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Isoflavone Content in Brazilian Soybean Cultivars

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Summary

Soybean is not well accepted as a human food source in the West because of its flavor. Isoflavones have been suggested as the cause of soybean bitterness and astringency. The isoflavone content of 22 Brazilian cultivars of different maturity groups was analysed by using High Performance Liquid Chromatography (HPLC). The content of daidzin and genistin in the Brazilian cultivars showed a considerable variability among cultivars of the same and different maturity groups and between consecutive years (1990 and 1991). Insect-resistant cultivar IAC-100 exhibited the highest isoflavone content in both years, while cultivar BR-36 had the lowest.

Key Words : soybean, isoflavones, daidzin, genistin, flavor, insect-resistance.

Introduction

Soybean is not widely consumed as human food source in the West because of its flavor. Three main flavors are distinguished in soybean ; beany, bitter and astringent (Maga 1973). The beany flavor results from the action of lipoxygenase enzymes. The enzyme activities and genetics of lipoxygenase have been exhaustively studied (Wolf *et al.* 1975, Axelrod *et al.* 1981, Nelson *et al.* 1979, Hildebrand and Hymowitz 1982, Hildebrand and Kito 1984, Kitamura *et al.* 1983 and 1985, Kitamura 1984, Davies and Nielsen 1986, and Hajika *et al.* 1991, among others). Bitterness and astringency are mainly due to saponins (Kitagawa *et al.* 1988, Okubo *et al.* 1992) and isoflavone (Huang *et al.* 1981, Matsuura *et al.* 1989, Tsukamoto *et al.* 1990, Kudou *et al.* 1991, and Ha *et al.* 1992). Because the flavor ascribed to the saponin can be reduced by mechanical removal of the seed hypocotyl, breeding soybean for improved flavor characteristics, should be particularly focused on the manipulation of lipoxygenase and isoflavone levels.

Genistin, daidzin and their aglycones, genistein and daidzein are the principal isoflavone compounds found in soybean (Murphy 1982). However, acetyl and malonyl forms of the isoflavone glycosides have also been identified (Ohta *et al.* 1979 and Kudou *et al.* 1991). Malonyl forms have been reported as the major isolavone constituents of soybean seeds, although they are thermally unstable and are converted into their corresponding isoflavone glycosides (Kudou *et al.* 1991). In general, concentrations of these compounds in soybean products decrease with increased processing (Murphy 1982). A significant variation is observed among different soybean cultivars as a result of different environmental conditions (Murphy 1982, Eldridge and Kwolek 1983, Kitamura *et al.* 1991, Kudou *et al.* 1991, and Tsukamoto *et al.* 1995). Environmental temperatures during seed development are a determining factor for isoflavone content in soybean seeds, and high temperatures decrease their amounts (Kitamura *et al.* 1991). These results were confirmed by Tsukamoto *et al.* (1995), who observed the effects of temperature during seed development of soybean plants grown in a temperature-controlled growth chambers.

Isoflavones are associated with the bitter and astringent taste. Kudou *et al.* (1991) observed the lowest threshold value for malonyl forms. Matsuura *et al.* (1989 and 1993) reported that daidzin and genistin glucosides are hydrolized by the enzyme β-glucosidase to daidzein and genistein aglycones, respectively, resulting in an increased objectionable after-taste in the soybean milk. Okubo *et al.* (1992) also observed stronger bitterness for daidzein and genistein. By using glossopharyngeal nerve of a frog to measure dry mouth feel (DMF) activities of soybean glucosides, they observed that genistin had a stronger DMF than daidzin fraction, as well as a lower threshold value for genistin than for daidzin.

These phytochemicals are a subject of current cancer research and have been suggested as possible cancer preventing agents (Messina and Barnes 1991, Caragay 1992, Coward *et al.* 1993 and Xu *et al.* 1994). It is possible that dietary isoflavones through soy foods are an important factor in the lower incidence of breast and prostate cancer in Asians as compared with Americans (Coward *et al.* 1993). Epidemiologic studies revealed low mortality in hormone-dependent cancers in Japanese women and men consuming a traditional diet, the high urinary excretion of isoflavone observed was correlated with a high intake of soybean-products (Adlercreutz *et al.* 1991). Isoflavones have also been reported as phytoalexins which are related to resistance to insects, pathogens and nematodes (Fisher *et al.* 1990, Rivera-Vargas *et al.* 1993, and Huang and Barker 1991).

