%±3.31%	14.68%
	Basic subunit(B)	603	10.23%–46.59%	27.98%±6.01%	21.46%
	11S/7S ratio	603	0.77–3.86	1.54±0.33	21.36%

Note: Group I and Group II represenseded the 103 cultivars released or great extended recently in China, and the 603 landraces, respectively.

2.3 Correlation analysis

The correlation analysis among the relative contents of the 11S, 7S protein fractions and their respective subunits showed that (Table 2).

2.3.1 Significant positive correlation among the relative contents of 7S protein fraction and its respective subunits, and among the relative contents of subunits of 7S protein fraction existed, except for no significant correlation between α′ and α subunit (r=0.048).

2.3.2 Significant correlation among the relative contents of 11S protein fraction and its respective subunits, and among the relative contents of the subunits of 11S globulin existed, except for between that of A_3 polypeptide and 11S protein fraction (r=−0.038). Moreover, the significant cor

relation between the relative contents of the basic subunit and the acidic subunits, A_3 polypeptide ($r = -0.585$, $P < 0.01$) and the polypeptide group of $A_{1a}A_{1b}A_2A_4$ ($r = -0.455$, $P < 0.01$), appeared to be negative.

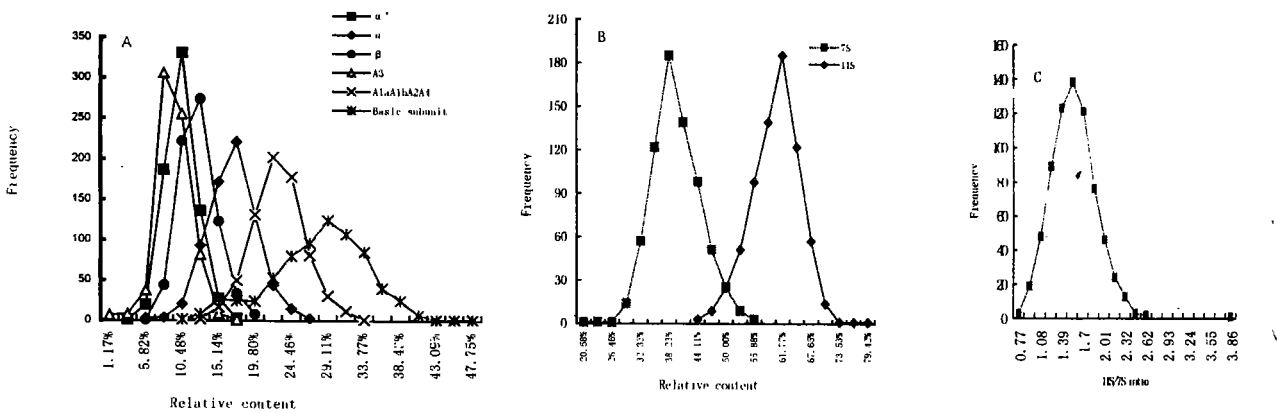

Fig. 2 The frequency distribution of relative contents of 11S and 7S globulin and their subunits for 706 germplasm

A: The relative content distribution of α′, α, β subunit, A_3 – polypeptide, a polypeptide group of $A_{1a}A_{1b}A_2A_4$, and the basic subunit ($B_{1a}B_{1b}B_2B_3B_4$)

B: The relative content distribution of 7S and 11S globulins

C: The distribution of the 11S/7S ratios

2.3.3 The correlation among the relative contents of the subunits of 7S fractions and 11S fractions appeared to be remarkably negative, but no significant correlation between the relative contents of A_3 polypeptide and α′ subunits ($r = 0.054$) was detected, and a remarkably positive correlation between the relative contents of A_3 polypeptide and β-subunit ($r = 0.260$, $P < 0.01$) existed.

Table 2 The correlation among the relative contents of the 11S, 7S fraction and their composing subunits

α′	α	β	7S	A_3	$A_{1a}A_{1b}A_2A_4$	Basic subunit	11S	
α	0.048							
β	0.276**	0.250**						
7S	0.542**	0.750**	0.718**					
A_3	0.054	−0.165**	0.260**	0.038				
$A_{1a}A_{1b}A_2A_4$ (A)	−0.197**	−0.227**	−0.162**	−0.290**	0.431**			
Basic subunit (B)	−0.360**	−0.447**	−0.591**	−0.685**	−0.585**	−0.445**		
11S	−0.542**	−0.750**	−0.718**	−1.000**	−0.038	0.290**	0.685**	
11S/7S ratio	−0.533**	−0.744**	−0.681**	−0.976**	−0.059	0.260**	0.688**	0.976**

Note: ** indicated significant difference at 0.01 level.

2.3.4 A remarkably negative correlation among the relative contents of 11S protein fraction and the subunits of 7S protein fraction, and among the relative contents of 7S protein fraction and the subunits of 11S protein fraction existed, except between A_3 polypeptide and 7S protein fraction ($r = 0.038$) was founded.

