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Research Note

Greater differences exist in seed protein, oil, total soluble sugar and sucrose content of vegetable soybean genotypes [*Glycine max* (L.) Merrill] in Northeast China

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Abstract

Understanding edamame seed compositions at edible stage is fundamental for developing cultivars that are considered as healthier diet. Thirty vegetable soybean genotypes were grown at the rate of 28 plants m^{-2} in a randomized complete block design with three replications in field condition in Northeast China. Protein, oil, total soluble sugar (TSS) and sucrose contents of the seeds at the edible stage (R6-R7) were determined. Cultivar Tai 292, a commercial vegetable soybean cultivar, was used as a standard cultivar for comparison of genotypes evaluation. The mean values in seed protein, oil, TSS and sucrose of all genotypes were 420 mg g^{-1}, 186 mg g^{-1}, 60 mg g^{-1} and 43 mg g^{-1}, respectively. Genotype variation in sucrose content was 187% and that of TSS was 163%, while the variations in oil and protein were only 39% and 26%, respectively. Sucrose content constituted 71% of TSS. In fresh seed of vegetable soybean, negative correlations were found between protein and TSS (r=-0.52), protein and sucrose (r=-0.43), while the determination coefficients (R^2) were only 0.17 for protein and sucrose. Every 10 mg·g^{-1} increase in seed protein was accompanied by 4.3 mg·g^{-1} decrease in sucrose. No significant correlation (p>0.05) was found between oil and TSS, and oil and sucrose. Sixteen out of 30 genotypes and 7 out of 30 genotypes had greater protein and sucrose contents than check cultivar Tai 292. Genotypes L-1, L66 and L-139/19 are the valuable genetic resources in developing vegetable soybean cultivars with higher sucrose, lower oil content without reduced protein content.

Keywords: Cultivars; Edamame; Fresh seed; Seed Physiology; Soybean breeding.
Abbreviations: total soluble sugar:TSS.

Introduction

Vegetable soybean [*Glycine max* (L.) Merrill] is large seed soybean which is harvested at R6-R7 stage when seeds are immature and pods are not turning yellow (Zhang et al., 2010). The protein and oil content are the main quality traits of vegetable soybean and are valued by edamame breeders. The protein content of vegetable soybean was 56% higher than green peas (*P. sativum*) (Masuda, 1991). Rao et al. (2002) reported that protein and oil content of fresh seed vegetable soybean ranged from 33% to 39% and 13% to 16%, respectively. In developed countries, vegetable soybean varieties with lower oil percentage and relatively higher protein content are more popular among young people who seek healthy diets (Brar and Cater, 1993). Saldivar et al. (2011) stated that vegetable soybean seed with more than 45% protein and seed oil of less than 18% are acceptable. Humans can recognize four basic flavors i.e. sweet, sour, bitter and salty (Defilippi et al., 2009). Sugar and volatile component are classified as the main chemical substances which affect the eating quality of fruit (Jouquand et al., 2008). Shanmugasundaram et al. (2001) reported that the cultivars of vegetable soybean with higher sucrose level were more popular than the cultivars with low sucrose in Japan. For fresh seed of vegetable soybean, the total soluble sugar content ranged from 6.0% to 7.4% in a four-year study in Taiwan (Tsou and Hong, 1991) and 7.5% to 12.5% during a three-year study in China (Zhang et al., 2006). An inverse relationship between seed protein and carbohydrate has been reported in grain soybean (Masuda, 1991; Wilcox and Shibles, 2001; Sitthiwong et al., 2005). Hartwig et al. (1997) analyzed forty soybean cultivars with various protein content and found that the correlation between protein and sucrose was negative (r=-0.78**) while correlation between oil and sucrose was positive (r=0.67**). For vegetable soybean, a significant positive correlation between seed sugar and oil contents has been reported (Openshaw and Hadley, 1981). Based on the results from these studies developing a high sucrose vegetable soybean with high protein but low oil content is a challenging task. However, the results reported by other researchers showed a negative relationship between sugar and oil. For example, Shanmugasundaram et al. (2001) reported that the correlation coefficient between sugar and oil content was -0.50 whereas between sugar and protein content was -0.15 but significant only in 6 out of 20 entries. Saldivar et al (2011) reported that the seed protein decreased 2%-6% during the first 3-5 weeks after flowering and then gradually increased until maturity, while the seed oil accumulated rapidly at 3-7 weeks after flowering and remained fairly constant afterward. In contrast, the sucrose content was high at initial stage but decreased as seeds developed.