Due to the importance of isoflavone, and the ongoing breeding program to obtain adapted soybean cultivars for human consumption at the National Soybean Research Center of EMBRAPA, these compounds in Brazilian soybean cultivars were chosen for analysis.

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Materials and Methods

Seed samples of 22 Brazilian soybean cultivars of different maturity groups, planted in November 29, 1990 and November 9, 1991 in Londrina, Paraná state (23° 12'S latitude), were analyzed for isoflavone content by using High Performance Liquid Chromatography (HPLC). The quantitative analysis of four kinds of isoflavone compounds (daidzin, genistin and their respective malonyl forms) were carried out by a modified method (Kudou *et al.* 1991). One hundred mg of milled seed cotyledons were extracted in screw capped test tubes with 4.0 m*l* of 70 % aqueous ethanol, for one hour at room temperature. After centrifugation, 10 μl aliquot of the supernatant was used directly for HPLC analysis on an ODS (TSK-gel) commercial packed column (Tosho Co. Ltd., Tokyo, ODS-80TM, 4.6×250 mm). The eluting solvent consisted of a linear gradient of acetonitrile 10-50 %, containing 0.1 % acetic acid, for 30 min at 40 °C. The solvent flow rate was 1.0 m*l*/min and the UV-absorption was measured at 260 nm. HPLC analysis was carried out at the Legume Breeding Laboratory of the National Agriculture Research Center (NARC) in Tsukuba, Japan.

Because malonyl forms of daidzin and genistin are unstable and gradually converted to daidzin and genistin (Kudou *et al.* 1991), the sum of daidzin and malonyldaidzin and genistin and malonylgenistin are reported as daidzin and genistin, respectively. Harvesting and flowering date were registrated for each cultivar to figure out days of isoflavone accumulation. According to Kudou *et al.* (1991), accumulation of isoflavone in soybean seeds starts 35 days after flowering. Average temperatures of this period through harvest were observed and regression coefficient analysis were performed to show a relationship between total isoflavone content and temperature during filling period. Total isoflavone content and maturation time (days from emergence to harvest) were also analysed by regression coefficient analysis. Analysis of variance (ANOVA) and Tukey's test, $P \geq 0.05$ were used to compare the total amounts of daidzin and genistin compounds for all samples.

Results and Discussion

Isoflavone content of soybean seeds in the 22 Brazilian cultivars, showed a substantial variability, in both years (Table 1). Isoflavone content ranged from ca. 12-94 mg/100 g seed meal for daidzin and ca. 28-278 mg/100 g seed meal for genistin. Kitamura *et al.* (1991) reported a significant difference in the isoflavone content of early and late maturity cultivars, that is, they observed that early maturity cultivars exhibited the lowest isoflavone content. However, isoflavone content increased, when early maturity cultivars had their seeding time delayed and were grown under conditions suited for late cultivars. The temperature variability during seed development was proposed as the major factor influencing levels of isoflavone in seeds. Tsukamoto *et al.* (1995) confirmed that high temperatures during seed development decrease isoflavone content significantly.

In this study, considering each year, all cultivars were sown in the same day and therefore were exposed to about the same temperature (Fig. 1). During the filling period no marked differences in temperature were observed, resulting in a non significant relationship between temperature and total isoflavone content (Fig. 2). This almost stable temperature could be responsible for the lack in variability in the isoflavone content among early and late maturity cultivars; the variability in isoflavone content was also independent of maturation time (Fig. 3). However, although no significant coefficient of determination for total isoflavone content, with temperature during filling period, and with maturation time, data is suggesting a linear tendency among these parameters (Fig. 2 and 3). Some late cultivars, which were exposed to lower temperature during filling period, had high levels of isoflavones, while early maturity cultivars showed low isoflavone contents at higher temperatures. Results showing variability suggest that additional factors, besides temperature could be affecting isoflavone content.

Isoflavone content was statistically different among cultivars and between years, with the genistin content being ca. 2 times greater in 1990 than 1991 (Table 1). Temperature and precipitation (Fig. 1) were lower in 1990 than in 1991, and harvest was delayed about 10 days in the first year (Table 1). These climatic differences could explain the observed variability in both years. Isoflavone content has been reported as being influenced by environment, cultivars, locations, and years (Eldridge and Kwolek 1983). The relation of daidzin and genistin showed no correlation ($r = 0.48$), in 1990. However, a significant correlation ($r = 0.84$) was observed in 1991 (Fig. 4). Cultivar IAC-100 had high isoflavone contents and cultivar BR-36 had low contents, in the two planting years (Table 1).

