Productivity and chemical composition of food-type soybeans sown on different dates

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ABSTRACT. The objective of this study was to investigate the effect of different sowing dates on the yield, 100-grain weight, oil, protein and isoflavone contents of food-type soybean breeding lines UEL 110, UEL 115 and UEL 123 and a cultivar BRS 257. The materials were seeded on four sowing dates, and the experiment was conducted in a randomized block design with four replications. The productivity and 100-grain weight varied with the sowing dates and soybean genotypes. The protein content ranged from 36.40 to 42.44%, and the oil content ranged from 18.29 to 22.71%. No significant interaction was found between the genotype and sowing dates for the protein content. The isoflavone content also varied with the sowing dates and soybean genotypes. The cultivar BRS 257 had the highest isoflavone content, including the β-glucoside, malonyl glucoside and aglycones forms. The different sowing dates influenced the productivity, 100-grain weight, oil and protein content and isoflavone levels. Higher temperatures and lower rainfall during the grain filling decreased the productivity and isoflavone content and increased the protein content. For all sowing dates, the BRS 257 soybean food-type cultivar showed the highest isoflavone content, indicating that the effect of genotype is more important.

Keywords: soybean, oil, protein, isoflavones.

Produtividade e composição química de soja tipo alimento em diferentes épocas de semeadura

RESUMO. O objetivo deste trabalho foi investigar o efeito das diferentes épocas de semeadura na produtividade, massa de 100 grãos, teor de proteínas, óleo e isoflavonas de linhagens de soja tipo alimento UEL 110, UEL 115 e UEL 123 e cultivar BRS 257. O material foi semeado em quatro épocas de semeadura e o experimento foi conduzido em blocos casualizados com quatro repetições. A produtividade e a massa de 100 grãos variaram com a época de semeadura e com os diferentes genótipos. O teor de proteínas variou de 36,40 a 42,44% e o de óleo de 18,29 a 22,71%. Não foi encontrada interação entre genótipo e épocas de semeadura para o teor de proteínas. O teor de isoflavonas variou com as épocas de semeadura e com os genótipos. A cultivar BRS 257 apresentou maior teor de β-glicosídeos, malonil-glicosídeos e agliconas. As diferentes épocas de semeadura influenciam a produtividade, massa de 100 grãos, conteúdo de óleo, proteínas e isoflavonas. Altas temperaturas e baixas precipitações durante o enchimento dos grãos reduzem a produtividade e teor de isoflavonas e aumentam o teor de proteínas. A cultivar BRS 257 apresenta o maior teor de isoflavonas indicando que o efeito genético é mais importante.

Palavras-chave: soja, óleo, proteína, isoflavonas.

Introduction

Soybean is a versatile grain due to its high protein content (40%), high lipid content (20%), high mineral content, especially of K, Na, Ca, Mg, S and P, and vitamin content, including thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, inositol and choline (Liu, 1997).

In addition to this rich chemical composition, soybean grains have isoflavones. Isoflavones are secondary plant metabolites, a group of natural bioflavonoids synthesized exclusively by the Leguminosae (Fritsche & Steinhart, 1999), and they are associated with the risk reduction or prevention of various diseases, such as breast cancer and prostate cancer (Liggins et al., 2000), cardiovascular disease (Rimbach et al., 2008), and estrogenic and antioxidant activity (Liu, Kanjo, & Mizutani, 2010; Ma et al., 2010). Twelve isoflavone forms have been reported in soybeans, including three aglycones:

genistein, daidzein and glycitein and their respective 7-O-β-D-glucosides (genistin, daidzin and glycitin), 6"-O-malonyl-7-O-β-D-glucosides (malonylgenistin, malonyldaidzin and malonylglycitin) and 6"-O-acetyl-7-O-β-D-glucosides (acetylgenistin, acetyldaidzin and acetylglycitin (Shao et al., 2009).