Table 1. Contents of protein, oil, TSS and sucrose in fresh seed of 30 vegetable soybean genotypes

Traits	Average mg g^{-1}	Range mg g^{-1}
Protein	426	379 to 478
Oil	186	144 to 217
TSS	60	35 to 133
Sucrose	43	21 to 102

Table 2. Linear regression analysis and correlation coefficients among protein, oil, TSS and sucrose for 30 vegetable soybean cultivars.

Dependent versus Independent variables	Regression mean squares	R^2	Constant (b)	Standard error of b	Correlation coefficient
Protein-TSS	3036.4*	0.16	279.8	92.5	-0.52*
Protein-sucrose	2092.7*	0.17	224.4	77.3	-0.43*
Oil-TSS	21.8	0.01	51.9	42.4	0.04
Oil-sucrose	118.2	0.11	25.0	32.7	0.10

* Significant level at P<0.05

The objectives of this research were: (a) to examine if there are differences in seed protein, oil and sugar contents at edible stage in vegetable soybean genotypes, and (b) to investigate the composition interrelationship and feasibility of breeding vegetable soybean cultivars with increased sucrose content while protein content remains unchanged.

Results and Discussion

Seed composition contents at edible stage in different vegetable soybean genotypes

As shown in Table 1, the average content of protein, oil, total soluble sugar (TSS) and sucrose in the fresh seed of 30 vegetable soybean genotypes were 426 mg g^{-1}, 186 mg g^{-1}, 60 mg g^{-1} and 43 mg g^{-1}, respectively. The range of protein, oil, TSS and sucrose content were 379-478 mg g^{-1}, 144-217 mg g^{-1}, 35-133 mg g^{-1}, and 21-102 mg g^{-1}. The results obtained in this study were consistent with earlier investigations that vegetable soybean contained 333 to 457 mg g^{-1} protein, 130 to 227 mg g^{-1} oil and 7.3 to 113 mg g^{-1} sugar at R6-R7 stage (Tsou and Hong, 1991; Brar and Cater, 1993; Rao et al., 2002). The present study showed that genotype variation in sucrose content was 187% and that of TSS was 163%, while the variations in oil and protein were only 39% and 26%, respectively (Table 1). Previous research highlighted the importance of sugar level in the fresh vegetable soybean seeds because the total soluble sugar content directly influences the organoleptic properties of edamame and determines consumer acceptability (Sugimoto et al., 2010), and there is a significant relationship between taste score and sugar content (Young et al., 2000; Shanmuga-sundaram et al., 2001; Kumar et al., 2011). The greater range of sucrose and TSS than protein and oil in vegetable soybean seed found in this study provides a valuable and promising resource for breeding desired vegetable soybean cultivars. The current study also revealed that sucrose content accounted for 71.7% of the TSS in average (Table 1), which clearly indicated that sucrose content constitutes the majority of TSS in fresh seed of vegetable soybean and thus could be the substantial factor influencing the taste of vegetable soybean. Soybean cultivars with larger seed size and higher sucrose content are considered as desirable in the production of vegetable soybean (Saldivar et al., 2011).

The correlations among protein, oil, TSS and sucrose in fresh seed

Negative correlation were found between protein and TSS (r=-0.52), protein and sucrose (r=-0.43) (Table 2 and Fig.1), while the determination coefficient (R^2) between protein and

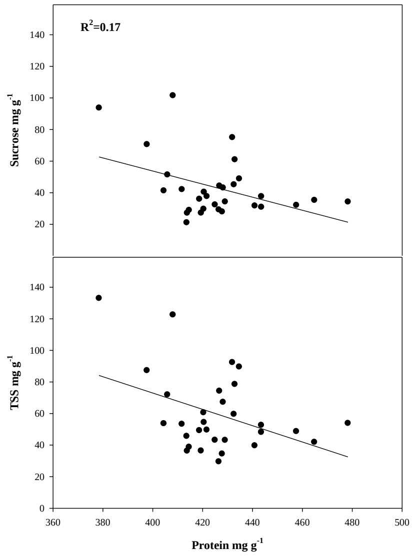

Fig 1. Regression of TSS and sucrose with protein for 30 vegetable soybean genotypes at fresh edible stage.

