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Profiling and relationship of water-soluble sugar and protein compositions in soybean seeds

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ABSTRACT

Sugar and protein are important quality traits in soybean seeds for making soy-based food products. However, the investigations on both compositions and their relationship have rarely been reported. In this study, a total of 35 soybean germplasms collected from Zhejiang province of China, were evaluated for both water-soluble sugar and protein. The total water-soluble sugar (TWSS) content of the germplasms studied ranged from 84.70 to 140.91 mg/g and the water-soluble protein (WSP) content varied from 26.5% to 36.0%. The WSP content showed positive correlations with the TWSS and sucrose contents but negative correlations with the fructose and glucose contents. The clustering showed the 35 germplasms could be divided into four groups with specific contents of sugar and protein. The combination of water-soluble sugar and protein profiles provides useful information for future breeding and genetic research. This investigation will facilitate future work for seed quality improvement.

1. Introduction

Soybean seeds contain about 40% protein, 20% oil, and 30% carbohydrates, providing a significant food source of protein, oil, and carbohydrates to humans. Although the whole soybean seeds can be eaten after being boiled or roasted, most of them are produced into a great variety of foods, such as tofu, soymilk, soy sauce, soy flour and others. The quality characteristics of traditional soy-based food products, namely soymilk and tofu, depend mainly on soybean variety and processing conditions (Cai & Chang, 1999; Esteves, Martino, Oliveira, Bressan, & Costa, 2010; Rajni, Dianne, & Perry, 2003; Saldivar, Wang, Chen, & Hou, 2011; Yang & James, 2013; Yoshikawa, Chen, Zhang, Scaboo, & Orazaly, 2014). Seed water-soluble sugars, especially sucrose, affect the taste and flavor of soyfoods. Increased seed sucrose and total sugar content could improve the sweetness of tofu and soymilk (Hou et al., 2009). Stachyose and raffinose, two other major sugar components in soybean seeds, are not readily digested in the gastrointestinal tract and easily cause flatulence or diarrhea (Kumar et al., 2010). Hence, it is important to increase sucrose content and reduce stachyose and raffinose contents for soybean quality breeding.

Soybean protein usually is fairly soluble in water and only water-soluble protein (WSP) can be processed and utilized in traditional soyfoods. Therefore, the seed WSP content is often applied to evaluate protein characteristic for soybean processing and storage instead of the total protein content (Li, Li, & Li, 2008). Different varieties result in a wide variation in the protein content and composition that impact soyfood yield and quality. The contents of 11S (glycinin) and 7S (β-conglycinin) globulins, two principal groups of seed WSP, were shown to have a significant effect on tofu yield and texture. The 11S content showed positive correlation with tofu hardness, whereas the 7S content showed negative correlation with tofu firmness (Kim & Wicker, 2005; Poysa, Woodrow, & Yu, 2006; Rajni et al., 2003). The ratios of 11S to 7S globulins (11S/7S ratio) were positively and significantly correlated with tofu yield (Sladjna, Miroljub, Mirjana, & Biljana, 2011). In addition, seed protein would be more balanced with a higher 11S/7S ratio because the content of sulfur-containing amino acids was higher in 11S than 7S (Jiang et al., 2010). Therefore, it is very attractive to increase WSP and 11S contents and improve seed protein for nutrition and processing.

Soyfoods and vegetable soybean are popular traditional food in Southern China, especially Zhejiang, Fujian, Jiangsu and Shanghai. The sugar and protein compositions affect the flavor and nutrition of soyfoods and vegetable soybean. High sucrose, high 11S, low stachyose and low 7S are desirable profiles and preferred by soyfood market. Hymowitz, Collins, Panczner, and Walker (1972) analyzed seed protein, total sugar and individual sugar contents of 60 soybean cultivars, and a significant positive correlation existed between protein and stachyose. Hartwig, Kuo, and Kenty (1997) evaluated 40 soybean cultivars for seed protein, sucrose, raffinose

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and stachyose contents and reported a significant negative correlation between protein and raffinose. These indicated that the combination of sugar and protein analyses demonstrated a potential to develop soybeans with desirable sugar and protein contents. However, more information is needed about the relationship between sugar and protein compositions, especially water-soluble components in soybean seeds.

In Southern China, many soybean varieties have seed protein contents higher than 45%. For example, Zheqiudou3, developed by Zhejiang Academy of Agricultural Sciences, had a protein content of 50.05%. Jiang et al. (2010) evaluated the seed protein composition of 321 germplasms from the Zhejiang soybean collection. The contents of 11S and 7S globulins showed a significant variation and the 11S/7S ratios were between 1.270 and 4.008. A total of 63 germplasms from the Zhejiang soybean collection were also analyzed to compare seed water-soluble sugar contents in order to evaluate their breeding value as vegetable soybean (Yu, Yuan, Fu, & Zhu, 2015). There were significant differences for total sugar content among different varieties, and the total sugar contents of most varieties were in the range of 80–120 mg/g. Although the investigations on both protein and sugar compositions have rarely been reported in this region, improving seed compositions by modifying sugar and protein contents together is becoming one of the important breeding objectives.