The yield and the isoflavone, protein and lipid contents can be influenced by the genetic and climatic conditions. Air temperature during the grain formation stage is the most critical environment factor affecting the final content of oil, protein and isoflavones. However, other factors also affect these characteristics, such as humidity, light intensity and rainfall (Carrão-Panizzi, Berhow, Mandarino, & Oliveira, 2009). Regarding the protein and oil level, Marques et al. (2011) observed that the oil content of the Brazilian soybean cultivars ranged from 14 to 24%, and the protein content ranged from 33 to 49%; they concluded that the sowing dates affected only the protein content of the cultivars. Carrão-Panizzi et al. (2009) observed that the total isoflavone content ranged from 12 mg 100 g^{-1} (cv. Embrapa 48) to 461 mg 100 g^{-1} (cv. CS 305) and concluded that the differences among the soybean cultivars were due to genetic effects. Ávila et al. (2007) observed that the oil and isoflavone content varied in range among the Brazilian soybean cultivars, but the protein content was not changed. Carrão-Panizzi, Simão and Kikuchi (2003) studied the effects of the genotype, environment and interactions on the isoflavone contents and reported significant genotype x environment interactions. The average of the total isoflavone content of the Brazilian soybean cultivars is approximately 150 mg 100 g^{-1} (Carrão-Panizzi & Kitamura, 1995).

Lipoxygenases (LOXs) (EC 1.13.11.12) are a class of enzymes that produce the volatile compounds associated with the rancid or beany soybean flavor (Fukushige, Wang, Simpson, Garden, & Hildebrand, 2005). Genetic elimination of lipoxygenase enzymes can improve the flavor of the soybean by reducing the formation of the volatile compounds associated with the characteristic flavor. The lipoxygenase-free soybean cultivars are classified as food-type soybeans because they show this special characteristic (Silva et al., 2012; Liu, 1999).

The selection of genotypes with high grain yield is the main goal of soybean breeding programs. In addition to high grain yield, currently, high-quality soybean grains must have a mild flavor and nutritive and functional properties. Therefore, the objective of this study was to investigate the effect of different sowing dates on the yield, oil, protein content and the isoflavone contents of food-type breeding lines and soybean cultivars and their interactions.

Material and methods

Soybean food types

Three lines (UEL 110, UEL 115, and UEL 123) of the breeding program of The State University of Londrina (UEL), located at Parana state, Brazil and the BRS 257 soybean cultivar supplied by Embrapa Soybean, which is located in the city of Londrina, Parana State, Brazil, were evaluated. All seeds of the breeding lines and the cultivar are lipoxygenase-free and classified as food-type soybeans (Silva et al., 2012). The materials differed in maturity, height, seed weight, growth habit, and other characteristics (Table 1).

Table 1. Agronomic characteristics of the genotypes studied.

Agronomic Characteristics	Lines/Cultivar			
	UEL 110	UEL 115	UEL 123	BRS 257
Florewing dates (days)	44	41	44	43
Maurity group	Early	Middle	Middle	Early
Stem lenght (cm)	97.08	109.39	106.56	67.97
Growth Type	Determined	Indeterminate	Indeterminate	Determined
Seed coat color	Yellow	Yellow	Yellow	Yellow
Hilum color	Yellow	Brown	Brown	Yellow
Lipoxygenases	Null	Null	Null	Null
Lipoxygenases	Null	Null	Null	Null

Grain yield (kg ha^{-1}) and 100-grain weight (g).

The experiment was laid out in a randomized block design with four replications and plot areas of 5 x 0.45 x 0.07 m. The sowing dates were October 7 (sowing date 1), October 14 (sowing date 2), October 29 (sowing date 3) and November 9 (sowing date 4) in the 2013/2014 crop season. The seeds were sown in the farm of The UEL's Agronomy School (23° 20' 23'' S, 51° 12' 32'' W, 535 m altitude), Londrina, Paraná State, Brazil. According to Instituto Agronômico do Paraná (IAPAR, 1987), the climate classification is Köppen Cfa, with an annual precipitation average ranging from 1400 to 1600 mm. The average rainfall and daily temperatures during the experimental period are shown in Figure 1.

After being manually harvested, the grain yield was calculated as productivity in kg ha^{-1}, with correction for 13% moisture. For the calculation of the 100-grain weight (g), four replicates of 100 grains were weighed.

