Planting Date Influences Fresh Pod Yield and Seed Chemical Compositions of Vegetable Soybean

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Abstract. Planting date influences grain soybean yield and quality, but no information is available regarding the responses of seed chemical compositions to delayed planting date in vegetable soybean [Glycine max (L.) Merr.]. Three vegetable soybean cultivars, CAS No. 1, Tai 292, and 121, were planted on 3 May, 15 May, 27 May, and 8 June in the field during the 2010 and 2011 growing seasons. The experiment was a randomized complete block design with three replications on a typical Mollisol (black soil). We found that late planting reduced fresh pod yield in all cultivars and years. The reduction in fresh pod yield to delayed planting was significantly correlated with the reduction in the number of two-seed pods per plant. Cultivars with strong capacity in retaining more two-seed pods may possess an advantage if planting is delayed. Planting after 15 May increased seed protein content by 4.1% to 7.5% and reduced oil content by 2.4% to 26.3% for different cultivars. The contents of free amino acid, sum of fructose and glucose, raffinose, and stachyose in seed were also increased by late planting. By contrast, late planting reduced the seed sucrose content ranging from 7.6% to 45.5% for the different cultivars. Planting on 3 May usually produced the greatest fresh pod yield and highest seed sucrose content. These results demonstrated that late planting after early May might have a negative impact on the eating quality of vegetable soybean.

Planting date is an important factor affecting soybean grain yield and grain quality. Early planting is recommended for soybean production in the northern and upper Midwest United States (De Bruin and Pedersen, 2008; Robinson et al., 2009). However, in some areas, early-planted soybean seed growth may be injured because of cool and wet soil conditions or late spring frost and thus result in reduced grain yield (Andales et al., 2000; Meyer and Badaruddin, 2001). Egli and Cornelius (2009) reported the effect of planting date on soybean yield varied substantially from year to year in concert with variation in environmental conditions (principally rainfall amounts and distribution); however, yield was always reduced with delayed planting according to a summa-rizing planting date researches over the past 35 years. De Bruin and Pedersen (2008) found soybean yield declined 70 kg·ha⁻¹·week⁻¹ when planting date was delayed between late April and early May in Iowa. Soybean yield was severely reduced when the planting date was delayed to late May or early June in the upper Midwest (Grau et al., 1994; Lueschen et al., 1992). Planting date affected the growth and development patterns of soybean and changed the content of protein, oil, carbohydrate, and other chemical components in seed (Hu and Wiatrak, 2012; Kumar et al., 2006; Muhammad et al., 2009). Bellaloui et al. (2011) showed that early planting increased seed oil content by 16% compared with late planting with irrigation and reduced oil content was reported with delayed planting date (Kumar et al., 2006; Tremblay et al., 2006). The different temperature during seed filling of various planting dates was strongly correlated with oil content (Kane et al., 1997; Muhammad et al., 2009). Increased protein content across years and cultivars was reported with delayed planting (Kane et al., 1997; Muhammad et al., 2009). However, Tremblay et al. (2006) showed that no effect of delaying planting date from mid-May to mid-June on soybean protein content. All these studies mentioned were focused on the yield and quality of grain soybean. There is less information available for the effect of planting date on fresh pod yield, particularly for seed quality of vegetable soybean. The vegetable soybean is a larger seed soybean harvested at the R5 to R6 stage when seeds are immature and before pods turn yellow (Zhang et al., 2010). Vegetable soybean is very popular in East Asian countries. The literature history of Chinese eating maodou (vegetable soybean) can be traced to the Song dynasty (960 to 1279), recorded in a poem and an agronomy document. The vegetable soybean, edamame, became more acceptable in many countries in recent years because of its pleasant taste and high nutrient quality. Our objectives of this research were to determine the responses of fresh pod yield of vegetable soybean with emphasis on changes in seed chemical composition to planting date in northeast China.

