

EFFECTS OF GENETICS AND ENVIRONMENT ON ISOFLAVONE CONTENT OF SOYBEAN FROM DIFFERENT REGIONS OF BRAZIL¹

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ABSTRACT - The effects of genetics and environmental factors on isoflavone content of soybean (*Glycine max* L.) cultivars grown in different locations in Brazil in 1993/94 were evaluated. Seeds of different cultivars were analysed by high performance liquid chromatography (HPLC). In Rio Grande do Sul (RS), Paraná (PR), and Mato Grosso do Sul (MS) States, a significant difference in the isoflavone total content average of the cultivars IAS 5 and FT-Abyara (163.9, 116.4 and 79.5 mg/100 g, respectively) was observed. In general, IAS 5 contained higher isoflavone than FT-Abyara. Cultivars IAS 5 and FT-Abyara grown at Vacaria, RS (28°30' S latitude) with temperature average of 19°C, had the highest isoflavone concentrations (218.7 and 163.8 mg/100 g, respectively). In Palotina, PR (24°27' S latitude), where temperature average was 24°C, the isoflavone concentrations were 105.9 and 86.8 mg/100 g, respectively. The lowest isoflavone contents were observed for FT-Estrela and FT-Cristalina, (27.6 and 46.5 mg/100 g, respectively) at Rondonópolis, MT (16°20' S latitude), where the temperature was 27°C.

Index terms: *Glycine max*, daidzin, genistin, crop location, HPLC.

EFEITOS DA GENÉTICA E DO AMBIENTE NOS TEORES DE ISOFLAVONÓIDES EM SOJA DE DIFERENTES REGIÕES DO BRASIL

RESUMO - Analisaram-se os efeitos da genética e de fatores ambientais nos teores de isoflavonóides em cultivares de soja (*Glycine max* L.) provenientes de diferentes locais do Brasil, na safra 1993/94. Sementes de diferentes cultivares foram analisadas por cromatografia líquida de alto desempenho (HPLC). Nos estados do Rio Grande do Sul (RS), Paraná (PR) e Mato Grosso do Sul (MS) observou-se diferença significativa entre as médias do conteúdo total de isoflavonóides das cultivares IAS 5 e FT-Abyara (163,9, 116,4 e 79,5 mg/100 g, respectivamente). Em geral, IAS 5 apresentou teores mais altos de isoflavonóides que FT-Abyara. As cultivares IAS 5 e FT-Abyara provenientes de Vacaria, RS (28°30' latitude S), com temperatura média de 19°C, apresentaram concentrações mais altas de isoflavonóides (218,7 e 163,8 mg/100 g, respectivamente). Em Palotina, PR (24°27' latitude S) onde a temperatura média foi de 24°C, as concentrações de isoflavonóides foram de 105,9 e 86,8 mg/100 g, respectivamente. Os menores teores de isoflavonóides foram observados nas cultivares FT-Estrela e FT-Cristalina (27,6 e 46,5 mg/100 g, respectivamente), em Rondonópolis, MT (16°20' latitude S), onde a temperatura foi de 27°C.

Termos para indexação: *Glycine max*, daidzina, genistina, locais de cultivo, HPLC.

INTRODUCTION

Potential health benefits of soy foods in the prevention and treatment of heart diseases and cancer warrant an increase in soybean consumption in the Western countries (Messina et al., 1994). Epidemiological data suggest that the low incidence of breast cancer in the Asian populations is related to their traditional diet, in which the soybean is an important component (Adlercreutz et al., 1991; Coward et al., 1993). The urinary excretion of

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isoflavones was highly correlated with soybean products intake (Adlercreutz et al., 1991). Anticancer effects of isoflavones place particular emphasis on soybean which is a good source of these compounds (Messina, 1995).

Isoflavones were found in soybean in the conjugated glucosides forms daidzin, genistin, and glycitin, their corresponding aglycones daidzein, genistein, and glycitein, and the malonyl glucosides: 6"-O-malonyl daidzin, 6"-O-malonyl genistin, and 6"-O-malonyl glycitin (Walter, 1941; Naim et al., 1973; Ohta et al., 1979, 1980; Kudou et al., 1991).