Cultivar IAC-100 consistently had a high daidzin and a high genistin content. IAC-100, a Brazilian soybean cultivar, has been reported as being resistant (antibiosis) to insects (Rosseto 1989). The resistance to soybean looper, *Pseudoplusia includens* (Walker) and to Mexican Bean Beetle, *Epilachna varivestis* (Mulsant) has been reported due to the antixenosic properties of some soybean isoflavone (Liu *et al.* 1992, and Burden and Norris 1992).

Glyceollin is one of isoflavone in legumes that contributes to a plant's antixenosis and antibiosis (Fisher *et al.* 1990, and Graham 1991). Daidzein may be one of the precursors for glyceollin, and therefore contribute to its accumulation (Graham 1991). However the hydrolysis of daidzin and genistin glucosides to daidzein and genistein requires activation by the enzyme β-glucosidase (Matsuura and Obata 1993). According to our data, a

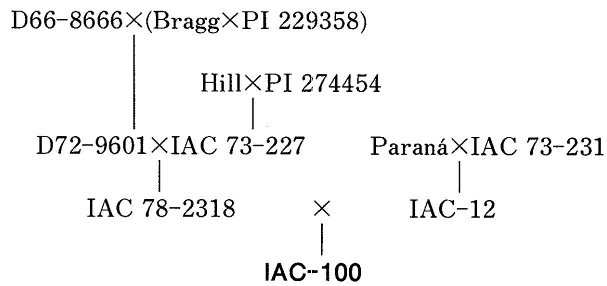
Table 1. Mean (± SD) of isoflavone content (mg/100 g seed meal) in Brazilian soybean cultivars sown in November, 1990 and 1991, in Londrina, Paraná, Brazil

Cultivars	1990/91				1991/92		
	Maturity group	Harvest date	Daidzin	Genistin	Harvest date	Daidzin	Genistin
BR-36 (1)	Early	29/03/91	12. 45*a* (±0. 49)	96. 15*ab* (±4. 03)	19/03/91	12. 60*a* (±0. 42)	28. 40*a* (±14. 00)
DAVIS (2)	Early	22/03/91	29. 55*bcdefgh* (±0. 21)	104. 05*ab* (±2. 62)	13/03/92	20. 60*ab* (±1. 84)	42. 40*ab* (±15. 98)
BR-7 (3)	Early	31/03/91	21. 15*abcd* (±2. 05)	82. 20*a* (±9. 62)	19/03/92	34. 80*bcde* (±3. 11)	49. 85*ab* (±8. 41)
BR-38 (4)	Early	27/03/91	22. 60*abcde* (±0. 42)	132. 55*abc* (±2. 19)	19/03/92	27. 00*abc* (±1. 41)	47. 15*ab* (±18. 60)
BR-29 (5)	Early	29/03/91	25. 75*abcdef* (±0. 92)	147. 55*abcd* (±3. 18)	13/03/92	32. 90*bcd* (±0. 42)	57. 45*ab* (±20. 58)
BR-16 (6)	Early	25/03/91	28. 15*bcdefg* (±1. 34)	178. 50*bcde* (±19. 52)	13/03/92	48. 35*efgh* (±0. 78)	71. 50*abc* (±24. 61)
