Journal of Soil Science and Plant Nutrition, 2014, 14 (4), 769-782
RESEARCH ARTICLE

Effect of phosphorus on the composition and accumulation of 7S and 11S globulin subunits during seed development of three soybean varieties

J. Han[1], W.G. Jie[2], B.Y. Cai [1,2],*

[1]*Key Laboratory of Microbiology, Heilongjiang Province, College of Life Sciences, Heilongjiang University, Harbin 150080, China.* [2]*Department of Food and Environment Engineering, Heilongjiang East University, Harbin 150086, China.* **Corresponding author: caibaiyan@126.com*

Abstract

Three soybean varieties (Dongnong 42, high-protein cultivar; Hefeng 25, medium-protein; and Dongnong 46, low-protein cultivar) grown under different P conditions were investigated in order to evaluate the effect of P on the composition and accumulation of 7S and 11S globulin subunits. The soybean seeds were planted in pots and fertilized with 0.033 g of N and K_2O per kg soil. Four levels of P treatment were designed, which were P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g of phosphorus pentoxide (P_2O_5) per kilogram of soil). The composition of extractable proteins was determined by conducting SDS-PAGE. No significant differences were observed between the varieties with regard to the molecular weights of the globulin subunits. After the subunits were formed, their concentrations gradually increased under different P treatments, reached a peak at 70 d post-anthesis (DPA), and then declined during maturity. The concentrations of the 7S and 11S globulins and other subunits in Dongnong 42 and Hefeng 25 were the highest under the P3 treatment, while they were the highest in Dongnong 46 under the P2 treatment. Under the same P treatment, the concentrations were high in Dongnong 42 and low in Dongnong 46 and Hefeng 25. Significant differences were observed in the concentrations of 7S and 11S globulin subunits between the varieties and levels of P treatments, especially under the P3 treatment. Alpha subunit was mainly in relation with the P-nutrition in the three varieties. These findings suggested that the optimal combination of cultivars and P treatment levels could contribute to the increased concentrations of both soybean globulins and their subunits.

Keywords: Soybean, phosphorus level, globulin, subunit, composition.

1. Introduction

Soybean seeds contain 40% protein and are one of the most important vegetable protein sources for humans. Approximately 90% of soluble proteins in soybean seeds are globulins, and more than 70% of globulins are glycinin (G) (11S globulin) and β-conglycinin (βc) (7S globulin). The properties of soybean proteins are influenced by the composition and structure of these proteins, which have become the main focus of soybean protein research (Wang and Chen, 1998). The 7S and 11S globulins are composed of different polypeptide subunits. Therefore, the variations in the properties of the two globulins can be primarily attributed to the differences in their subunits. Previous studies reported 7S globulin was found to comprise three subunits, i.e., α´, α, and β (Utsumi *et al*., 2002). The 11S globulin was found to comprise acidic and basic subunits (Ma *et al*., 2006; Jiang *et al*., 2006). Currently, studies on the 7S and 11S globulins are mainly focused on the effect of their concentration on the functional properties of soybean proteins (Cheng *et al.*, 2006a, 2006b; Mujoo *et al.*, 2003; Morita and Shimoyamada, 2013; Ortiz *et al*., 2004; Poysa *et al*., 2006), accumulation of globulin subunits, analysis of globulin subunit loss (Kawakatsu *et al*., 2010; Guo *et al*., 2006), methods of globulin extraction and separation (Molina *et al*., 2002; Liu *et al*., 2007; Ryan *et al*., 2005; Manjaya *et al*., 2007; Chen *et al*., 2012). The percentage concentration of each soybean globulin subunit is a hereditary trait of soybean cultivars, which is not liable to other factors (Cheng *et al*., 2006a). Furthermore, the concentration of the same subunit in the total amount of proteins determined by electrophoresis remains unchanged at two times of independent experiments. Therefore, the results obtained by applying the subunit percentage composition as analysis data are more reliable and accurate than applying the scanned subunit percentage, area, and mean density. Gel scanning can also be used to obtain data on the concentration of various subunits of storage proteins in soybean cultivars; however, the method has many shortcomings because there are many factors affecting SDS-PAGE and the data for the subunit concentration derived from electrophoretograms of the same soybean cultivar (Krishnan *et al*., 2009; Natarajan *et al*., 2009; Speroni *et al*., 2009).

Soybean globulin proteins can be phosphorylated and affect their food functional properties (Katano *et al*., 2005; Kaviani, 2008; Morales *et al*., 2010). However, little is known about the effect of fertilization on the composition of soybean 7S and 11S globulin subunits, except for reports on the effect of nitrogen (N) and sulfur (S) nutrition on globulin and its subunits (Sexton *et al*., 1998; Zhou *et al*., 2006; Salvagiotti *et al*., 2008). Phosphorus (P) is an essential component in plant N metabolism and is closely associated with protein metabolism (Kaviani, 2008; Beltrano *et al*., 2013; Pigna *et al.* , 2010). The studies on the relationship between P and the subunit composition of the 7S and 11S globulins of different high-quality soybean cultivars shall provide further guidance for in-depth research on soybean phosphate fertilizer application, soybean protein quality improvement, and high-quality, cost-effective cultivation techniques for various soybean cultivars. In this study, we used three typical soybean varieties (high-protein, middle-protein, and low-protein cultivar) grown under different P conditions to understand the effect of P on subunit composition of the 7S and 11S globulins. Furthermore, we investigate the optimal P fertilization for soybean cultivars with different genotypes at the molecular level in order to improve the soybean quality.