One efficient way to modify the sugar and protein compositions in soybean seeds and thus improve product marketability is through conventional breeding and genetic enhancement. Therefore, collection, evaluation, and utilization of diverse germplasms with desirable sugar and protein traits are becoming increasingly important for soybean breeding and genetic enhancement. In this study, we evaluated both water-soluble sugar and protein profiles of soybean seeds in a collection of 35 germplasms in Zhejiang province of China. The correlations between the water-soluble sugars and proteins in soybean seeds were also studied. The data provide insights into the relationship between sugar and protein as it correlates with seed quality. This research provides useful information for genetic research and facilitates future breeding for seed quality improvement.

2. Materials and methods

2.1. Plant materials

A total of 35 soybean cultivars, landraces, and elite breeding lines were selected to represent the soybean germplasms grown in Zhejiang province of China. Among them, 13 varieties came from the institutes of Zhejiang province in China; 8 were the landraces initially collected and grown in Zhejiang province; and the other 14 were introduced from other provinces of China or other countries. Each germplasm was grown in a single plot (1 × 1.5 m) in the research fields of Zhejiang Academy of Agricultural Sciences, Hangzhou, China in July 17th, 2013. All these germplasms were harvested at natural maturity. About 15 g seeds from each germplasm were finely ground using a portable high-speed grinder (Dade, China) and screened through a 250-μm standard testing sieve (Huakang, China).

2.2. Sugar extraction, identification and quantification

Sugar extraction was performed according to the procedures described by Hou et al. (2009) and Song, Liu, Li, and Gu (2013) with some modifications. About 0.15 g fine powder of each germplasm was weighed into a 2.0-ml centrifuge tube, and then 1.5 ml distilled water was added. The tube was shaken at 180 rpm at room temperature for 2 h, and then centrifuged at 12,000 rpm for 10 min. The 750 μl supernatant was transferred into a 2.0-ml centrifuge tube, and then 750 μl of acetonitrile was added. The tube was mixed and incubated at room temperature for 10 min, and then centrifuged at 12,000 rpm for 10 min. A volume of 500 μl supernatant was filtered through a 0.45-μm membrane disc filter (Sangon, China).

Sugar identification and quantification was conducted using Agilent 1100 HPLC system with a refractive index detector (RID) (Agilent, USA). An Agilent ZORBAX carbohydrate analysis column (4.6 × 150 mm, 5 μm) was used for sugar separation. A 20-μl aliquot of each sugar sample was injected, and sugars were eluted with 75% acetonitrile at a flow rate of 1.0 ml/min. The temperatures of column and detector were 30 and 35 °C, respectively.

Five sugars including fructose, glucose, sucrose, raffinose, and stachyose were purchased from Sigma–Aldrich, USA. The sugars were used as external standards to identify and quantify each sugar. Sugar identification was done by comparing their retention times to those of the standard sugars. The sugar standards, each at concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 mg/ml, were prepared to create calibration curves for quantifying each of the five sugars (Supplementary materials). The sugar concentration in soybean seeds is presented as milligrams per gram (mg/g) of dry seed matter.

2.3. Protein extraction, separation and quantification

The total of water-soluble protein (WSP) was extracted from 20 mg fine powder with 1.5 ml distilled water at room temperature for 2 h. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant (20 μl) was transferred into a 1.5-ml tube and diluted with 180 μl double distilled water. The content of the diluted protein extract was then determined using the Bradford assay.

Protein extraction was performed following the procedures optimized by Yang and James (2013) and Liu et al. (2007). Briefly, about 50 mg fine powder was weighed into a 1.5-ml centrifuge tube and then 1 ml of 0.03 mol/L Tris–HCl (pH 8.0) with 10 mmol/L dithiothreitol (DTT) was added. The tube was shaken at 180 rpm at room temperature for 2 h, and then centrifuged at 12,000 rpm for 10 min. A volume of 50 μl supernatant was transferred into a 1.5-ml centrifuge tube, and then diluted with 200 μl distilled water. The diluted protein extract was diluted with Laemmli sample buffer (Bio-Rad, USA), and then denatured by heating at 100 °C for 5 min.

Protein separation by electrophoresis was conducted in 1.5-mm thick gels. The separating gels contained 120 g/L 30% acrylamide/bisacrylamide (29:1), 375 mmol/L Tris–HCl (pH 8.8), 1 g/L sodium dodecyl sulfate (SDS), 1 g/L ammonium persulfate (APS) and 0.4 ml/L N,N,N′,N′-tetramethylethylenediamine (TEMED). The stacking gels contained 50 g/L 30% acrylamide/bisacrylamide, 125 mmol/L Tris–HCl (pH 6.8), 1 g/L SDS, 1 g/L APS and 1 ml/L TEMED. The gels were run in a buffer solution containing 1 g/L SDS with 3 g/L Tris-base and 14.5 g/L Glycine at 200 V. The gels were stained overnight by shaking with 1 g/L Coomassie Brilliant Blue R250 in 400 g/L methanol and 100 g/L acetic acid. The gels were destained in 100 g L methanol with 100 g/L acetic acid.

The destained gels were analyzed using Quantity One, version 4.6.2 (Bio-Rad, USA). Protein molecular weight markers (Precision Plus Protein Standards, Bio-Rad) included bands for 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa. The bands representing 7S and 11S globulin subunits were identified according to their relative mobility against the protein ladder and published data. Quantitative estimation of each band was measured as the percentage of the corresponding intensity of the band with respect to the total intensity of the lane.