BR-5 (7)	Early	03/04/91	31. 15*cdefgh* (±0. 78)	143. 05*abcd* (±3. 75)	19/03/92	39. 50*cdef* (±1. 56)	57. 10*ab* (±17. 25)
OCEPAR-4 (8)	Early	25/03/91	31. 50*defgh* (±1. 27)	103. 00*ab* (±1. 13)	13/03/92	25. 10*abc* (±4. 10)	61. 45*abc* (±32. 60)
BR-23 (9)	Early	31/03/91	32. 60*defghi* (±4. 38)	176. 65*bcde* (±21. 99)	19/03/92	45. 65*defg* (±0. 49)	71. 95*abc* (±21. 00)
BR-37 (10)	Early	23/03/91	33. 00*defghi* (±0. 14)	202. 70*cdef* (±0. 99)	13/03/92	67. 15*ij* (±5. 44)	91. 35*abc* (±17. 61)
IAS-4 (11)	Early	29/03/91	41. 45*ghij* (±0. 07)	127. 35*abc* (±1. 20)	13/03/92	23. 25*ab* (±2. 47)	52. 90*ab* (±21. 50)
IAC-100 (12)	Early	30/03/91	60. 35*k* (±8. 70)	278. 60*f* (±21. 78)	26/03/92	80. 65*j* (±6. 43)	122. 50*cd* (±32. 81)
BR-12 (13)	Inter-mediated	03/04/91	16. 20*abc* (±0. 28)	133. 60*abc* (±3. 54)	26/03/92	23. 35*ab* (±0. 49)	45. 25*ab* (±17. 61)
BABR-31 (14)	Late	24/04/91	28. 95*bcdefg* (±0. 49)	132. 05*abc* (±37. 69)	17/04/92	28. 20*bc* (±1. 84)	47. 15*ab* (±16. 90)
EMGOPA-301 (15)	Late	02/05/91	30. 50*bcdefgh* (±2. 54)	98. 55*ab* (±7. 99)	08/05/92	23. 80*ab* (±7. 78)	52. 55*ab* (±22. 41)
BR-15 (16)	Late	26/04/91	37. 50*efghij* (±0. 28)	229. 30*def* (±0. 42)	23/04/92	25. 35*abc* (±0. 21)	29. 90*a* (±5. 80)
GOBR-33 (17)	Late	05/05/91	40. 45*fghij* (±0. 78)	263. 95*ef* (±0. 78)	08/05/92	61. 95*hi* (±6. 72)	133. 15*abc* (±48. 15)
GOBR-26 (18)	Late	08/05/91	44. 25*hij* (±0. 64)	184. 10*bcde* (±1. 41)	30/04/92	58. 80*ghi* (±2. 40)	148. 30*d* (±68. 45)
BR-28 (19)	Late	12/05/91	47. 50*ijk* (±3. 67)	143. 50*abcd* (±12. 73)	30/04/92	30. 75*bcd* (±0. 92)	64. 15*abc* (±31. 47)
FT-CRIST. (20)	Late	01/05/91	49. 85*jk* (±7. 99)	197. 65*cdef* (±12. 94)	24/04/92	23. 15*ab* (±1. 91)	78. 45*abc* (±0. 78)
IAC-8 (21)	Late	18/04/91	75. 70*l* (±8. 77)	152. 85*abcd* (±15. 63)	16/04/92	54. 45*fghi* (±3. 04)	87. 80*abc* (±29. 70)
MSBR-34 (22)	Late	27/04/91	94. 60*m* (±12. 58)	196. 20*cdef* (±17. 64)	23/04/92	50. 85*fgh* (±0. 07)	85. 45*abc* (±34. 15)