Materials and Methods

Plant materials and treatment conditions. Field experiments were conducted at the agronomy farm of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China (lat. 45°77′N, long. 126°61′E, altitude 128 m) in the 2010 and 2011 growing seasons; daily maximum/minimum temperature and precipitation of July, August, and September in 2010 and 2011 are shown in Figures 1 and 2. The experimental design was a randomized complete block design with three replications on a typical Mollisol (black soil). Four planting dates, 3 May, 15 May, 27 May, and 8 June, were established in the 2-year experiment. In 2010, one vegetable soybean cultivar, CAS No. 1, was planted, whereas in 2011, two additional vegetable soybean cultivars, Tai 292 and 121, were included. Each plot consisted of five rows with 0.65 m between rows. The row length was 5.0 m. Seeds were planted with a grain drill at a depth of 3 cm and a rate of 280,000 seeds/ha. Based on earlier studies at this location, 50 kg·ha⁻¹ carbamide [46% nitrogen (N)], 50 kg·ha⁻¹ diammonium phosphate (18% N, 46% P₂O₅), and 150 kg·ha⁻¹ of composite fertilizer (18% N, 16% P₂O₅, 16% K₂O) were applied before seeding (Zhang et al., 2010). Weeds were controlled by hand. Plants in the middle...
two rows of each plot were harvested when the plants were at the R6 stage (Fehr et al., 1971). Fresh pods in 1.3 m² from each plot were weighed for marketable yield. All plants were separated into one-seed, two-seed, and three-seed pods. Immediately after harvest, 150 fresh seeds were randomly selected from manually shelled pods. The fresh seed samples were weighed before being placed in a forced-air oven at 105 °C for 30 min and then at 80 °C for 72 h. Ten grams of dried seeds were ground in a grinder (IKA-WERKE, Finland) to pass through a 147-µm sieve for chemical composition analysis.

**Measurement of protein, oil, and free amino acid content in seed.** The crude protein of vegetable soybean was determined using the method of combustion N analysis by Elementar-Vario (Elementar Analysensysteme GmbH E-III, Germany). Total N was converted to the crude protein content using a conversion factor of 6.25 (Saldivar et al., 2011). The ninhydrin method was used to measure the total free amino acids. Oil content was determined using the Soxhlet extractor method where 0.5 g of dried sample was weighed and wrapped tightly using a weighted piece of filter paper and placed into Soxhlet apparatus (Shuniu, China) in a water bath maintained at 60 °C. Then 200 mL of ethyl ether was added to the Soxhlet apparatus to extract oil. After a 48-h extraction period, the defatted sample was placed in a forced-air oven at 45 °C for 12 h and the weight was used to calculate the oil content by a difference method.

**Analysis of sugars in seed.** For content of sugars, 0.4 g of ground seed was extracted with 4 mL 80% ethanol in a 10-mL centrifuge tube, which was then placed in an 80 °C water bath for 40 min and then homogenized on a vortex for 10 min. The mixture was then centrifuged at 4500 g for 3 min and the supernatant was removed to a new 10-mL centrifuge tube and condensed to 1 mL using a rotary evaporator, after which it was filtered through a 0.2-µm membrane for high-performance liquid chromatography (HPLC) analysis. A HPLC with Waters Refractive Index detection (HPLC-RID) was used for separation and measurement of each sugar. Samples were injected using a Waters autosampler. The sugars were separated on a BENSON polymeric™ 1000-0 BP-100 Ca++ Carbohydrate Column (250 mm × 4 mm i.d.) at a flow rate of 0.5 mL·min⁻¹. The mobile phase was distilled water. The detection was accomplished using a RI2414 detector. Commercial sugars, including glucose, fructose, sucrose, raffinose, and stachyose, purchased from Sigma-Aldrich (St. Louis, MO), were used as external standards to identify and quantify each sugar based on their retention times and peak areas.

**Data analysis.** Data were presented as mg·g⁻¹ of dry weight and were the averages of triplicate determinations. One-way analysis of variance tests were applied to confirm significant data for the same cultivar among different planting dates and different cultivars in the same planting date. The mean data
were compared according to the Duncan’s multiple-range test at 5% significant level. A partial-correlation model was used to describe the response of yield components to planting date. All data differences were analyzed at 5% significant level across years. SPSS 13.0 software (SPSS Inc., Chicago, IL) was used to analyze for variance and mean comparison.