Kudou et al. (1991) reported that in soybean seeds, the total isoflavone content in the hypocotyl was 5.5 to 6.0 times higher than that of the cotyledons; the glycitin forms occurred only in the hypocotyl part and no isoflavones were observed in the seed coat. The accumulation of isoflavones occurred during seed filling (between 35 and 60 days after flowering), with genistin and malonyl genistin content increasing at the end of the seed development stage, while daidzin and malonyl daidzin increased during the entire period.

Variability on isoflavone concentration was observed among soybean cultivars grown in different locations and crop years (Eldridge & Kwolek, 1983; Wang & Murphy, 1994). Early maturing soybean cultivars, the so-called "summer-type soybeans", had a stable low concentration of isoflavones (Kitamura et al., 1991). High temperature during the seed filling stage was related as a major factor in determining the levels of isoflavones in seeds (Tsukamoto et al., 1995). Differences in isoflavone content were also observed among Brazilian soybean cultivars (Carrão-Panizzi & Kitamura, 1995; Carrão-Panizzi, 1996).

The objective of this study was to evaluate the effects of genetic and crop location on isoflavone content in Brazilian soybean cultivars, which are grown in different environments, from Equator to 32° S latitude.

MATERIAL AND METHODS

Seed samples were collected from the 1993/94 final yield soybean trials, from different locations of Brazilian soybean production regions. The three states considered

in the first experiment with 14 different locations were the following: Rio Grande do Sul (RS): Vacaria, Pelotas, Cruz Alta, Passo Fundo, Santa Rosa; Paraná (PR): Londrina, Ponta Grossa, Mariópolis, Palotina, Cascavel, Campo Mourão; and Mato Grosso do Sul (MS): Campo Grande, São Gabriel do Oeste, Sidrolândia. In these states, the effects of genetic and environmental factors on isoflavone content were evaluated in seeds of cultivars IAS 5 and FT-Abyara. Since within each state, different locations and number of locations were considered, the experiment was analysed as a complete randomized block design with hierarchical classification. Cultivar EMBRAPA 4 was evaluated just for conditions of Paraná State sowed in Londrina, Ponta Grossa, Mariópolis, Palotina, Cascavel, and Campo Mourão.

The second experiment was arranged in the same experimental design and the following states and locations were considered: Mato Grosso do Sul (MS): Campo Grande, São Gabriel do Oeste, Sidrolândia; Minas Gerais (MG): São Gotardo, Conceição das Alagoas; and Goiás (GO): Goiânia, Rio Verde. The cultivars FT-Cristalina and FT-Estrela were used in this experiment.

For both experiments the mathematical model is of mixed effects and the values for F-test were calculated according to Hicks (1973) methodology.

$$Y_{ijkl} = m + S_i + L_{j(i)} + B_{k(ij)} + C_l + C * L_{jl(i)} + S * C_{il} + e_{ijkl}$$

where, Y is the observations; m is the effect of mean; S_i is the effect of state; $L_{j(i)}$ is the effect of location_j within state_i; $B_{k(ij)}$ is the effect of block_k within state_i and location_j; C_l is the effect of cultivar_l; $C * L_{jl(i)}$ is the effect of interaction between cultivar_l and location_j within state_i; $S * C_{il}$ is the effect of interaction between State_i and cultivar_l; and e_{ijkl} is the residual effect with normal distribution $N \sim (0, \sigma^2)$.

The third experiment was carried out at Maranhão State as a complete randomized block design with treatments arranged in factorial: two locations, three cultivars and three blocks. The locations were Balsas and Tasso Fragoso with cultivars BR-35 (Rio Balsas), EMBRAPA 9 (Bays), and EMGOPA 312 (Potiguar). The mathematical model for this experiment was the following:

$$Y_{ijk} = m + L_i + B_{j(i)} + C_k + C * L_{ik} + e_{ijk}$$

where, Y is the observations; m is the effect of mean; L_i is the effect of location_i; $B_{j(i)}$ is the effect of block_j within location_i; C_k is the effect of cultivar_k; $C * L_{ik}$ is the effect of interaction between cultivar_k and location_i; e_{ijk} is the residual effect with normal distribution $N \sim (0, \sigma^2)$.