* Clarification according to average days from emergence to maturity in 1990 and 1991.
Means followed by the same letter in each column are not significantly different using Tukey's test (P>0.05). Average of two replications.

high daidzin content could be related with resistance to insects or pathogens.

IAC-100 was the first Brazilian soybean cultivar released with insect resistance (Rosseto 1989). By its genealogy, showed below, it is possible to recognize which parents contributed for this characteristic.



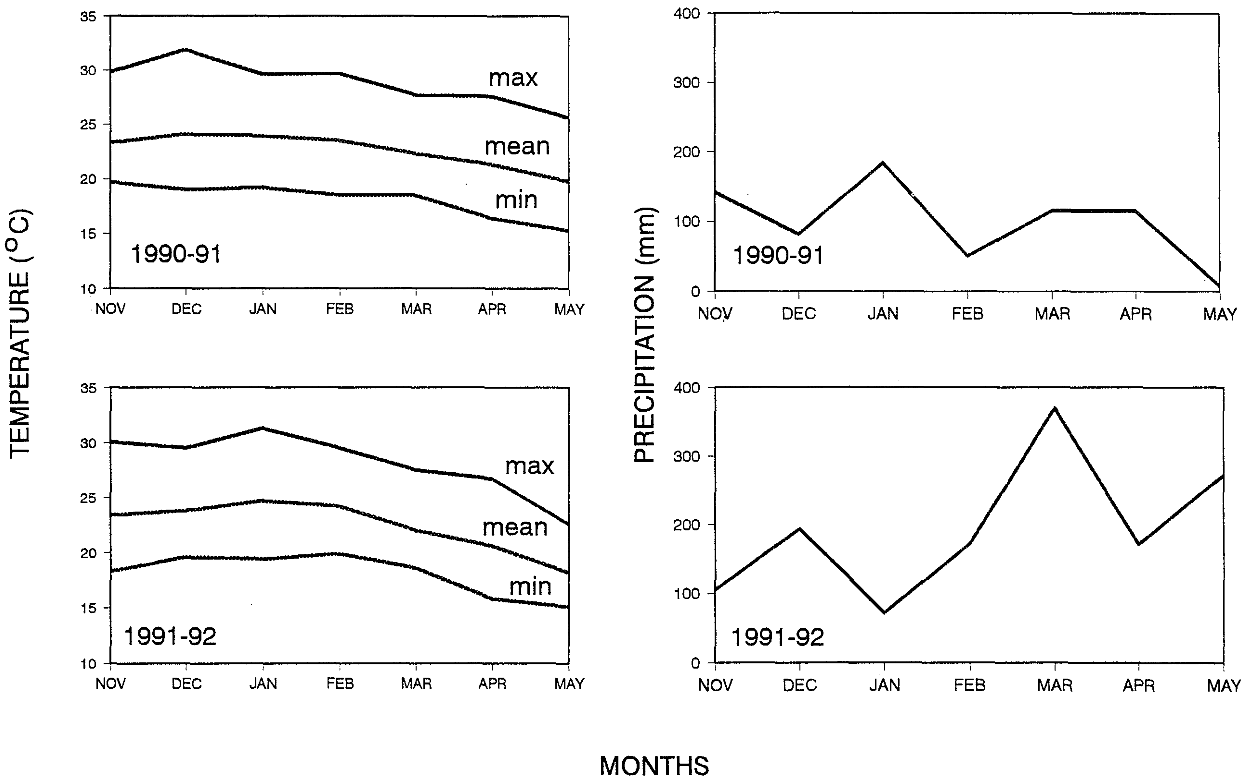


Fig. 1. Climatic data during soybean season in Londrina, PR, Brazil (23° 12'S latitude).

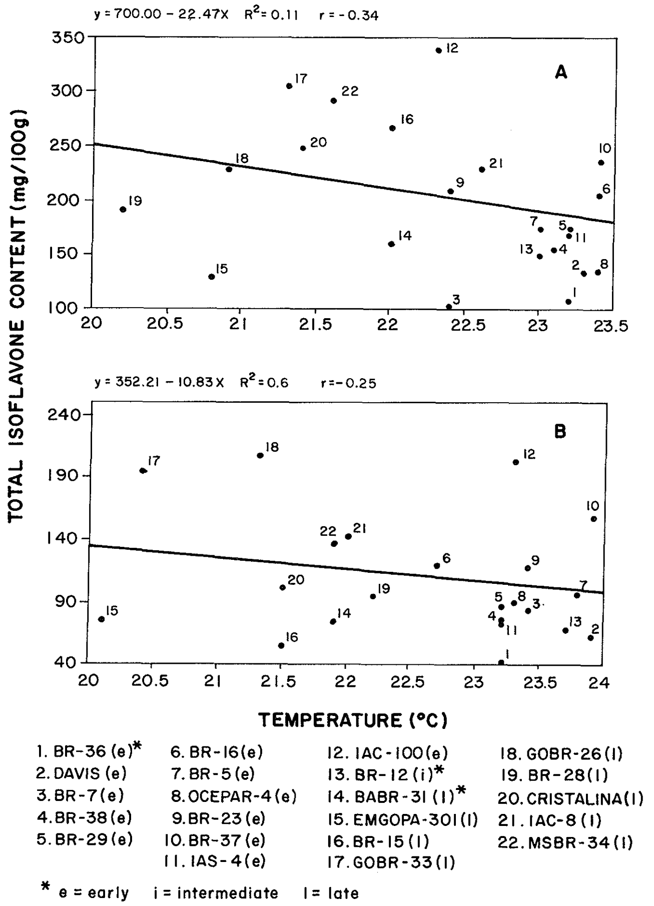


Fig. 2. Effect of temperature in the period of 35 days after flowering to harvest on total isoflavone content, in Brazilian soybean cultivars, in 1990 (A) and in 1991 (B).