Results

Yield and yield components. Fresh pod yield of vegetable soybean decreased with delayed planting date (Table 1). Although a significant difference was observed between 2010 and 2011 for fresh pod yield for cv. CAS No. 1, the effect of delaying planting date on fresh pod yield was invariable. The highest fresh pod yield was consistently found in earlier planting dates in both years for all cultivars.

There was a large decrease in the number of two-seed pods per plant with delayed planting (Table 2). Further analysis demonstrated that only the decrease in the number of two-seed pods per plant was significantly correlated with fresh pod yield reduction (Table 3).

Seed protein, oil, and free amino acid content. Significant differences were observed among planting dates for seed protein content (Tables 4 to 6).

Planting date, year, cultivar, and the interaction of planting date × year had a significant impact on vegetable soybean seed oil content (Tables 4 and 5). Overall, a significant decrease in seed oil content was recorded after delaying planting date (Table 6).

The seed free amino acid content was significantly affected by planting dates and cultivar (Tables 4 to 6).

Seed sugar content. As a pair of isomeride, fructose and glucose were not discriminated by the HPLC-RID system, and it has been shown they had similar content and development trends during the seed filling stage (Saldivar et al., 2011). Therefore, we combined fructose and glucose for analysis. The sum of the two sugars was significantly affected by planting date, year, cultivar, the interaction of planting dates × year, and the interaction of planting dates × cultivar (Tables 4 and 5). However, we found that late planting always increased the sum of the two sugars at the fresh edible stage (Table 7). Likewise, delayed planting increased the seed content of raffinose and stachyose, although the cultivars differed in their seed raffinose and stachyose contents (Table 7). The seed sucrose content was more than 15 times higher than all other sugars. However, unlike other sugars, delayed planting decreased the seed sucrose content (Table 7), and the seed sucrose content was unaffected by the interaction of planting dates × year (Table 4). Although the cultivars differed in sucrose content, planting on 3 May always resulted in higher sucrose content than planting on later dates; the cultivar 121 exhibited a different response to delayed planting.

Table 1. The response of fresh pod yield of vegetable soybean to planting date.

| Yr      | Planting date | Cultivars | Fresh pod yield (kg·ha⁻¹) |
|---------|---------------|-----------|---------------------------|
| 2010    |               |           |                           |
| 3 May   | CAS No. 1     | 10,980 a* |
| 15 May  | CAS No. 1     | 8,352 b*  |
| 27 May  | CAS No. 1     | 6,750 c*  |
| 8 June  | CAS No. 1     | 6,588 c*  |
| 3 May   | CAS No. 1     | 15,876 AA |
| 15 May  | Tai 292       | 16,866 AA |
| 27 May  | Tai 292       | 13,644 AB |
| 8 June  | Tai 292       | 13,158 BB |
| 3 May   | Tai 292       | 13,014 AB |

2011

| Yr      | Planting date | Cultivars | Fresh pod yield (kg·ha⁻¹) |
|---------|---------------|-----------|---------------------------|
| 3 May   | CAS No. 1     | 13,860 BA |
| 15 May  | Tai 292       | 11,682 bA|
| 27 May  | Tai 292       | 8,694 bb  |
| 8 June  | Tai 292       | 10,152 CA |
| 3 May   | Tai 292       | 9,594 bb  |

*For the same cultivar, means followed by the same lowercase letter in the same year are not significantly different among planting dates (P = 0.05). For the same planting date, means followed by the same uppercase letter in the same year are not significantly different among cultivars (P = 0.05).

Table 2. The response of fresh pod number of ‘CAS No. 1’ to planting date in 2010 and 2011.

| Yr      | Planting date | One-seed pod | Two-seed pod | Three-seed pod |
|---------|---------------|--------------|--------------|----------------|
| 2010    |               | (pod number/plant) |               |               |
| 3 May   | 8.3 a         | 20 a         | 4.2 b        |
| 15 May  | 2.8 b         | 14 b         | 3.3 b        |
| 27 May  | 1.2 b         | 9.1 c        | 6.0 a        |
| 8 June  | 3.4 b         | 9.2 c        | 4.0 b        |
| 2011    |               | (pod number/plant) |               |               |
| 3 May   | 7.0 a         | 33 a         | 6.4 a        |
| 15 May  | 7.8 a         | 29 b         | 9.2 a        |
| 27 May  | 6.8 a         | 21 c         | 9.3 a        |
| 8 June  | 7.0 a         | 20 c         | 6.0 a        |

*Means followed by the same letter in the same year are not significantly different among planting dates (P = 0.05).