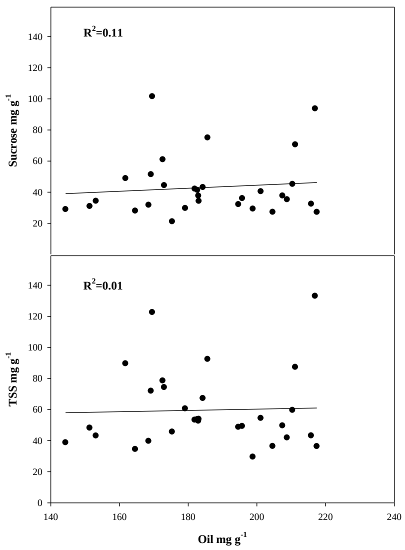

Fig 2. Regression of TSS and sucrose with oil for 30 vegetable soybean genotypes at fresh edible stage.

Table 3. Contents of protein, oil, TSS and sucrose and quantity protein and quantity sucrose in the seeds of the evaluated genotypes.

Genotype	Dry basis				Quantity protein compared with Tai 292	Quantity sucrose compared with Tai 292
	Protein mg·g^{-1}	Oil mg g^{-1}	TSS mg g^{-1}	Sucrose mg g^{-1}		
Tai 292	409 ± 3.4fg	177 ± 5.8de	64.4 ± 3.8ef	41.2 ± 3.4fg	100fg	100f
L-37	478 ± 4.9a	183 ± 4.7d	53.9 ± 4.8fg	34.3 ± 1.8hi	117a	83hi
L-107	465 ± 7.3b	209 ± 4.0ab	41.9 ± 4.1hi	35.3 ± 2.1hi	114b	86hi
L-72	458 ± 8.8b	195 ± 4.6c	48.7 ± 2.7gh	32.1 ± 1.7i	112b	78i
L-17	444 ± 3.3c	183 ± 3.8d	52.7 ± 3.3g	37.7 ± 1.8h	109c	91h
L-100	444 ± 4.1c	151 ± 4.2g	48.2 ± 3.4gh	30.9 ± 1.6ij	109c	75ig
L-57	441 ± 7.8cd	168 ± 4.5ef	39.7 ± 3.1i	31.7 ± 1.8ij	108cd	77ij
L-39	433 ± 3.7d	210 ± 3.3ab	59.6 ± 2.9f	45.1 ± 3.5ef	106d	110ef
L-66	433 ± 3.3d	173 ± 3.9e	78.6 ± 3.3d	61.0 ± 5.1d	106d	148d
L-139/19	432 ± 1.8d	186 ± 3.4d	92.4 ± 3.7c	75.0 ± 5.3c	106d	182c
L-1	435 ± 4.1d	162 ± 4.4f	89.6 ± 3.4c	48.9 ± 2.7e	106d	119e
L-104	428 ± 3.4de	184 ± 3.9d	67.2 ± 4.6e	43.2 ± 2.3f	105de	105f
L-105	428 ± 4.2de	165 ± 3.3f	34.5 ± 4.9i	28.0 ± 1.8j	105de	68j
L-156	429 ± 2.4de	153 ± 3.8g	43.1 ± 2.5h	34.3 ± 1.7hi	105de	83hi
L-50	427 ± 1.5e	199 ± 3.4bc	29.5 ± 6.8i	29.3 ± 1.4j	104e	71j
L-103	427 ± 1.6e	173 ± 3.6e	74.3 ± 3.8de	44.3 ± 1.8f	104e	108f
L-11	425 ± 3.5e	216 ± 5.2a	43.2 ± 2.4h	32.4 ± 1.5i	104e	79i
L-108	421 ± 5.6ef	201 ± 3.1bc	54.5 ± 4.5fg	40.4 ± 1.6g	103ef	98g
L-61	419 ± 7.6ef	196 ± 4.0c	49.3 ± 2.9gh	36.0 ± 1.7h	103ef	87h
L-7	422 ± 3.5ef	207 ± 3.6b	49.7 ± 2.4gh	37.7 ± 1.4h	103ef	91h
L-25	419 ± 6.8ef	205 ± 3.4bc	36.4 ± 2.5i	27.2 ± 2.1j	103ef	66i
L-57	420 ± 5.3ef	179 ± 6.2de	60.6 ± 3.7ef	29.7 ± 2.3ij	103ef	72ij
L-4	415 ± 5.1f	144 ± 5.3h	38.8 ± 3.3i	29.0 ± 1.0j	102f	70j
L-106	414 ± 6.3f	217 ± 3.4a	36.3 ± 2.4i	27.2 ± 1.8j	101f	66j
L-109	412 ± 3.4fg	182 ± 4.1d	53.4 ± 5.1fg	42.1 ± 2.9fg	101fg	102fg
L-77	414 ± 3.7f	175 ± 4.5de	45.7 ± 2.2h	21.1 ± 2.5k	101f	51k
L-52	408 ± 3.2fg	170 ± 4.8e	123.1 ± 5.3b	102 ± 3.9a	100fg	246a
L-43	406 ± 1.7g	169 ± 5.2ef	72.1 ± 4.8de	51.4 ± 4.8e	99g	125e
L-60	404 ± 3.6g	183 ± 3.5d	53.7 ± 5.4fg	41.3 ± 2.6fg	99g	100fg
L-101	398 ± 1.5h	211 ± 3.9ab	87.3 ± 4.9c	70.6 ± 4.9c	97h	171c
L-123	379 ± 3.7i	217 ± 2.7a	133.3 ± 4.5a	93.7 ± 3.7b	93i	228b