2. Materials and Methods

2.1. Plant material

Three representative soybean varieties, which have been extensively cultivated in recent years in Heilongjiang Province, China, were used in this study. They were Dongnong 42 (high-protein cultivar with an average protein concentration of 46.04% and a fat concentration of 19.33%), Dongnong 46 (low-protein cultivar with an average protein concentration of 37.17% and a fat concentration of 23.32%), and Hefeng 25 (medium-protein with an average concentration of 40.07% and a fat concentration of 19.26%) (Ning *et al*., 2003; Zhao *et al.*, 2007; Chen, 2009; Guan *et al*., 2009).

2.2. Experimental design

The experiments were conducted in a greenhouse at the Test Station of the Sugar Research Institute of Harbin Industry University. The basic soil productivity was relatively high, and the nutrient composition of the soil was as follows: organic matter, 25.57 g/kg; total N, 1.73 g/kg; total P, 5.6 g/kg; total potassium (K), 23.2 g/kg; alkali-soluble N, 140.1 mg/kg; available P, 13.44 mg/kg; available K, 201 mg/kg; and pH 6.9. The soybean seeds were sown in different pots, filled with mixture of 12.5 kg air-dried soil containing urea, potassium sulfate, and diammonium phosphate, including phosphorus pentoxide, P_2O_5, which is used as a source of P. Different concentrations of the P source, P_2O_5 were used to alter the nutrient components in these pots. The plants were cultivated in soil fertilized with 0.033 g/kg of N and K_2O and with 4 concentrations of P, namely, P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times. Two lines of protection pots were placed around the planting pots in order to reduce the edge effects. On April 30, 2006, the seeds were sown. Three sprouts were placed in each pot. All the plants were managed in the same manner as field plants and were watered and weeded regularly during the growing season. They were harvested on September 10, 2006. Samples were obtained at 30, 40, 50, 60, 70 DPA and maturity. For each level of treatment, three pots of legumes were randomly selected for sampling and were taken to the laboratory where deactivation of enzymes was performed at 80ºC for 30 min. The samples were dried to constant weight at 60–70ºC. Each seed processed during maturity was dried, blended, and stored at room temperature for further analysis. The experiment duration was between October 2006 and June 2007.

2.3. Determination of soybean seed globulin subunits

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (Ma *et al*., 2006). In order to obtain soluble proteins, weighed soybean was milled to a powder with a protein concentration of 0.5–1% proteins. Soybean powder (1 mL) was transferred to a 1 mL sample buffer containing 0.36 g urea, 20 μL 2-mercaptoethanol, 20 μL bromophenol blue (BPB) and was incubated at 60ºC for 2 h or at room temperature overnight. The supernatant was harvested after centrifugation at 5000 × g for 5 min. The total protein of the extract was evaluated using Lowry test. Aliquots of 50 μg of the sample proteins or the protein standard (MBI, Fementas, Germany) were separated on 12% SDS-polyacrylamide gel (5 mA through the stacking gel, 10 mA through the separating gel) and were then stained using Coomassie Brilliant blue R-250. The bands were scanned using a Shanghai Tianneng GIS gel imaging system (Shanghai, China). The results were quantified using a quantification software from the manufacture (Tianneng, Version GIS 3.74).

The concentration of globulin subunits in this experiment is expressed as the percentage of the concentration for each globulin subunit in the total amount of soybean storage proteins (Cheng *et al*., 2006b; Guo *et al*., 2006). The relative percentage composition of the subunit was obtained by multiplying the peak area with the mean density to derive mass, which then divides gross mass of standard samples of proteins (Ma *et al.*, 2006). Different electrophoretograms were then employed to compare the subunit percentage composition and provided data for comparative analysis.

2.4. Statistics

All values were expressed as mean ± SD. Analysis of variance (ANOVA) was used to determine the significance of the differences between the three groups using the data processing system (DPS) Version 2.c. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. The effect of P on subunit composition of 7S and 11S globulin

The electrophoresis banding pattern of the storage proteins in seeds of the three soybean cultivars distinguished 7S globulin from 11S globulin. The 7S globulin comprised of 4 subunits of α´, α, γ, and β; and 11S globulin comprised of acidic and basic subunits (5 each) (Figure1). It shows the electrophoretogram of 7S and 11S globulins in the mature seeds of three soybean cultivars that were treated under different concentrations of P treatment.

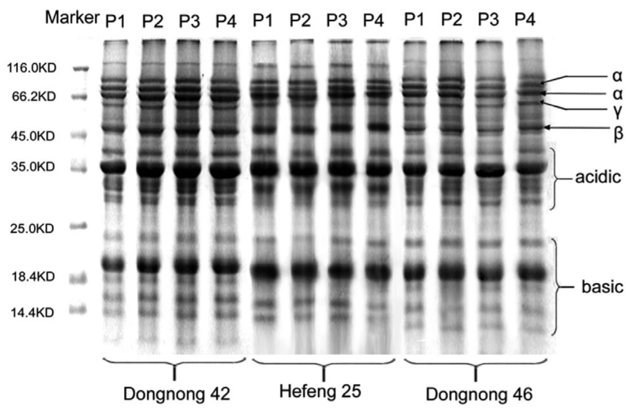

Figure 1. Effect of various phosphorous levels P1, P2, P3 and P4 treatment on the composition and accumulation of 7S and 11S globulin subunits in mature soybean grain, Dongnong42, Hefeng25 and Dongnong46. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times.

Table 1 presents the molecular weights of various subunits in the electrophoretogram. The molecular weights were calculated using the gel imaging system. The analysis revealed that the molecular weights of the subunits did not differ significantly according to the concentration of P applied and the type of cultivar used. This suggests that the effect of the applied N, P, and K fertilizers on molecular weights of 7S and 11S globulin subunits was minimal, and that the changes in the molecular weights of the globulin subunits between the cultivars were insignificant.