Table 3. Analysis of correlation between fresh pod yield and different pod of ‘CAS No. 1’ after delayed planting.

| Yr       | Planting date | One-seed pod | Two-seed pod | Three-seed pod |
|----------|---------------|--------------|--------------|----------------|
| 2010     |               | (pod number/plant) |               |               |
| 3 May    | 0.402         | 0.616        | 0.003        |
| 15 May   | 0.604         | 0.002        | 0.989        |
| 2011     |               | (pod number/plant) |               |               |
| 3 May    | -0.510        | 0.828        | -0.334       |
| 15 May   | 0.850         | 0.000        | 0.206        |

Table 4. Analysis of variance (F-value and level of significance) of the effect of year (2010, 2011), planting date, and their interactions on ‘CAS No. 1’ chemical composition.

| Protein | Oil | FAA | F + G | Sucrose | Raffinose | Stachyose |
|---------|-----|-----|-------|---------|-----------|-----------|
| Year (Y) |     |     |       |         |           |           |
| 2010    |     |     |       |         |           |           |
| 2011    |     |     |       |         |           |           |
| Planting date (P) |     |     |       |         |           |           |
| 2010    |     |     |       |         |           |           |
| 2011    |     |     |       |         |           |           |
| P × Y   |     |     |       |         |           |           |
| 2010    |     |     |       |         |           |           |
| 2011    |     |     |       |         |           |           |

FAA = free amino acid; F + G = fructose + glucose.

Table 5. Analysis of variance (F-value and level of significance) of the effect of cultivars, i.e., CAS No. 1, Tai 292, and cv. 121, in 2011, planting date, and their interactions on chemical composition.

| Protein | Oil | FAA | F + G | Sucrose | Raffinose | Stachyose |
|---------|-----|-----|-------|---------|-----------|-----------|
| Cultivar (C) |     |     |       |         |           |           |
| 2010    |     |     |       |         |           |           |
| 2011    |     |     |       |         |           |           |
| P × C   |     |     |       |         |           |           |
| 2010    |     |     |       |         |           |           |
| 2011    |     |     |       |         |           |           |
Changes in the planting dates are known to influence the yield of soybean. The results from this study support previous conclusions in grain soybean that earlier planted soybean produced more grain yields (Egli and Cornelius, 2009; Oplinger and Philbrook, 1992). The reduction in fresh pod yield of vegetable soybean induced by delayed planting date might be in part attributable to the shortened duration of seed filling in later planting dates (Weaver et al., 1991). Shorter duration of vegetative and reproductive stages could result in yield reduction because of decreased pod number and seed number per unit area (Egli and Bruening, 2000; Chen and Wiatrak, 2010). Zhang et al. (2010) proposed that the decline in marketable yield of vegetable soybean by 34 to 55 kg ha\(^{-1}\) d\(^{-1}\) with delayed sowing date was mainly related to the decrease in two-seed and three-seed pods. Results of our study further revealed that the number of two-seed pods might be the major yield determinant of vegetable soybean in a changing environment. Because delayed planting date involved environment change (Egli and Cornelius, 2009), cultivars with greater capacity for maintaining more two-seed pods may possess an advantage in a late planting environment.

Delayed planting influence on the level of protein in mature grain soybean seed has been demonstrated in many studies (Kane et al., 1997; Muhammad et al., 2009; Tremblay et al., 2006). However, no investigation has been conducted on vegetable soybean. Our results revealed an advantage of delayed planting to protein accumulation at the fresh edible stage in vegetable soybean. It is consistent with the results of Bellaloui et al. (2011) and Dardaneli et al. (2006) who indicated that late planting increased the level of protein in grain soybean. The variation of protein content are attributed to temperature during the reproductive stage. The protein content of soybean increased for higher temperature during R6 to R7 but decreased for higher temperature during R5 to R6 (Mishra and Cherkauer, 2010). In the present study, the early-planted soybean (18 to 28 °C) was exposed to higher temperature than late-planted soybean (16 to 26 °C) during the R5 to R6 stage. This might be the reason for the higher seed protein content to the later-planted vegetable soybean. Because protein and oil in mature soybean seed are negatively correlated (Watanabe and Nagasawa, 1990), the response of oil content of vegetable soybean would be expected to be opposite the response of protein, i.e., it would be reduced by delayed planting as found in this study. The exposure to lower temperature during the R5 to R6 stage with late planting in this study might also be the reason for the reduction of oil content in the vegetable soybean.