The fourth experiment arranged in a complete randomized block design, considered cultivars FT-Cristalina and FT-Estrela, sowed in Rondonópolis at Mato Grosso State, according to the following model: $Y_{ij} = m + C_i + B_j + e_{ij}$

where, Y_{ij} is the observations; m is the effect of mean; C_i is the effect of cultivar; B_j is the effect of block; ε_{ij} is the residual effect with normal distribution $N(0, \sigma^2)$.

To enhance efficiency of the estimatives and precision of the significance of the F-test, the assumptions to validate the analysis of variance (ANOVA) were evaluated throughout the following exploratory methods: normal distribution (Shapiro & Wilk, 1965), homogeneity of variance (Hartley, 1940; Burr & Foster, 1972), and model additivity (Tukey, 1949). Differences among treatment mean values were determined by Tukey's test at $P \leq 0.05$ (Cochran & Cox, 1957). Statistical Analysis System (SAS, 1995), and Sistema de Análise Estatística - SANEST (Zonta et al., 1982) were used for data analysis.

Daily local temperature average during the soybean season (November 1993 to April 1994) was recorded in order to identify temperature differences among locations.

For the isoflavone analysis, 100 g samples of soybean seeds containing 11% to 13% moisture were taken; 30 g were weighted to form subsamples, with 5 g milled for 40 seconds in a vibrating sample mill (Heiko mod. TI-100). Since traditional soybean processing into food products does not separate seed parts (seed coat, hypocotyl, and cotyledon), the isoflavone analysis was performed on the whole seed.

The quantitative analysis of isoflavones was carried out by high performance liquid chromatography (HPLC) (Kitamura et al., 1991; Kudou et al., 1991). One hundred mg of milled samples were extracted with 4.0 mL of 70% aqueous ethanol containing 0.1% acetic acid, in screw-capped test tubes, for one hour, at room temperature. After centrifugation (4 minutes at 16,000 g, at 4°C), a 40 μ L aliquot of the supernatant was used for HPLC analysis. Analyses of isoflavones were performed in a Tosoh chromatograph model UV 8011, equipped with UV detector. The separation was carried out on a reversed phase ODS-C18 column (Tosoh Corp., Tokyo, Japan) TKS-gel ODS-80TM (i.d. 4.6 x 250 mm).

Elution of isoflavones compounds was performed by a linear gradient system. The initial gradient system consisted of 20% acetonitrile and 80% of 0.1% acetic acid solution. After 30 minutes of elution, the proportion of 45% of acetonitrile and 55% of acetic acid solution was reached and all the isoflavones were detected. The solvent flow rate was 1.0 mL/minutes, and the UV absorption was measured at 260 nm. Purified soybean isoflavones

(daidzin and genistin) were used as standards (Kudou et al., 1991). Isoflavone contents were calculated as mg/100 g soybean flour (Eldridge & Kwolek, 1983; Kitamura et al., 1991). Total isoflavone content was the sum of isoflavone glucosides genistin, daidzin, malonyl daidzin and malonyl genistin, on wet weight basis.

RESULTS AND DISCUSSION

In different locations of the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul, cultivar IAS 5 was consistently different from cultivar FT-Abyara considering isoflavone content, which suggested that genetic plays a significant role on isoflavone accumulation (Table 1). Cultivar EMBRAPA 4, tested just for locations in Paraná State, showed the lowest levels of isoflavones (58.1 mg/100 g) compared to cultivars IAS 5 (131.4 mg/100 g) and FT-Abyara (101.5 mg/100 g).