3.2. The effect of P on accumulation of 7S and 11S globulin subunits

The electrophoretogram derived after SDS-PAGE of the globulins in the treated soybean cultivars of the three genotypes at 30, 40, 50, 60, and 70 DPA was analyzed and the relative concentration of various subunits were obtained (Figures 2–4).

Figures 2, 3, and 4 indicate the concentrations of the subunit components of the 7S and 11S globulins at various time points after anthesis in each the 4 levels of P treatments. The α and basic subunits accumulated earlier, i.e., approximately 30–40 DPA; α´Subunits emerged at 50 DPA, then disappeared, and later re-emerged to stay until maturity. The α´ globulin subunits of Dongnong 42 under P3 treatment and Dongnong 46 under P2 treatment appeared at 70 DPA, which was earlier than under the other 3 levels of treatment, suggesting that appropriate P treatment promoted earlier accumulation of the α´ subunits. The γ globulin subunits were observed at maturity. The γ

773

subunits of Dongnong 42 and Hefeng 25 globulins were observed at 50 DPA under P3 treatment and then disappeared. The γ subunits of Dongnong 46 globulins were observed at 50 DPA under P4 treatment and then disappeared. The β globulin subunits of P1- and P4-treated Dongnong 42 formed at 50 DPA. The P2-treated β globulin subunits of this cultivar formed at 30 DPA, then vanished and reappeared at 60 DPA. The β globulin subunits of the P3-treated Dongnong 42 cultivar was formed at 30 DPA, and its concentration increased gradually in a time-dependent manner. β globulin subunits of P1-, P2- and P3-treated Hefeng 25 appeared at 30 DPA, and the β globulin subunits of P4-treated Hefeng 25 appeared at 50 DPA. The β globulin subunits of P1-treated Dongnong 46 appeared at 40 DPA and the β globulin subunits of P2-, P3- and P4-treated Dongnong 46 cultivar emerged at 30 DPA. The emergence sequences of the 7S and 11S globulins in all the 4 levels of P treatment indicated that P fertilization exerted certain effects on the subunit formation. The subunits of some P1- or P4-treated cultivars formed relatively late, while some subunits of the P2- or P3-treated cultivars emerged relatively early, indicating that the emergence of the subunits was closely related to the amount of fertilized P and that appropriate amounts of P fertilization could promote the accumulation of various subunits. The phenomenon of the appearance and the subsequent disappearance of the α´ and γ subunits may have resulted from external environmental factors or on internal changes occurring during seed development.

Table 1. Effect of 4 phosphorus treatments on subunit molecular weights of 3 soybean cultivars

Cultivars	Treatments	(Subunits)					
		α′ kD	α kD	γ kD	β kD	Acidic kD	Basic kD
Dongnong42	P_1	80±1	68±1	62.2±1.2	49.2±1.2	41±1～30±1	24±1～14.4±1.1
	P_2	81±2	69±1	62.2±1.3	49.2±1.1	41±2～30±1	24±2～14.4±1.2
	P_3	81±2	70±2	62.2±1.1	49.2±1.2	41±1～30±1	24±1～14.4±1.1
	P_4	81±1	68±1	63.2±1.2	50.2±1.3	41±1～31±1	24±1～14.4±1.3
Hefeng25	P_1	79±1	67.2±1.2	60.2±1.3	49.2±1.4	41±1～31±1	25±1～14.4±1.2
	P_2	80±1	66.2±1.3	61.2±1.2	51.2±1.1	41±2～31±1	24±1～15.4±0.9
	P_3	80±2	67±1	61.2±1.5	52.2±1.0	42±2～31±2	25±2～13.4±0.8
	P_4	79±1	68±1	62.2±1.2	49.2±1.2	40±2～29±1	23±1～12.4±0.7
Dongnong46	P_1	80±1	66±2	62.2±1.3	50.2±1.3	41±1～31±1	24±1～14.4±1.1
	P_2	80±2	67±1	61.2±1.2	48.2±1.2	41±2～30±1	24±1～14.4±1.2
	P_3	79±1	66.2±1.2	60.2±1.1	48.2±1.1	40±1～30±1	24±2～14.4±1.1
	P_4	80±1	67±1	61.2±1.2	49.2±1.6	41±1～31±1	24±1～14.4±1.3
Average molecular weight		80.00±1.17	67.46±1.37	61.62±1.26	49.62±1.25	40.92±1.55～30.42±1.07	24.08±1.21～14.23±1.17

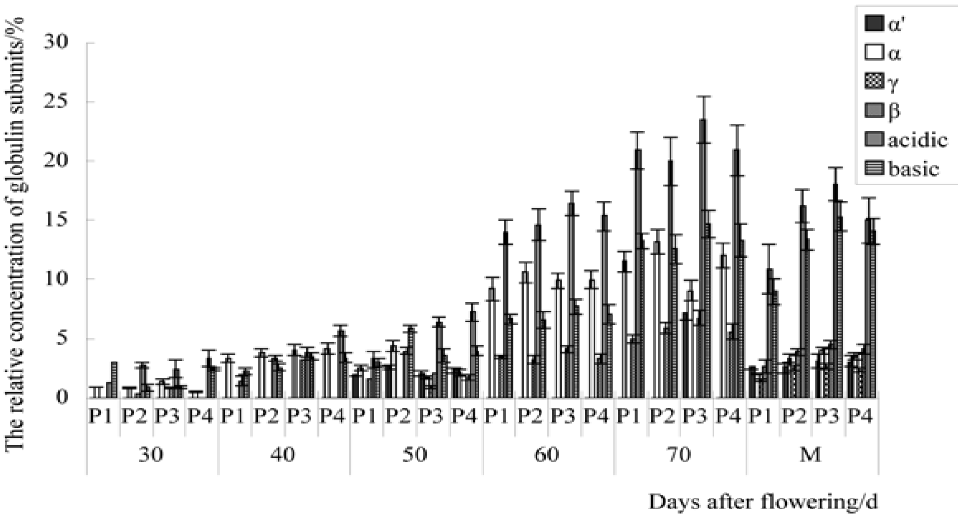

Figure 2. Effect of different phosphorus treatments on the relative concentration (%) of globulin subunits in Dongnong 42. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times. M (maturity)