Oil and protein content

Oil and protein contents were determined in the whole soybean grains by near-infrared spectroscopy (NIR) according to the Heil (2012) method using an Antaris™ II FT-NIR analyzer from Thermo Scientific (Thermo Electron Co., USA). The determinations were performed in triplicate, with a resolution of 2.0 cm^{-1} and an average of 32 scans per determination. The predictions were made by mathematical models for oil (R = 0.9487) and crude protein (R = 0.9675).
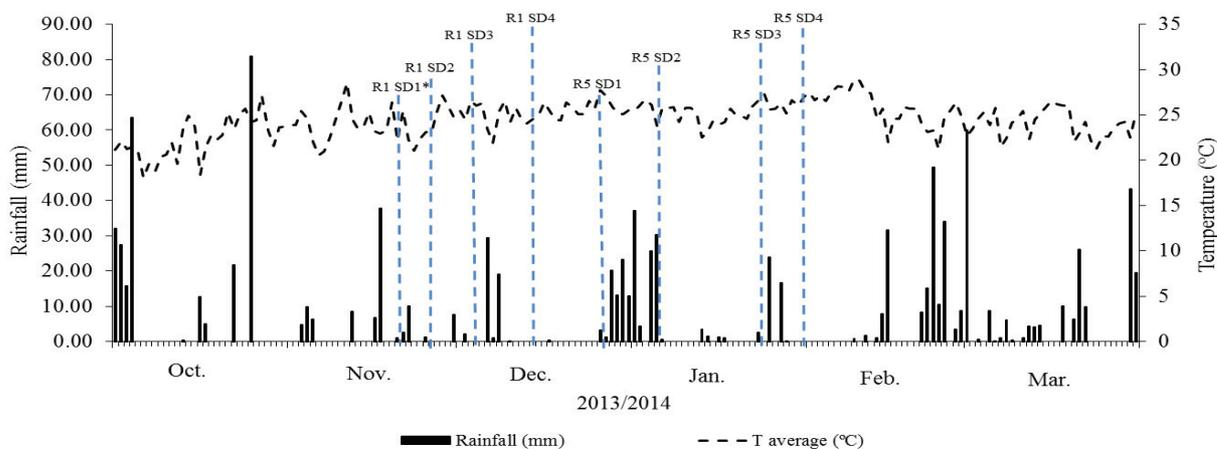
Figure 1. Rainfall and daily temperatures averages observed during the experiment and soybean stages of development.

*Reproductive stage R1 and R5 (Fehr & Caviness, 1977) in the first, second, third and fourth sowing date (SD).

Isoflavone analysis

For the isoflavone content determination, the soybean grains were ground in a TE 631 knife grinder mill from Tecnal, Brazil and defatted with hexane in a 1:10 ratio (w/v) for 1 hour at room temperature by continuous and rotary agitation, followed by vacuum filtration. The extraction was performed in duplicate with 0.5 g of each sample ground with 6 mL of organic solvent containing 70% ethanol acidified with 0.001% glacial acetic acid and then stirred once every 15 min. for 1 hour at room temperature. Then, the mixture was placed in an ultrasonic bath for 15 min. at room temperature, centrifuged at 21,000 xg at 4°C for 15 min. in an Eppendorf 5804 R Centrifuge (Hamburg, GE) and filtered in a 0.22 μm Millex filter. The separation and quantification of isoflavones were performed in duplicate according to Berhow (2002) using an Ultra-Performance Liquid Chromatography (UPLC) system. The column was a reversed-phase type (model ACQUITY UPLC BEH C18, Waters, United States) with dimensions of 2.1 mm (ID) x 50 mm length and a particle size of 1.7 μm. Elution was performed in a linear binary gradient system using mobile phase A, with methanol with 0.025% trifluoroacetic acid, and mobile phase B with ultrapure deionized water with 0.025% trifluoroacetic acid. The flow rate applied was 0.35 mL min.[-1] at 24°C. The gradient begins with 25% eluent A; at the 7 min gradient, the elution ratio ultimately reached 80% A, and then, the initial conditions had returned by 8 min., with a total run time of 10 min. The detector was a diode array (Waters) with a 254 nm wavelength. Standard solutions for the calibration curve construction (peak area of isoflavone contents) were made for each of the twelve isoflavone forms: daidzin, genistin, glycitin, malonyl daidzin, malonyl genistin, glycitin malonyl, acetyl daidzin, acetyl genistin, acetyl glycitin, glycitein and genistein, which were purchased from Sigma and Fluka (0.5, 1, 2, 4, 6, 8, and 10 μg mL^{-1}). The standards were injected in triplicate to yield the corresponding chromatograms for the isoflavone forms, each with its corresponding retention time. The peaks for each isoflavone in each sample were identified for the comparison of the retention times and UV spectrum of the respective reference standard regions. The application was coupled in the chromatograph-generated calibration curves, and the isoflavone concentrations were calculated and expressed in mg of each isoflavone per 100 g of sample (mg isoflavone 100 g^{-1}).