In addition to genetic differences among cultivars, variation was also observed in isoflavone contents of seeds harvested in different locations (Table 1), indicating that the isoflavone content was strongly affected by environmental conditions. Results from analysis of variance (ANOVA) indicated significant F-test responses for the interactions between locations cultivar (Table 1). Location and crop year also affected isoflavone content (Eldridge & Kwolek, 1983; Wang & Murphy, 1994). At Rio Grande do Sul State (Table 1), the greatest differences for both cultivars, FT-Abyara and IAS 5 were observed between Vacaria (163.8 and 218.7 mg/100 g, respectively) and Santa Rosa (123.7 and 126.9 mg/100 g, respectively), where temperature average during the soybean growing season (November to April) were 19°C and 23°C, respectively. The highest temperature average observed in Santa Rosa may have reduced the isoflavone content of the cultivars, fact also observed by Kitamura et al. (1991). In Kyushu, Japan, Tsukamoto et al. (1995) reported that cultivar Lee had isoflavone concentration 5.8 times higher (142.3 mg/100 g) when sowed in July (temperature average 21°C) compared to that sowed in May (24.6 mg/100 g) when temperatures were 25.9°C.

In Paraná State, the same trend as in Rio Grande do Sul State was observed (Table 1). Cultivars

TABLE 1. Total isoflavone glucosides (mg/100 g, \pm SD) in seeds of soybean cultivars IAS 5 and FT-Abyara, as a function of sowing location within the state in the 1993/94 crop season (n=3, CV=7.93%)¹.

State	Location	Latitude	Altitude	Temperature	Isoflavones (mg/100 g)	
					South	(m)
Rio Grande do Sul	Vacaria	28°30'	971	19	163.8aB (\pm 16.2)	218.7aA (\pm 25.1)
	Passo Fundo	28°17'	687	20	155.6aB (\pm 10.3)	196.0bA (\pm 10.6)
	Pelotas	31°45'	17	21	162.9aB (\pm 12.2)	185.3bA (\pm 4.3)
	Cruz Alta	28°38'	452	21	132.9bB (\pm 16.2)	173.7cA (\pm 8.5)
	Sta. Rosa	27°53'	277	23	123.7bA (\pm 7.3)	126.9dA (\pm 8.3)
Mean					147.8aB (\pm 19.6)	180.1aA (\pm 28.4)
Paraná	Mariópolis	26°21'	800	20	130.4aA (\pm 10.5)	145.1aA (\pm 9.7)
	Ponta Grossa	25°05'	975	21	116.7abB (\pm 13.6)	143.4aA (\pm 2.4)
	Cascavel	24°58'	800	22	102.9bcB (\pm 2.7)	136.5aA (\pm 18.4)
	Londrina	23°11'	585	23	86.3dB (\pm 6.2)	142.6aA (\pm 17.1)
	Campo Mourão	24°02'	630	22	85.8dB (\pm 7.3)	115.0bA (\pm 6.3)
	Palotina	24°27'	376	24	86.8cdB (\pm 6.1)	105.9bA (\pm 13.6)
Mean					101.5bB (\pm 19.0)	131.4bA (\pm 19.0)
Mato Grosso do Sul	Campo Grande	20°28'	532	25	96.3aA (\pm 4.5)	93.2aA (\pm 3.7)
	S. Gabr. Oeste	19°21'	658	-	55.5bB (\pm 2.6)	93.8aA (\pm 8.0)
	Sidrolândia	20°56'	484	-	68.5bA (\pm 5.1)	69.6bA (\pm 9.5)
Mean					73.5cB (\pm 18.4)	85.5cA (\pm 13.5)

¹ Values of the interaction between location and cultivar, fixed within the state, followed by the same small letters in the columns and capital letters in the lines, are not significantly different (Tukey $P \leq 0.05$); values from the interaction between state and cultivar, in bolding, followed by the same small letters in the columns and capital letters in the lines are not significantly different (Tukey $P \leq 0.05$).