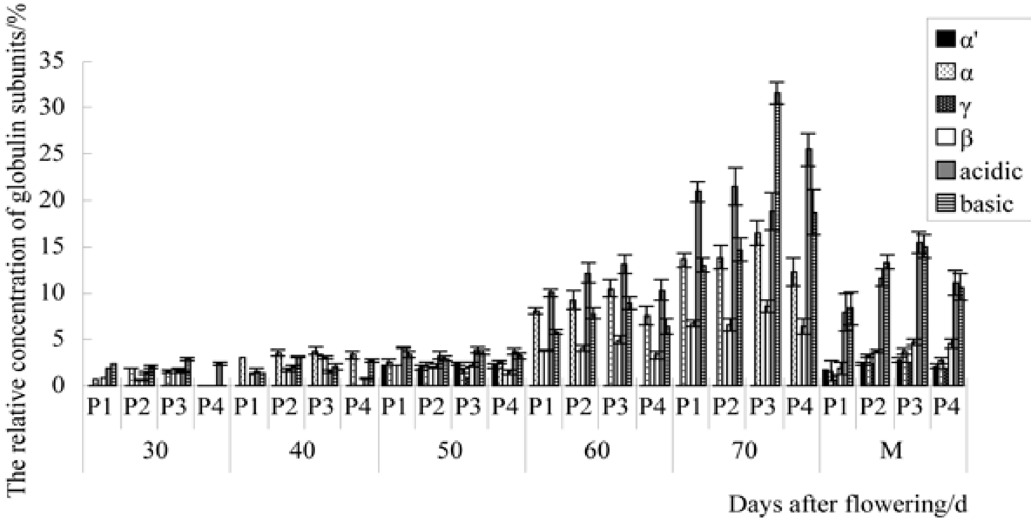

Figure 3. Effect of different phosphorus treatment on the relative concentration (%) of globulin subunits in Hefeng 25. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times. M (maturity)

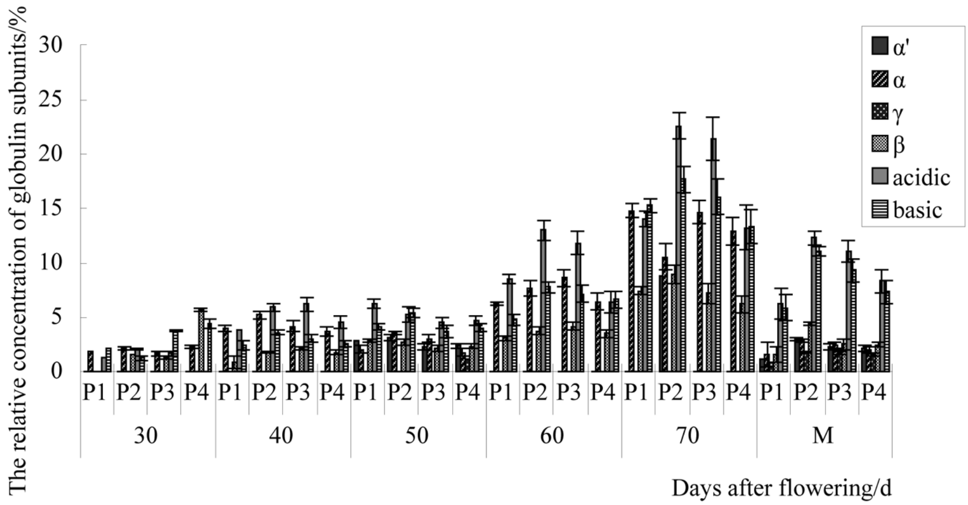

Figure 4. Effect of different phosphorus treatments on the relative concentration (%) of globulin subunits in Dongnong 46. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times. M (maturity)

3.3. The effect of P on the concentration of 7S and 11S globulin subunits

The concentration of the various subunits of the same cultivar differed with the levels of P treatment (Figures 2–4). The concentrations of the α globulin subunits of Dongnong 42 reached the highest value at 30 DPA under the P3 treatment, at 40 DPA under the P4 treatment, and between 50 and 70 DPA under the P2 treatment. The pattern of changes in the concentration of β subunits was unclear before 60 d, and the concentration of this subunit reached the highest value after 60 d under the P3 treatment. The concentrations of acidic and basic subunits increased with the amount of P fertilization between 30 and 50 DPA, and it increased to the peak value from 50 d up to maturity under P3 treatment. The concentrations of the various subunits indicated that the P3 treatment was responsible to enhance the concentration of various Dongnong 42 subunits.

The concentrations of α globulin subunits of Hefeng 25 reached the highest value at 30 d under P2 treatment and from 40 d to maturity under P3 treatment. The concentrations of β subunits reached the highest value between 30 d and maturity under the P3 treatment. The pattern of changes in the concentrations of acidic subunits was not clear before 70 DPA under different P treatments and the concentrations of these subunits reached the highest level during maturity under P3 treatment. The concentrations of basic subunits reached the highest level during the initial period of formation under P3 treatment. The concentrations of the subunits in Hefeng 25 indicated that the P3 treatment was conducive to enhance the subunit concentration of this cultivar.

The concentrations of the α subunits Dongnong 46 reached the highest level at the initial period of formation under the P4 treatment, between 40 and 50 DPA under P2 treatment, and between 60 and 70 d under the P3 treatment.