Statistical analysis

The data were submitted to analysis of variance (ANOVA), and the means were compared by Tukey's test at 5% probability using the SISVAR program (Ferreira, 2011). The Pearson correlation using the GENES program was applied to the contents of the protein and oil (Cruz, 2013).

Results and discussion

According to the analysis of variance (ANOVA), the genotype x sowing date interaction showed a significant difference (p < 0.05) in grain yield, 100-grain weight, oil content and for the content of all isoflavone forms, suggesting different responses of the genotypes with regard to the sowing date. However, the protein content showed no significant interaction.

The breeding lines and the BRS 257 soybean cultivar did not differ in productivity for the first and second sowing date (Table 2). In the third sowing date, a higher grain yield was observed for the BRS 257 soybean cultivar, but this cultivar showed lower grain productivity from the fourth sowing date. The highest productivity was obtained for all breeding lines and for the cultivar BRS 257 from sowing date 1 (SD 1) and 3

(SD 3), and this productivity did not differ among the soybeans. The lowest grain yields were observed from sowing date 4 for the breeding line UEL 110 and for the cultivar BRS 257. The higher 100-seed weight was observed for the UEL 110 and UEL 123 breeding lines in SD 1 and for UEL 115 and UEL 110 in SD 3 and SD 4. Within the genotypes, UEL 110, BRS 257 showed the higher 100-seed weight for SD 1 and SD 4. During the experiment, a prolonged drought period was associated with high temperatures (Figure 1), which coincided with the grain filling in SD 2 and SD 4 and the flowering in the SD 4. The productivity and 100-grain weight are well known to be influenced by genetic and climatic factors. The interaction between genotype and climatic conditions results in significant differences in the performance of each genotype according to climatic conditions such as temperature and rainfall (Gauch & Zobel, 1997). Hydric stress in the flowering stage can cause flower abortion and anthesis, and during the seed filling, it can affect the 100-seed weight, reducing the productivity in both cases (Avila et al., 2007; Barbosa et al., 2013). The lower availability of water promotes a decrease in the photosynthesis and grain filling (Marcos Filho, 2005). High temperatures, especially when associated with periods of low rainfall during ripening, can cause early maturation under these conditions of low vigor, as these conditions do not support the natural deposition of carbohydrates, lipids and proteins (Marques et al., 2011; Zhe, Lauer, Borges, & Leon., 2010; França Neto, Krzyzanowski, Henning, West, & Miranda, 1993) because there is a reduction of photosynthate translocation to the grains.

No significant difference at p < 0.05 was observed in the genotype x sowing date interaction for protein content, indicating that the temperature variation among the sowing dates was not enough to cause differences in protein. However, some differences were observed when each factor was considered for genotype and sowing date (Table 3). Protein content ranged from 36.40 to 42.44%, with cultivar BRS 257