IAS 5 and FT-Abyara presented higher isoflavone content in Mariópolis and in Ponta Grossa than in Palotina, which could be due to the local temperature average of 20°C, 21°C and 24°C, respectively (Table 1). Mariópolis and Ponta Grossa are located at higher altitude (800 m and 975 m, respectively) than Palotina (376 m). Exactly the same trend observed for IAS 5 e FT-Abyara was also found for cultivar EMBRAPA 4, which isoflavone concentration was 74.6 mg/100 g in Mariópolis and 48.8 mg/100 g in Palotina.

At Londrina (PR), although the high temperature average of 23°C, the cultivar IAS 5 presented a high isoflavone concentration (142.6 mg/100 g), compared to the concentrations observed in Mariópolis and Ponta Grossa, where temperature average was lower (20°C) (Table 1). Cultivar EMBRAPA 4 showed a similar response. According to Koeppen climate classification (Strahler, 1975), Londrina (23°11'50" S latitude; 585 m altitude) presents a subtropical climate with hot summer and high precipitation. Despite this classification, Londrina is located in a transition zone between temperate and subtropical regions, which confers to Londrina's climate an unstable behavior, ranging from temperate to subtropical. Therefore, results observed in Londrina for isoflavone contents were probably influenced by this climate variation. During seed filling, IAS 5 and EMBRAPA 4, which are early maturing cultivars, could be exposed to temperature variations that could occur in Londrina and were not detected. Cultivar FT-Abyara (intermediate maturing) had similar isoflavone concentration at Londrina (86.3 mg/100 g) and at Palotina (86.8 mg/100 g) (Table 1), as expected since both locations have similar high temperatures.

No differences in isoflavone content were observed between cultivars IAS 5 e FT-Abyara grown at Campo Grande and Sidrolândia, both located in Mato Grosso do Sul State. At São Gabriel do Oeste, however, the difference was significant (Table 1). Cultivar FT-Abyara was greatly influenced by sowing location, while IAS 5 showed this effect only in Sidrolândia (Table 1). In Mato Grosso do Sul State high temperatures are very common. This situation could have reduced isoflavone content of the cultivars, that showed similar concentrations to those

observed in Palotina (PR), where local temperatures were also high (Table 1).

In general, isoflavone content was lower as latitude decreased (Table 1). Although Santa Rosa is located at higher latitude (27°53'S), the local altitude is low (277 m), which increases local temperature average (23°C). These conditions reduced isoflavone content of the cultivars compared with other locations of Rio Grande do Sul State (Table 1). In Mariópolis (PR), soybean cultivars contained high isoflavone content as a result of lower local temperature average and higher latitude among the locations in the State of Paraná, as well as seeds from locations at Mato Grosso do Sul State contained low average concentrations of isoflavones as a result of low latitude and high temperature average (Table 1).

A significant ($P \leq 0.05$) effect of location was observed for total isoflavone content of the cultivar IAS 5 and FT-Abyara in the States of Rio Grande do Sul, Paraná, and Mato Grosso do Sul (Table 1). The interaction between states and cultivars was significant ($P \leq 0.05$) (Table 1). Cultivar IAS 5 had the highest average levels of total isoflavones in all states. Both cultivars IAS 5 and FT-Abyara had isoflavone contents in decreasing order in the States of Rio Grande do Sul, Paraná, and Mato Grosso do Sul (Table 1). American and Japanese cultivars also exhibited genetic variations and effects of sowing location and year (Wang & Murphy, 1994).

Differences for concentrations of daidzin, genistin, malonyl daidzin, and malonyl genistin were similar (Table 2) to differences observed for total isoflavones in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul (Table 1). The highest concentrations of these compounds were always observed in Rio Grande do Sul (Table 2). Cultivar IAS 5 had higher concentration of genistin and malonyl genistin than cultivar FT-Abyara suggesting that IAS 5 would be suitable to produce genistein when properly processed. Genistein, a compound formed by hydrolysis of the glucoside genistin (Matsuura et al., 1989), has properties similar to the antiestrogen drug tamoxifen, which is used in breast cancer therapy (Peterson & Barnes, 1991). Indeed, anticancer activity of genistein in cancers of prostata (Peterson & Barnes, 1993) and colon (Steele et al.,

TABLE 3. Average (\pm SD) concentration of total isoflavone glucosides (mg/100 g) in seeds of soybean cultivars FT-Estrela and FT-Cristalina as a function of interaction between sowing location within the state and cultivar in the 1993/94 crop season (n=3, CV = 10.08%)¹.