The concentrations of the β subunits reached the highest level at the initial period of formation under the P4 treatment, between 40 and 60 d under the P3 treatment, and from 70 d to maturity under the P2 treatment. The concentrations of the basic subunits reached the highest value at the initial period of formation under treatment with high levels of P and between 40 and 70 DPA under the P2 treatment. The concentrations of the above subunits indicate that the P2 treatment was conducive to increase the concentration of various Dongnong 46 subunits.

In summary, the pattern of changes in the subunit concentrations of different cultivars remained the same at different levels of treatment and after emergence, all the concentrations of various subunits increased gradually until 70 DPA when it attained the peak value and later declined during maturity. To further confirm the effect of P treatment on the subunit concentrations of 7S and 11S globulins, ANOVA was performed on the 12 combinations among the three cultivars and the four levels of treatment. The results showed that the concentrations of soybean seed globulins and various subunits significantly differed among the different cultivars. Moreover, the concentrations varied with P treatment levels (Table 2). The optimal combination of cultivars and P treatment levels were P3 treatment for Dongnong 42 and Hefeng 25 and P2 treatment for Dongnong 46. This indicated that to improve soybean protein quality and enhance globulin concentration, cultivars with high concentrations of globulin subunits should be selected based on hereditary traits, and then appropriate P fertilizers in addition to N and K fertilizers must be applied to soybean cultivars of different genotypes. Thus, ecological zones of high-quality soybean cultivation and systems of cost-effective intensive fertilizing should be established by taking into account the physiological needs of the cultivar as well as the soil, and climatic conditions, in order to achieve optimal high-quality characteristics of specific soybeans.

Table 2. Results of statistical analysis showing effects of cultivar and P treatment on protein subunit concentration

Items	α′	α	γ	β	acidic	basic	7S	11S	Total Globulin
Cultivars	4.17*	5355.6**	1605.6**	1396.2**	1439.4**	386.6**	12875**	1095**	1725.9**
Treatments	29.33**	500**	294.4**	869.6**	46.3**	174.4**	2771.9**	165.1**	358.9**

Notes: * and ** indicate significance at the $p<0.05$ and $p<0.01$ level, respectively.

3.4. The effect of P on the 7S and 11S globulin concentrations

In Dongnong 42, the highest concentration of 7S globulin under the P3 treatment and different levels of P treatment was observed during development, and that of 11S globulin was observed 50 DPA under the P4 treatment and 50 DPA under the P3 treatment (Figure 5). In Hefeng 25, the highest concentration of 7S globulin was observed at 30 DPA to maturity under the P3 treatment. The 11S globulin concentration of this cultivar remained relatively unchanged prior to 50 DPA and reached the highest level from 50 d to maturity under the P3 treatment (Figure 6). In Dongnong 46, the highest concentration of 7S and 11S globulins was observed during the initial period of formation under the P2 treatment (Figure 7). Clearly, the P3 treatment enhanced the globulin concentration in the high-protein and medium-protein cultivars, and the P2 treatment enhanced the globulin concentration in low-protein cultivars. Based on the changes in the concentrations of 7S and 11S globulins during the initial period of formation among the soybean cultivars of three genotypes, we observed that the 7S and 11S globulins of Dongnong 42 and Hefeng 25 accumulated relatively slowly 50 d before anthesis and rapidly between 50 and 70 DPA. The 7S and 11S globulins of Dongnong 46 were accumulated relatively slowly 60 d before anthesis and rapidly between 60 and 70 d. The period of rapid increase in the globulin concentration in the high-protein and medium-protein cultivars was longer than that in low-protein cultivars; this result was consistent with the protein concentrations in the cultivars.

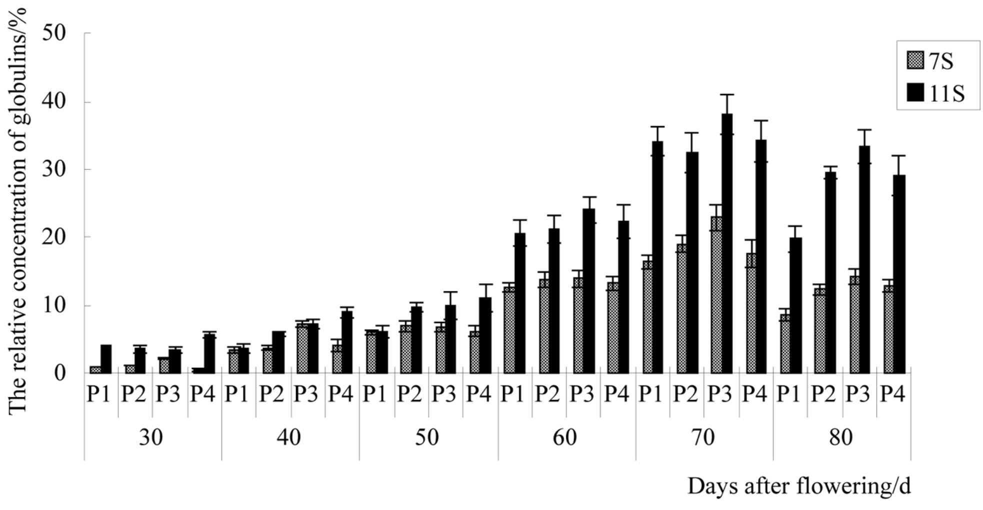

Figure 5. Effect of different phosphorus treatments on the relative concentration (%) of globulins in Dongnong 42. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times.