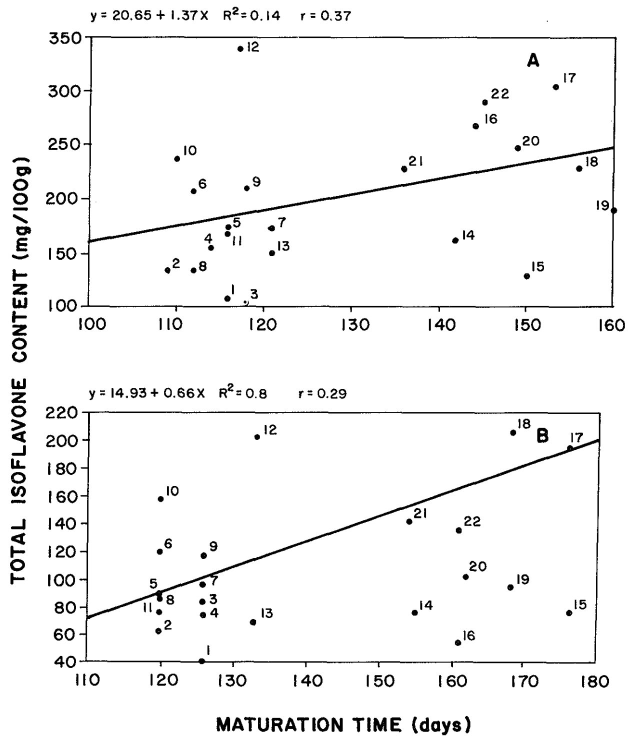


Fig. 3. Effect of maturation time (days to maturity) on total isoflavone content, in Brazilian soybean cultivars, in 1990 (A) and in 1991 (B) ; numbers correspond to the cultivars appearing in Fig. 2.

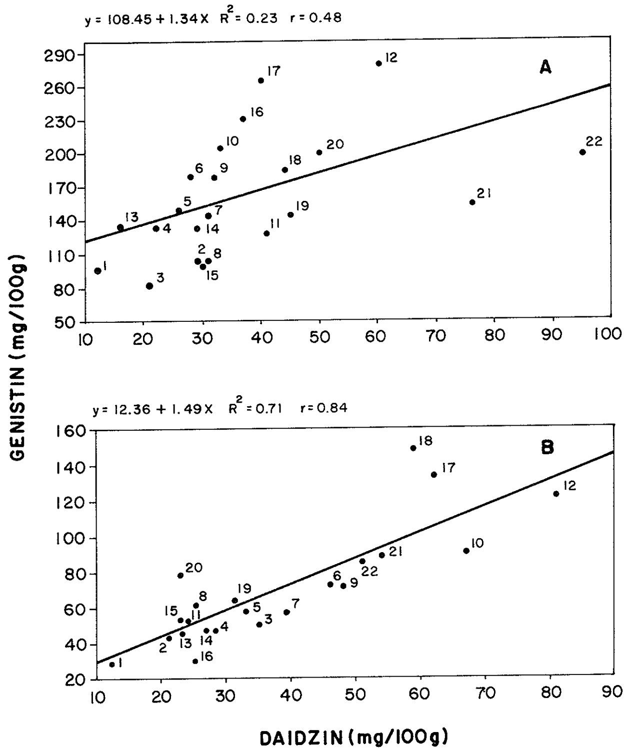


Fig. 4. Genistin and daidzin content (mg/100 g seed meal) in seeds of Brazilian soybean cultivars in 1990 (A) and in 1991 (B) ; numbers correspond to the cultivars appearing in Fig. 2.

The resistance of IAC-100 probably comes mostly from PI 274454 and PI 229358 (Rosseto 1989). In order to validate the relationship of isoflavone content with insect resistance, seeds from PI 227687 and PI 229358, which are known to be resistant to insects (Turnipseed and Sullivan 1976) were also analyzed. PI 227687 exhibited high content of isoflavone (82.6 mg/100 g and 185.5 mg/100 g daidzin and genistin, respectively), similar to IAC-100 (Table 1), which is also insect resistant. However, PI 229358 showed a low isoflavone content (16.0 and 46.5 mg/100 g daidzin and genistin, respectively). These data suggest that factors other than isoflavone are inducing resistance in this genotype. Differences in resistance could also be due to a specific distribution of various isoflavone compounds in a given organ and that this distribution is highly affected by the age of the plant organ (Graham 1991).

Liu *et al.* (1992) reported that the genotype PI 227687 produces significantly more phytoalexins than a relatively insect-susceptile variety 'Davis'. In our analysis, 'Davis' showed low daidzin and low genistin content for both years (Table 1). It has been observed that 'Davis' has a mild flavor (Carrão-Panizzi 1987), which may be related to the low isoflavone content found in this study. 'Davis' is also preferred by leaf feeding insects compared to other cultivars grown in Brazil (A. R. Panizzi, personal communication).

In conclusion, Brazilian soybean cultivars exhibit variable isoflavone contents which are independent of their maturity group. The observed variations in the isoflavone content between consecutive years indicate that environmental conditions affect these compounds. Further investigations to acquire additional information on the interactions of genotypes and environmental factors influencing the isoflavone content of soybean cultivars grown under Brazilian conditions are recommended.

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