Values followed by the same letter within each column are not significantly different ($P<0.05$)

sucrose was only 0.17 (Table 2). The results confirmed the earlier report by Hartwig et al. (1997) who found a negative correlation, although not significant, between protein content and levels of raffinose and stachyose. In contrast to the conclusion that sucrose and raffinose contents were positively correlated with oil content, but negatively correlated with protein content in grain soybean (Hymowitz et al., 1972), we found no significant correlations between oil (mg g^{-1}) and TSS (mg g^{-1}) as well as between oil (mg g^{-1}) and sucrose (mg g^{-1}) in vegetable soybean. Linear regression revealed that every 10 mg g^{-1} increase in seed protein was accompanied by 4.3 mg g^{-1} decrease in sucrose (Fig.1). This indicated that (a) the main carbon skeleton of protein is supplied from sucrose; and (b) it is possible to develop cultivars with higher sucrose but lower oil content although ATP and carbon skeleton requirement for amino acid assimilation may lead to a negative relationship between protein and sucrose in plants (Paul and Foyer, 2001). The later statement supports the results by Shanmugasundaram et al. (2001) who observed a highly significant negative correlation between sugar content and protein plus oil in a 514 samples study. Obviously, carbohydrate accumulation is a factor involved in seed protein production during the seed-filling period, and C and N metabolism are not completely independent (Hayati et al., 1996).

Diversity of quality traits in vegetable soybean genotypes

In current study, 16 out of 30 genotypes tested for quantity protein, 7 out of 30 genotypes for quantity sucrose were significantly higher than that of check cultivar Tai 292 (Table 3, $P<0.05$). The sucrose content in genotype L-1, L-66 and L-139/19 was 19%, 48% and 82% higher ($P<0.05$) while their protein content was 6% higher consistently than that of check cultivar Tai 292 (Table 3). The highest protein content, 478 mg g^{-1}, was achieved in L-37 which was 17% higher than that of check cultivar Tai 292. The highest sucrose content obtained in L-52 was 4.8 times higher than that in L-77, the genotype with the lowest sucrose content (Table 3). The quantity sucrose in L-52 and L-43 were 146% and 25% higher than that of Tai 292 while their quantity protein were relatively in the similar range. Though the quantity protein in L-101 and L-123 was 3 and 7% lower than Tai 292, they produced 71% and 127% more sucrose respectively, which could be translated to important genetic resources for high sucrose content in vegetable soybean. Mebrahtu and Devine (2009) concluded that the sucrose content level could be passed to progeny and different

Table 4. The characters of vegetable soybean genotypes in the study.