showing the highest content (40.77%). Sowing dates (SD) 2 and 4 were more appropriate for an increase in protein content, and the lowest content was observed when the grains were cultivated in the SD 3 (37.05%). High temperature reduced the protein content (Pípolo, Sinclair, & Camara, 2004). The higher temperatures occurred in the SD 2 and SD 4 (grain filling); however, these temperatures were probably not high enough to reduce the protein content. Similar results were shown by Bellaloui, Reddy, Gillen, Fischer, and Mengistu (2011). Some works showed a relation between higher temperatures and lower rainfall on the increase in protein content (Rangel, Minuzzi, Braccini, Scapim, & Cardoso, 2007). Within each genotype, UEL 110, UEL 115 and BRS 257 showed the lowest oil content for SD 2. Pearson's correlation between the protein and oil contents was negative and significant (-0.79), proving that when the protein content increased, the oil content decreased. These results are in accordance with those reported by Pípolo et al. (2004) and Kandil, Sharief, Morsy, and El-Sayed (2013).

Concerning the isoflavones from all sowing dates (Table 4), BRS 257 showed the highest β-glucosides content, suggesting the effect of genotype on these isoflavone forms. The breeding line UEL 110 did not differ from the cultivar BRS 257 in the second sowing date. Among the breeding lines, the observed β-glucosides content was greater in the third sowing date. The lower β-glucosides content were found in the SD 2. Regarding the malonyl glucosides, the same results were observed with BRS 257, which exhibited the highest content of malonyl glucosides in all sowing dates. All genotypes showed the highest malonyl content for SD 3 and the lowest for SD 2. Aglycone contents varied from 8.92 to 0.72 mg 100 g^{-1} of isoflavones. BRS 257 showed the higher aglycone content for SD 1, and no difference between the breeding lines UEL 110 and UEL 115 was observed for SD 2 and SD 3. The highest content of aglycones was observed for SD1, and the lowest contents were observed for all genotypes in SD 2.

Table 2. Grain yield (kg ha^{-1}) and 100 grains weight (g) of the studied genotypes in different sowing dates.

Genotype	Sowing date[1]				
	SD 1	SD 2	SD 3	SD4	
	Grain yield (Kg ha^{-1})				Mean
UEL 110	3649.08 Aa	2745.82 Ba	3526.02 Aab	1854.05 Cab	2943.74
UEL 115	3794.17 Aa	2563.26 Ba	3305.63 Ab	2136.65 Bab	2949.93
UEL 123	3876.00 Aa	2240.85 Ba	3642.03 Aab	2214.77 Ba	2993.42
BRS 257	3443.04 Aa	2412.48 Ba	4051.92 Aa	1450.03 Cb	2839.36
Mean	3690.57	2490.60	3631.40	1913.87	
	100 grains weight (g)				
UEL 110	16.46 Aa	13.68 Bab	13.77 Bab	17.39 Aa	15.33
UEL 115	15.21 Ab	13.08 Bb	14.63 Aa	13.44 Bc	14.09
UEL 123	16.40 Aa	12.89 Db	14.04 Cab	15.34 Bb	14.67
BRS 257	16.11 Aab	14.18 Ba	13.49 Bb	15.65 Ab	14.86
Mean	16.05	13.46	13.98	15.45	

[1]Sowing date at October 7, 2013 (SD 1), October 14, 2013 (SD 2), October 29, 2013 (SD 3) and November 9, 2013 (SD 4). Means followed by the same letter (capital in the line and small in the column) do not differ by Tukey at 5% probability. C.V (%) = 13.39 (Grain yield (kg ha^{-1})) and = 3.73 (100 grains weight (g)).

Table 3. Protein and Oil Content (%) of the studied genotypes sowing dates.

Genotype	Sowing dates[1]				
	SD 1	SD 2	SD 3	SD 4	
			Protein		Mean
UEL 110	39.85	40.49	36.65	39.43	39.11 b
UEL 115	38.24	39.92	36.41	39.16	38.43 b
UEL 123	39.89	39.32	39.60	36.40	38.80 b
BRS 257	40.33	42.44	38.72	41.60	40.77 a
Mean	39.58 B	40.54 A	37.05 C	39.95 AB	
			Oil		
UEL 110	21.39 Bab	20.40 Ca	22.66 Ab	20.86 Bcb	21.33
UEL 115	22.27 Aa	20.52 Ba	22.71 Aa	22.19 Aa	21.92
UEL 123	21.11 Bbc	20.99 Ba	22.62 Aa	21.82 Aba	21.63
BRS 257	20.31 Ac	18.29 Bb	20.38 Aa	20.33 Ab	19.83
Mean	21.27	20.05	22.09	21.30	

[1]Sowing date at October 7, 2013 (SD 1), October 14, 2013 (SD 2), October 29, 2013 (SD 3) and November 9, 2013 (SD 4). Means followed by the same letter (capital in the line and small in the column) do not differ by Tukey at 5% probability. C.V (%) = 2.58 (protein) and = 2.84 (oil).