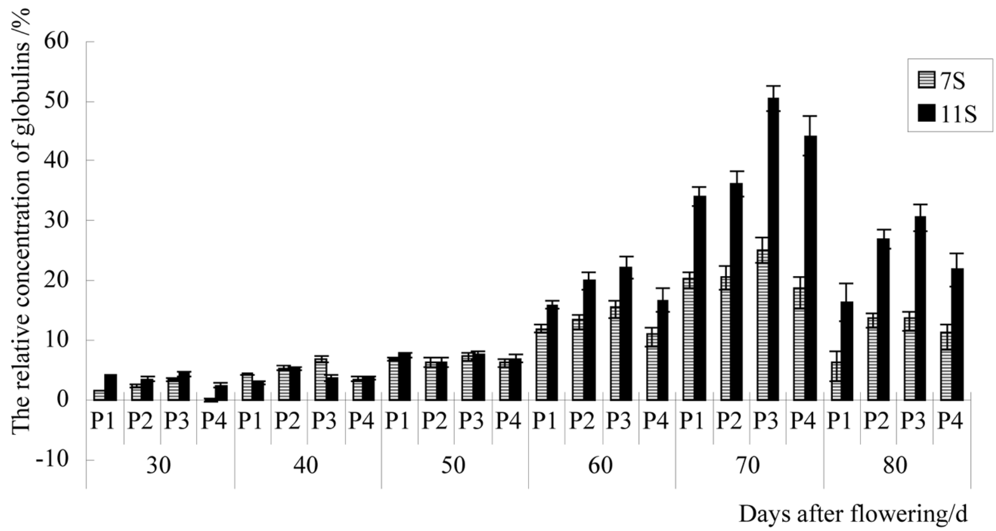

Figure 6. Effect of different phosphorus treatment on the relative concentration (%) of globulins in Hefeng 25. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times.

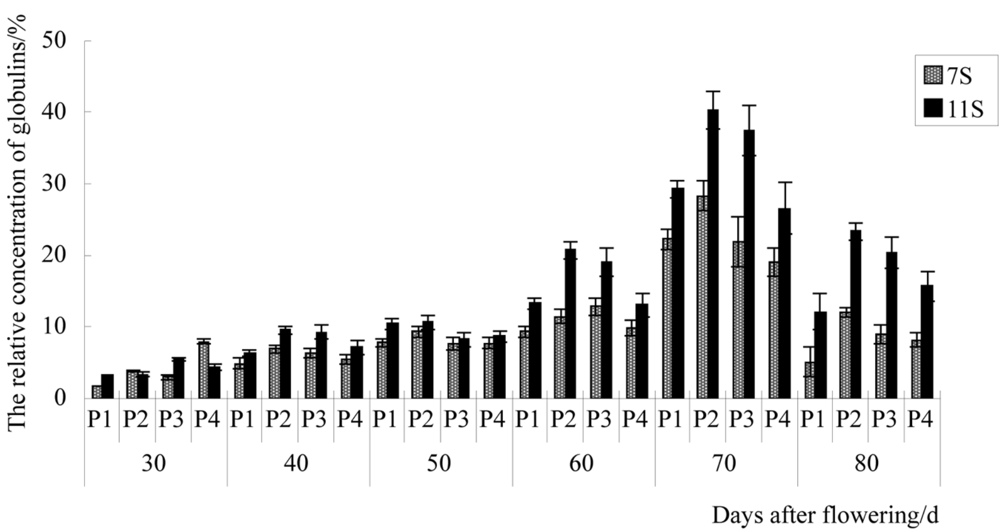

Figure 7. Effect of different phosphorus treatments on the relative concentration (%) of globulins in Dongnong 46. P1, P2, P3 and P4 (i.e., 0, 0.033, 0.067, and 0.100 g/kg of P_2O_5). Each of the soybean cultivars was treated with four levels of P treatment and each treatment was repeated 30 times.

4. Discussion

In this study, it was found that the 7S globulin of soybean seeds contained 4 subunits, namely, α′, α, γ, and β. Since linear SDS-PAGE gradient was used in this study, the 7S globulin subunits could be clearly separated, and broad bands of γ subunits were obtained. The 11S globulin was found to comprise acidic and basic subunits. However, the composition of the acidic and basic subunits of this subunit was inconsistent with the results of local and international studies. In addition, the definition of broad bands has not been standardized. In this study, we observed that both acidic and basic subunits yielded 5 bands each on SDS-PAGE, which indicates that they are each composed of 5 subunits. Further investigation is needed to identify the subunits.

The findings of this study indicated that the molecular weights of globulin subunits in soybean seeds did not vary greatly with the type of cultivars or the levels of P fertilization. In fact, they remained relatively unchanged, suggesting that subunit molecular weights varied imperceptibly between different cultivars. The findings of this study did not completely correlate with those from other studies. Molecular weights of the α′, α, and β subunits of 7S globulin measured by Nishizawa *et al*. (2004) were 71, 67 and 50 kDa, respectively. The molecular weights measured by Jiang *et al*. (2006) were 77.45, 66.72 and 47.60 kDa, respectively. The molecular weights of the 4 basic subunits of the 11S globulin were 20.88 kDa and 35.96kDa, respectively. The differences in the molecular weights measured in different studies may have resulted from variations in testing approaches, and procedures or methods of calculation. Therefore, efforts should be put forward in this regard, to formulate unified standards of measurement.

The time progression of the accumulation of the 7S and 11S globulin subunits in soybean seeds has been extensively reported. However, the results differ between the studies. Zheng *et al*. (1992) reported that 7S globulin began to synthesize between 15 and 20 DPA, 11S globulin began to accumulate between 3 and 5 DPA, and later, the concentrations of both the globulins clearly increased. Using immunofluorescence techniques, Ling *et al*. (1997) observed that 7S and 11S globulins accumulated between 15 and 30 DPA. Ochiai-Yanagi *et al*. (1978) reported that the 7S globulin began to be synthesized at 40 DPA, while the 11S globulin began to accumulate 10 d later. The results of this study showed that α and β subunits of the 7S globulin from the 3 cultivars began to accumulate at 30 DPA, the α′ and γ subunits formed later, and the acidic and basic subunits of the 11S globulin began to accumulate at 30 DPA. These findings are not consistent with those of the former studies. These differences in the results may be associated with variations in the test cultivars used or in the growing conditions.