However, the temperature during the R5 to R6 stage was different between 2010 and 2011 (Fig. 1). This may explain the interaction effect of planting date × year on seed oil content of ‘CAS No. 1’ (Table 4). In mature soybean, seed oil content was always decreased in a high-temperature environment (Rotundo and Westgate, 2009). These findings on delayed planting date in changing the quality trait of vegetable soybean could be meaningful because a high protein with relatively low oil content is an important quality trait for healthy food.

Free amino acid of soybean seed not only confers health benefits to humans, but also affects food taste (Bandegan et al., 2011). In the present study, the response of free amino acid content of seed to planting date was similar to seed protein content. The positive correlation (\(P < 0.05\)) between free amino acid of soybean seed and seed protein content has been reported (Hernandez-Sebastia et al., 2005), and free amino acid level in vegetable soybean seed was controlled by the balance between protein synthesis activities and amino acid supplied from different parts of the plant. Fresh seed free amino acid concentration was different among vegetable soybean cultivars, whereas crop conditions and ripe stage also affected the accumulation of free amino acid (Song et al., 2013). Thus, the changed environment during the seed filling stage caused by delayed planting date may influence the fresh seed free amino acid concentration significantly.

The accumulation of sugars is affected by the temperature during the seed filling stage and cultivars differ in sugar accumulation. Delayed planting date in the present studies did not have a similar effect on cultivars. Table 5 shows the planting date × cultivar interaction was significant for fructose + glucose, sucrose, and stachyose. Table 4 shows the planting date × year interaction with ‘CAS No. 1’ was significant only for fructose + glucose and sucrose. The present study confirmed that sucrose is the predominant sugar in the seed of vegetable soybean (Mebraltu and Devine, 2009) and that delayed planting reduced sucrose content. Sucrose is a major product of photosynthesis and the main substrate for sink metabolism. Shorter post-flowering photoperiod by delayed planting might be responsible for the reduction in sucrose content because sucrose is transported from synthesizing (source) organs to sink organs where it is stored.
(Lemoine, 2000), and any reduced type of source organs would influence sucrose accumulation. Furthermore, sucrose is a signal molecule for regulating the source-sink relationship. The weaker sink strength after delayed planting date may feed back in reducing the photosynthetic ability and thus decrease sucrose supply (Chiou and Bush, 1998).

Although the most abundant sugar was sucrose, and fructose and glucose are not considered important in mature seed of grain soybean, Santana et al. (2012) reported these sugars may be associated with the unique and distinct flavor of the vegetable soybean seed. Fructose and glucose levels were found to be higher in vegetable-type soybean than that in grain soybean (Silva et al., 2009). Although delayed planting increased the sum of fructose and glucose in this study, the maximum sum of the two sugars was only 2.09 mg g\(^{-1}\) and its influence on flavor of vegetable soybean is likely quite limited. Raffinose and stachyose are undesirable constituents of food because they result in flatulence and abdominal discomfort for humans and animals (Kumar et al., 2012). The present study found that the content of raffinose and stachyose in vegetable soybean seed was between 0.37 to 2.58 mg g\(^{-1}\) and 1.30 to 2.58 mg g\(^{-1}\), respectively, which was much lower than that in mature grain soybean seed (10 to 20 mg g\(^{-1}\), respectively, which was much lower than that in mature grain soybean seed). Therefore, the relative increase in raffinose and stachyose with delayed planting would not influence the characteristics of vegetable soybean as a healthy food. However, because sucrose is intensely associated with the eating quality of vegetable soybean and the cultivar with higher sucrose level is preferred in the market, the potential negative impact on the eating quality of vegetable soybean induced by delayed planting date deserves more attention.

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