Table 4. β-glucosides, malonylglucosides, aglycones and total isoflavones contents (mg 100 g^{-1}) of the studied genotypes.

Genotypes	Sowing dates[1]				
	SD 1	SD 2	SD 3	SD 4	
			β-Glucosides		Mean
UEL 110	76.70 Bb	34.16 Da	84.87 Ab	42.92 Cb	59.66
UEL 115	57.86 Ac	22.47 Cb	57.05 Ad	30.63 Bc	42.00
UEL 123	53.47 Bd	20.53 Db	74.00 Ac	29.16 Cc	44.29
BRS 257	92.84 Aa	35.26 Da	88.35 Ba	49.06 Ca	66.38
Mean	70.21	28.10	76.07	37.94	
			Malonylglucosides		
UEL 110	113.76 Bb	75.89 Da	155.67 Ab	87.40 Cb	108.63
UEL 115	88.89 Bc	45.35 Db	125.65 Ad	64.06 Cd	80.98
UEL 123	81.90 Bd	37.67 Dc	150.71 Ac	68.58 Cc	84.71
BRS 257	165.12 Ba	72.28 Da	195.22 Aa	97.91 Ca	132.63
Average	112.42	57.79	156.81	79.48	
			Aglycones		
UEL 110	7.80 Ab	0.99 Db	5.90 Bb	2.32 Ca	4.25
UEL 115	4.96 Ac	1.26 Da	3.38 Bd	1.78 Cb	2.84
UEL 123	5.13 Ac	0.72 Dc	4.05 Bc	1.70 Cb	2.90
BRS 257	8.92 Aa	1.47 Da	6.77 Ba	2.49 Ca	4.91
Average	6.70	1.11	5.02	2.07	

[1]Sowing date at October 7, 2013 (SD 1), October 14, 2013 (SD 2), October 29, 2013 (SD 3) and November 9, 2013 (SD 4). Means followed by the same letter (capital in the line and small in the column) do not differ by Tukey at 5% probability. CV (%) = 2.18 (Isoflavones total); 3.09 (Aglycones); 2.11 (β-Glucosides); 2.27 (Malonylglucosides).

These results indicate that the temperature affects the contents of these isoflavones. The average temperature during the months from October to March was 24.4ºC. February had the highest temperature average with 25.82ºC and the highest thermal variation 3.98ºC, which coincided with the beginning of grain filling. The period of the early grain filling (R5 stage) to physiological maturity (R8 stage) for genotypes sown on October 14, 2013 (SD 2), had the highest temperature average with 26.04ºC. There have been many studies proving the influence of environmental conditions and genetic factors on the isoflavone contents (Tsukamoto et al., 1995; Carrão-Panizzi et al., 2003; Lee et al., 2003; Riedl et al., 2007). Tsukamoto et al. (1995) and Kim, Kim, Kim, and Chung (2012) observed that the isoflavone content was lower when the temperature was high during the grain filling stage. Carrão-Panizzi et al. (2009) observed that total isoflavone content ranged among the genotypes studied and concluded that the differences in the isoflavone content were due to genetic effects because all cultivars were grown at the same location and in the same growing season.

Conclusion

The different sowing dates influence the productivity, 100-grain weight, oil and protein content and isoflavone levels.

Higher temperatures and lower rainfall during grain filling decreased the productivity and isoflavone content and increased the protein content.

For all sowing dates, the BRS 257 food-type soybean cultivar showed high isoflavone content, indicating that the effect of genotype is more important.

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