The concentrations of the 7S and 11S globulins and of their various subunits in soybean cultivars of different genotypes are sensitive to P fertilizers, and appropriate P fertilization enhances of the subunit concentrations of high, middle, and low-protein cultivars. One of the major reasons for the increase in the concentration of globulin subunits is the interaction between N and P. Appropriate P fertilization could promote N metabolism because P is the one of the constituents of the aminotransferase and nitratase, which promote amination, deamination, transamination, and nitrate deoxidization. P is also one of the constituents of various enzymes facilitating respiration (such as dehydrogenase, cytochrome oxidase, and flavine enzyme), and may play a role in promoting plant respiration. The organic acids (such as pyruvic acid, α-ketoglutaric acid, fumaric acid, and oxaloacetic acid) produced during respiration can be regarded as ammonia receptors that are required to synthesize amino acids. The concentrations of globulins and subunits decreased under relatively higher P levels, suggesting that the concentration is not dependent on the levels of P fertilization, because the mineral elements like N, P, and K play different roles. In this experiment, the levels of N, K fertilization meet the requirements of normal growth and development of soybean. An appropriate ratio of mineral elements ensures the metabolic coordrination and optimal growth of soybean cultivars.

fertilization meet the requirements of normal growth and development of soybean. An appropriate ratio of mineral elements ensures the metabolic coordrination and optimal growth of soybean cultivars. The damage resulting from the lack or overabundance of a certain element is not only dependent on the absolute amount of the element itself but also greatly dependent on its proportion relative to that of other elements. Therefore, the amount of P fertilization should be regulated and should depend on the physiological need and the fertilizer requirement of different cultivars to achieve high-quality characteristics of special soybean cultivars.

The final concentrations of the 7S and 11S globulins in soybean seeds are determined by the concentrations of their various subunits in soybean seeds. The concentrations of the various globulin subunits of soybean seeds rapidly increased from 60 to 70 DPA. This is a critical period for determining the concentrations of various soybean globulin subunits of soybean seeds. It is important to create appropriate environmental conditions for soybean growth during this period. During later stages of growth, fertilizer application, water and temperature conditions should be regulated to provide favorable conditions for protein accumulation and to achieve high-quality soybean cultivars.

5. Conclusions

P fertilization has no effect on the 7S and 11S globulin subunits of soybean cultivars and has only a slight effect on the molecular weights of these subunits. The molecular weights of various subunits vary insignificantly between the different cultivars. In soybean cultivars of different genotypes, 7S globulin comprises the 4 subunits α’, α, γ, and β; 11S globulin consists of acidic and basic subunits. P fertilization has some effect on the concentrations of the 7S and 11S globulins and on their subunits in the 3 soybean cultivars. Appropriate P fertilization increases the concentrations of both globulins and their subunits. These findings show that optimal combination of cultivars and P treatment levels could contribute to the increased concentrations of both soybean globulins and their subunits. These findings show that optimal combination of cultivars and P treatment levels could contribute to the increased concentrations of both soybean globulins and their subunits. It could make a contribution to improve the yield and quality of protein used as ingredient in food and feed industry.

Acknowledgments

This work was supported by a grant from Natural Science Foundation of China (31170466), Science and Technology Project from Heilongjiang Provincial Education Department (12521394), High-level Personnel Supported Program of Heilongjiang University (ecological restoration team Hdtd 2010-12), and Open Projects Foundation of Key Laboratory of Microbiology, Heilongjiang Province, Heilongjiang University (2012MOI-7).

References

Beltrano, J., Ruscitti, M., Arango, M.C., Ronco, M. 2013. Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. Journal of Soil Science and Plant Nutrition. 13, 123-141.

Chen, L.J. 2009. The research on soybean quality property state changeable regular of dongnong 42 in different seeding dates. Chinese Agricultural Science Bulletin. 25, 122-127.

Cheng, C.L., Shi, Y.G., Wang, Z.Y., Zhao, H.T. 2006a. Effect of protein subunits and 7S/11S on functional properties of SPI. Food Science. 27, 70–74.Cheng, C. L., Wang, Z.Y., Shi, Y.G., and Zhao, H.T. 2006b. Effect of soy protein subunits composition and ratio of 7S to11S on the quality and productivity of tofu. China Oils and Fats. 31, 16–19.

Chen, J., Chen, X.Y., Zhu, Q.J., Chen, F.L., Zhao, X.Y., Ao, Q. 2012. Determination of the domain structure of the 7S and 11S globulins from soy proteins by XRD and FTIR. J. Sci. Food Agr. 93, 1687–1691.

Guan, R.X., Qin, J., Hu, J.S., Chen, W.X., Chang, R.Z., Liu, Z.X., Qiu, L.J. 2009. Genetic composition of elite soybean cultivar Hefeng 25. Acta Agronomica Sinica. 35, 1590-1596.

Guo, S.T., Meng, Y., Zhang, X.M., Zhang, X.L., Qi, J., Qiu, L.J., and Chang, R.Z. 2006. Analysis of protein subunit composition of Chinese soybean cultivars and screening of soybean (*Glycine max* (L.) Merrill) cultivars lacking some subunits. Acta Agronomica Sinica. 32, 1130–1134.

Jiang, Z.F., Chen, Q.S., Yang, Q.K., Li, W.B. 2006. Analysis of subunits of storage protein of soybean germplasms resources. Journal of Northeast Agricultural University. 37, 596–603.