Genotype	Plant height (cm)	Node number	Branch number	Growth duration (days)
Tai 292	57.0 ± 4.3	12.0 ± 1.3	3.0 ± 1.2	107
L-37	62.4 ± 2.4	13.9 ± 1.2	1.5 ± 1.2	115
L-107	67.1 ± 4.7	15.6 ± 1.5	2.0 ± 0.9	115
L-72	61.3 ± 8.8	11.7 ± 2.0	1.9 ± 1.0	121
L-17	56.7 ± 7.8	11.3 ± 2.1	2.3 ± 1.4	113
L-100	63.9 ± 5.1	11.2 ± 1.4	1.6 ± 1.2	113
L-57	39.2 ± 2.8	10.9 ± 0.9	2.6 ± 1.7	101
L-39	49.0 ± 2.6	13.0 ± 0.8	6.0 ± 1.5	110
L-66	50.8 ± 5.6	13.2 ± 1.7	4.4 ± 1.1	122
L-139/19	38.8 ± 4.7	9.6 ± 1.4	0.2 ± 0.7	106
L-1	44.6 ± 2.9	9.8 ± 0.7	0.6 ± 0.9	100
L-104	68.1 ± 3.1	12.4 ± 1.6	2.4 ± 1.0	113
L-105	69.3 ± 4.2	14.9 ± 1.5	0.4 ± 1.0	113
L-156	119.0 ± 10.3	23.0 ± 3.2	4.0 ± 2.7	130
L-50	60.0 ± 3.9	13.8 ± 0.5	0.0 ± 0.0	123
L-103	32.1 ± 2.8	8.9 ± 0.6	1.9 ± 1.3	108
L-11	58.0 ± 5.5	14.7 ± 0.9	0.2 ± 0.7	113
L-108	39.3 ± 6.6	11.8 ± 0.8	2.8 ± 1.0	117
L-61	59.4 ± 7.1	13.3 ± 1.1	0.6 ± 0.8	114
L-7	42.5 ± 8.5	10.3 ± 1.0	2.9 ± 0.4	115
L-25	58.6 ± 1.7	13.7 ± 2.2	1.2 ± 1.0	113
L-157	53.0 ± 2.8	11.6 ± 1.1	5.0 ± 1.6	122
L-4	55.0 ± 5.3	12.0 ± 0.8	1.0 ± 1.3	115
L-106	53.1 ± 7.6	12.9 ± 1.8	1.5 ± 1.4	114
L-109	61.0 ± 4.5	16.7 ± 0.5	3.0 ± 1.9	115
L-77	67.0 ± 4.3	15.0 ± 0.0	3.2 ± 0.8	122
L-52	50.6 ± 5.3	12.6 ± 1.1	2.6 ± 1.1	130
L-43	54.9 ± 1.9	13.3 ± 2.3	1.5 ± 0.8	120
L-60	45.0 ± 2.5	12.9 ± 1.7	3.6 ± 1.8	115
L-101	61.6 ± 5.0	12.4 ± 3.2	1.6 ± 1.0	108
L-123	80.0 ± 8.0	14.7 ± 1.6	1.0 ± 1.0	110

genotypes possessed different combining ability. The differences of quantity protein and quantity sucrose in present study were other proofs for feasibility of developing cultivars with higher protein as well as higher sucrose. Thus, the information provided from this research might be helpful for vegetable soybean breeders to develop desired traits in vegetable soybean production.

Materials and methods

Plant material

Thirty vegetable soybean genotypes from China were selected in this study. The genotype characters including plant height, node number, branch number and growth duration days were shown in Table 4.