2.3.5 A remarkably negative correlation between the relative contents of 7S and 11S protein fractions ($r = -1.00$, $P < 0.01$) was identified. This result was consistent to that of Ogawa et al. (1989). Ogawa et al. supposed that there existed reciprocal compensatory relationship between 11S globulin and 7S globulin, and the relationship ensured that the content of the total seed storage protein remained steady. This reciprocal compensatory rela

tionship between 11S globulin and 7S globulin was very interesting and would been very important for soybean improvement on protein subunit quality.

Table 3 The polymorphism of the subunit relative contents of 11S, 7S globulins

Type	Characteristics	Note
1	High level of 7S globulin (3)	
2	Low level of a', a subunit (1)	
3	Absence of a', a subunit (1)	
4	High level of β subunit (2)	
5	Low level of β subunit (20)	
6	The separation of β subunit into β and β' subunit (3)	reported(2004)
7	Low level of 11S globulin (22)	
8	Low level of A_3 polypeptide (5)	
9	Absence of A_3 polypeptide (6)	

Note: The number in brackets was the accession number.

2.3.6 There existed remarkably negative correlation between 11S/7S ratio and the relative contents of 7S globulin and its respective subunits. There existed remarkably positive correlation between 11S/7S ratio and the relative contents of 11S protein fraction and its polypeptide group of $A_{1a}A_{1b}A_2A_4$(A), and the basic subunit (B). No significant correlation was found between 11S/7S ratio and A_3 polypeptide ($r = -0.059$).

2.4 Polymorphism analysis of subunit contents

63 accessions screened from 706 germplasm showed polymorphism of band type for the subunit contents, and the result was listed in Table 3 and Fig. 3. Some electrophoretic variants of soybean seed storage protein have been used in genetic and breeding research, and the results will be reported later.

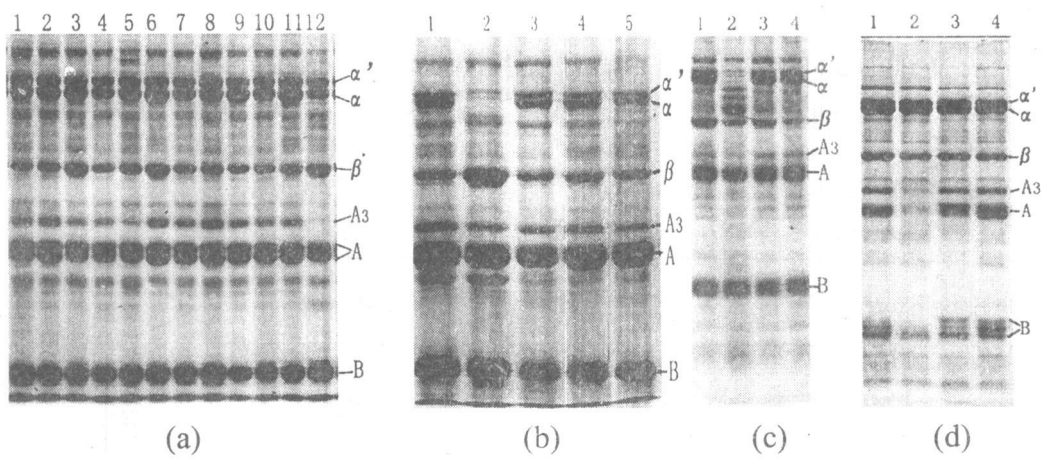

Fig. 3 Profiles of relative content variation of 11S, 7S globulins and their subunits of soybean seed storage protein
(a): lane 1, 2: low level of β subunit; lane 3, 6, 12: high level of β subunit; lane 3, 4, 5: low level of A_3 – polypeptide; lane 12: absence of A_3 polypeptide (b): lane 2: low level of α′, α subunit, high level of β subunit
(c): lane 2: absence of α′, α subunit (d): lane 2, 3: low level of 11S globulin

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706 份中国大豆种质贮藏蛋白 7S 和 11S 组分及其亚基相对含量的研究

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摘要 大豆籽粒贮藏蛋白 7S 和 11S 组分及其亚基含量、11S/7S 比值与大豆蛋白的营养价值和加工特性密切相关。获得具不同 7S 和 11S 组分及其亚基含量、不同 11S/7S 比值的种质材料是对大豆蛋白的营养价值和功能特性进行遗传育种改良的重要材料基础。本研究利用 SDS – PAGE 技术, 对 706 份中国大豆种质资源 7S、11S 组分及其亚基相对含量进行了研究。结果表明: 706 份我国大豆种质资源中 7S、11S 组分及其亚基相对含量具有丰富的遗传变异; 7S 和 11S 组分含量间存在极显著的负相关($r = -1.00$, $P < 0.01$); 603 份地方品种和 103 份新育成品种或主栽品种的 7S、11S 组分相对含量的平均值和变异幅度分别为 40.00%, 20.58% ~ 56.65%, 60.00%, 43.35% ~ 79.42%和 38.21%, 30.33% ~ 52.67%, 61.79%, 47.33% ~ 69.67%; 11S/7S 比值的平均值和变异幅度分别为 1.54, 0.77 ~ 3.86 和 1.65, 0.90 ~ 2.30; 筛选获得了 63 份 7S、11S 组分或亚基含量变异种质。

关键词 大豆; 种质资源; SDS – PAGE; 11S、7S 组分及其亚基; 相对含量