Ling, Z.P. 1997. 11S and & 7S protein accumulation during soybean development using immunofluorescent method. Science in China (Series B). 3, 285–288.

Liu, C., Wang, H.L., Cui, Z.M., He, X.L., Wang, X.S., Zeng, X.X., Ma, H. 2007. Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein. Food Chem. 102, 1310–1316.

Katano, T., Kamata, Y., Ueno, T., Furuya, T., Nakamura, T., Ohtsuki, K. 2005. Biochemical characterization of an effective substrate and potent activators of CK_2 copurified with Bowman-Birk-type proteinase inhibitor from soybean seeds in vitro. Biochimica et Biophysica Acta (BBA) - General Subjects. 1725, 47–56.

Kaviani, B. 2008. Modification of subunits composition of storage protein in soybean to improve seed quality in response to phosphorus and potassium treatments. J. Biotechnol. 136, S273.

Krishnan, H.B., Natarajan, S.S. 2009. A rapid method for depletion of Rubisco from soybean (*Glycine max*) leaf for proteomic analysis of lower abundance proteins. Phytochemistry. 70, 1958–1964.

Ma, H., Wang, X.S., Liu, C., Yu, T., Hao, X.Y., Gao, W.R., He, X.L. 2006. The concentration variation of 7S,11S globulins and their subunits of seed storage protein of 706 Chinese soybean germplasm. Soybean Science. 25, 11–17.

Manjaya, J.G., Suseelan, K.N., Gopalakrishna, T., Pawar, S.E., and Bapat, V.A. 2007. Radiation induced variability of seed storage proteins in soybean (*Glycine max* (L.) Merrill). Food Chem. 100, 1324–1327.

Molina, E., Defaye, A.B., Ledward, D.A. 2002. Soy protein pressure-induced gels. Food Hydrocolloids. 16, 625–632.

Morales, A.G., Moyano, J.F. 2010. Application of an in vitro gastrointestinal model to evaluate nitrogen and phosphorus bioaccessibility and bioavailability in fish feed ingredients. Aquaculture. 306, 244–251.

Morita, K., Shimoyamada, M. 2013. Proposal of mechanism of the freeze–thaw fractionation of 7S and 11S globulins in soymilk. Food Chem. 140, 39–43.

Mujoo, R., Trinh, D.T., and Ng, P.K.W. 2003. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chem. 82, 265–273.

Natarajan, S.S., Krishnan, H.B., Lakshman, S, Garrett, W.M. 2009. An efficient extraction method to enhance analysis of low abundant proteins from soybean seed. Anal. Biochem. 394, 259–268.

Ning, H.L., Yang, Q.K., Xu, C.Q. 2003. Effect of different transplanting location on the content of soybean protein and fat in dongnong 163. Soybean Bulletin. 2, 13.

Nishizawa, K., Maruyama, N., Satoh, R., Higasa, T., and Utsumi, S. 2004. A vacuolar sorting determinant of soybean β-conglycinin β-subunit resides in a C-terminal sequence. Plant Sci. 167, 937–947.

Ochiai-Yanagi, S., Fukazawa, C., Harada, K. 1978. Formation of storage protein components during soybean seed development. Agr. Biol. Chem. 42, 697–702.

Ortiz, S.E.M., Puppo, M.C., and Wagner, J.R. 2004. Relationship between structural changes and functional properties of soy protein isolates–carrageenan systems. Food Hydrocolloids. 18, 1045–1053.

Pigna, M., Cozzolino, v., Glandonato Caporale, A., Mora, M.L., Di Meo, V., Jara, A.A., Violante, A. 2010. Effects of phosphorus fertilization on arsenic uptake by wheat grown in polluted soils. Journal of Soil Science and Plant Nutrition. 10, 428-442.

Poysa, V., Woodrow, L., Yu, K. 2006. Effect of soy protein subunit composition on tofu quality. Food Res. Int. 39, 309–317.

Ryan, K.J., Brewer, M.S. 2005. Purification and identification of interacting components in a wheat starch-soy protein system. Food Chem. 89, 109–124.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann A. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans. Field Crops Res. 108, 1–13.

Sexton, P.J., Paek, N.C., Shibles, R.M. 1998. Effects of nitrogen source and timing of sulfur deciency on seed yield and expression of 11S and 7S seed storage proteins of soybean. Field Crops Res. 59, 1–8.

Speroni, F., Beaumal, V., Lamballerie, D.M., Anton, M., Añón, C.M., Puppo, C.M. 2009. Gelation of soybean proteins induced by sequential high-pressure and thermal treatment. Food Hydrocolloids. 23, 1433–1442.

Utsumi, S., Maruyama, N., Satoh, R., and Adachi, M. 2002. Structure-function relationships of soybean proteins revealed by using recombinant systems. Enzyme Microb. Tech. 30, 284–288.

Wang, J.L., and Chen, C.L. 1998. Survey of soybean storage protein composition of 11S/7S. Journal of Shandong Agricultural Science. 1, 48–50.

Zhao, X.M., Song, X.J., Wang, X.Y., Ning, H.L., Li, W.B., Li, W.X. 2007. Regression model of effects of nitrogen, phosphorus and potassium fertilizer on oil content in soybean variety Dongnong 46. Chinese Agricultural Science Bulletin 23, 332-336.

Zheng, Y.Z., Hen, M.Y., Hao, S. 1992. The relationship between soybean cotyledon formation and storage protein accumulation. Chinese Plant Report. 34, 641–644.

Zhou, E.Y., Liu, L.J., Zu, W., Sun, C.S. 2006. Research on the concentration of the protein subunits with sulfur in the soybean plants. Journal of Northeast Agricultural University. 37, 582–587.