Experimental layout and cultural practices

Field experiment was conducted at the agronomy farm of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin (45°73′N, 126°61′E, and altitude 128 m). Thirty vegetable soybean cultivars were planted on 4 May 2010 in a randomized complete block design with three replications on a typical Mollisol (Black soil). Each plot consisted of five rows with 0.65 m spacing and 5.0 m length. Seeds were planted with a grain drill at 3 cm depth at the rate of 280,000 seeds ha^{-1}. Based on earlier studies at this location, 50 kg ha^{-1} carbamide (46% N), 50 kg ha^{-1} diammonium phosphate (18% N, 46% P_2O_5), and 150 kg ha^{-1} of composite fertilizer (18% N, 16% P_2O_5, 16% K_2O) were applied before seeding (Zhang et al., 2010; Liu et al., 2011). Weeds were controlled by hand.

Measurements

Plants from the middle two rows of each plot were harvested when the plants were at R6 stage (Fehr et al, 1971). Immediately after harvest, 150 fresh seeds were randomly selected from manually shelled pods. The fresh seed samples were weighed before placed in an air oven at 105°C for 30 minutes and then at 80°C for 72 hours to dehydrate. Ten grams of dried seeds were grounded in a grinder (IKA-WERKE, Finland) to pass 147μm sieve for chemical composition analysis. The crude protein of vegetable soybean was determined using the method of combustion nitrogen analysis by Elementar-Vario (Elementar Analysensysteme GmbH E-Ⅲ, Germany). Total nitrogen was converted to the crude protein content using a conversion factor of 6.25 (Saldivar et al., 2011). Oil content was determined using the Soxhlet extractor method by weighing 0.5 gram of dried sample and wrapped tightly using a weighted piece of filter paper into Soxhlet apparatus, and then adding 200 ml ethyl ether to extract oil in a 60°C water bath. After 48-hour extracting, the defatted sample was kept in an air oven at 45°C for 12 hours and then the weight was used to calculate the oil content by difference method (Wang, 2011). For sugar content, 0.5 g of ground seed was extracted by 4 ml 80% ethanol with a 10-ml centrifuge tube in 80°C water bath for 40 minutes and was homogenized on a vortex for 10 minutes. Then the mixture was centrifuged at 4500×g for 3 minutes and supernatant was removed to a 50-ml volumetric flask with 80% ethanol to dilute to the 50-ml volume. The supernatant was used to quantify the content of total soluble sugar and sucrose. Total soluble sugar content was determined according to Wang (2002) using anthrone sulphuric acid method. The content of sucrose was analyzed by resorcinol hydrochloric acid method (Huber and Israel, 1982) with minor modification where1 ml of 1% resorcinol and 3 ml of 30% HCl was added in the tube to be incubated at 80°C for 10 minutes and then measured at 480 nm. For ease of comparison, the concept of quantity protein and quantity sucrose by Hartwig et al. (2000) was introduced. Quantity protein and quantity sucrose were calculated by following formulas:

$$\text{Quantity protein} = \frac{\text{protein content of a given genotype}}{\text{protein content of check cultivar}} \times 100$$

$$\text{Quantity sucrose} = \frac{\text{sucrose content of a given genotype}}{\text{sucrose content of check cultivar}} \times 100$$

In the current study, Tai 292, a commercial vegetable soybean cultivar released by Asian Vegetable Research and Development Center was used as a standard check cultivar.

Statistical Analyses

Data were presented as percent as mg g^{-1} of dry weight and are the averages of triplicate determinations. The mean of protein, oil, TSS and sucrose among different genotypes were compared according to the Duncan's multiple range tests at 5% significant level. The relationship among protein, oil, TSS and sucrose was tested in linear regression model. The SPSS 16.0 software was used to analyze variance and mean comparison.

Conclusion

Greater differences in seed sucrose and TSS contents are observed in vegetable soybean genotypes in Northeast China. Sucrose content constitutes the majority of TSS in fresh seed of vegetable soybean, which is a substantial factor influencing the taste of vegetable soybean. Although negative correlations were found between protein and TSS, protein and sucrose, the greater range of sucrose and TSS than protein and oil in vegetable soybean seed, several genotypes could be used as important genetic resources for high sucrose and protein content in vegetable soybean and thus provide a valuable and promising resource for breeding desired vegetable soybean cultivars.

Acknowledgments

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