State	Location	Latitude South	Altitude (m)	Temperature (°C)	Isoflavones (mg/100 g)	
					FT-Estrela	FT-Cristalina
Goiás	Goiânia	16°40'	749	23	67.1bA (\pm 9.4)	55.4bB (\pm 3.4)
	Rio Verde	17°26'	715	-	112.7aA (\pm 5.3)	70.2aB (\pm 4.1)
Mean					89.9aA (\pm 8.9)	62.8bB (\pm 25.7)
Minas Gerais	C. Alagoas	19°55'	509	26	61.7bA (\pm 7.8)	44.3bB (\pm 10.3)
	São Gotardo	19°20'	1055	23	90.8aA (\pm 1.8)	98.3aA (\pm 0.3)
Mean					76.2bA (\pm 30.3)	71.3aA (\pm 16.7)
Mato Grosso do Sul	Campo Grande	20°28'	532	25	91.0aA (\pm 5.5)	43.2bB (\pm 2.3)
	S. G. Oeste	19°21'	658	-	49.0bA (\pm 1.4)	56.3aA (\pm 3.5)
	Sidrolândia	20°56'	484	-	45.7bA (\pm 14.3)	42.6bA (\pm 5.5)
Mean					61.9cA (\pm 2.8)	47.4cB (\pm 23.2)

¹ Values of the interaction between location and cultivar, fixed within the State, followed by the same small letters in the columns and capital letters in the lines, are not significantly different (Tukey $P \leq 0.05$); values from the interaction between state and cultivar, in bolding, followed by the same small letters in the columns and capital letters in the lines are not significantly different (Tukey $P \leq 0.05$).

TABLE 4. Average (\pm SD) concentration of total isoflavone glucosides (mg/100 g) in seeds of soybean cultivars as a function of the interaction between sowing location and cultivar at the State of Maranhão, in the 1993/94 crop season (n=3, CV = 10.86%)¹.

Location	Latitude South	Altitude (m)	Isoflavones (mg/100 g)			Mean
			BR-35	EMBRAPA 9 (Bays)	BR/EMGOPA 312	
Balsas	07°27'	250	43.3aA (\pm 5.9)	34.7aB (\pm 3.5)	52.2aA (\pm 6.1)	43.4a (\pm 8.8)
			Tasso Fragoso	08°27'	242	46.4aA (\pm 4.8)

¹ Mean values followed by the same small letter in the columns and capital letter in the lines are not significantly different (Tukey $P \leq 0.05$).

Our results demonstrate that isoflavone concentration is influenced by genetic and environmental factors. Thus breeding soybean cultivars for high or low isoflavone concentration is not an easy task. As observed in the literature, ingestion of foods with higher levels of these compounds could help in the treatment or prevention of breast and prostate cancer. In this case, Brazilian Southern states, where temperatures are cooler and concentrations of these compounds are higher, would be more suitable for soybean production aiming at using in medicine development. On the other hand, soybean cultivars with a reduced isoflavone concentration occur in locations of warmer temperatures (low latitudes). Hence, these locations could be suitable for production of soybean with better flavor since isoflavones are responsible for the astringency sensation observed in soybean products.

CONCLUSIONS

1. Isoflavone concentration in soybean cultivars is genetically determined and affected by environment factors.
2. Cultivar IAS 5 has higher isoflavone content than cultivar FT-Abyara in all locations of Mato Grosso do Sul, Rio Grande do Sul, and Paraná states.
3. High local temperature average decreases isoflavone concentration of soybean cultivars.
4. Depending on sowing location it is possible to produce soybean for medicine uses or less astringent soybean products.

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