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ORIGINAL PAPER

Environmental and Genetic Variation of Soybean Tocopherol Content Under Brazilian Growing Conditions

Mercedes Concórdia Carrão-Panizzi · Sevim Z. Erhan

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Abstract Information about the chemical composition of soybean cultivars (cvs) and environmental impact on their composition is important for processors and exporters to meet the demand of niche markets. Tocopherol composition (α, β, γ, δ, and total), was analyzed in seeds of 89 Brazilian soybean cultivars grown under fertile soil in Ponta Grossa, Paraná state, Brazil, in 2001. A large range of variability was observed: for α-tocopherol, 11 ppm (cv. Davis) to 191 ppm (cv. IPB-T); for β-tocopherol, 6 ppm (cv. IAC 1) to 64 ppm (cv. IPB-T); for γ-tocopherol, 304 ppm (BR62 Carla) to 1333 ppm (cv. Bienville); for δ-tocopherol, 174 ppm (cv. UFV 15) to 580 ppm (cv.IAS-5; and for total tocopherols, 561 ppm (cv. BRS62 Carla) to 1,983 ppm (cv. BR4-RC). For comparison of different growing locations, cv. MG/BR 46 Conquista was grown in 16 different locations of the Minas Gerais and Goiás states (Central Region, ca. 17° South latitude). Higher content of total tocopherol was found in Conquista, Uberaba, Sacramento, and Cerrado, while lower contents were observed in Alvorada, Iraí, and Uberlândia. In the South region (ca. 23° South latitude), the cultivar IAS 5, grown in 12 different locations of Paraná and São Paulo states, showed high total tocopherol content in Londrina, Pedrinhas, Ponta Grossa, and Nuporanga, and lower amounts in Cascavel, Pirassununga, Luiziana, and Morro Agudo. Tocopherol content in soybean seeds varied due to genetic differences as well as to the local environmental factors of different growing locations.

Keywords Soybean cultivars · Growing locations · Seeds · Tocopherol composition

M. C. Carrão-Panizzi
Embrapa Labex-EUA, Embrapa Soybean,
86970-001 Londrina, PR, Brazil

S. Z. Erhan (✉)
USDA/ARS, National Center for Agricultural Utilization Research (NCAUR), Food and Industrial Oil Research (FIO),
1815 N. University St, Peoria, IL 61604, USA
e-mail: sevim.erhan@ars.usda.gov

Introduction

Tocopherols are natural antioxidants and stabilizers that can inhibit lipid degradation. Along with other nutritional compounds in soybean seeds, they also have effects on health, by preventing cardiovascular diseases and cancer, and improving the immunological system [1]. Alpha tocopherol is the primary precursor to vitamin E in the human body and is a good antioxidant in vivo. Gamma and delta tocopherols are better in vitro antioxidants [2]. Four tocopherol homologues (α-, β-, γ-, and δ-tocopherols) are found in soybean oil, whose relative concentrations are: 4–10, 1–3, 60–66 and 24–29%, respectively [3]. Tocopherols can have both prooxidant and antioxidant activities in oils, depending on the concentration of each individual tocopherol homologue. At low levels, tocopherols inhibit hydroperoxide formation, but beyond an optimal concentration, which is different for each tocopherol homologue, they appear to promote hydroperoxide formation. In contrast, tocopherol mixtures exhibit a dose-dependent inhibition of hydroperoxide decomposition [4]. Thus, the optimal tocopherol profile for increasing oil stability should be determined by the concentration where they inhibit hydroperoxide formation. Optimal antioxidant activities are observed at concentrations of 100–250 ppm for α-tocopherol [5], 250–500 ppm for γ-tocopherol [6], and 500–1,000 ppm for δ-tocopherol [7]. In in vitro determinations, a comparison of the antioxidant activity of the individual tocopherols at their optimal concentrations revealed that α-tocopherol (∼100 ppm) was 3–5 times more potent than γ-tocopherol (∼300 ppm) and 16–32 times more potent than δ-tocopherol (∼1,900 ppm) [8].

Breeding soybean to enhance tocopherol composition, mainly α and γ-tocopherols is important for their nutritional and functional effects on the stability of food systems and on human health. Genetic variability for tocopherol content has been studied. Ujiie et al. [9] analyzed more than 1,000 genotypes and found three varieties with high α-tocopherol content. Weather and seasonal changes or differences in climate conditions over each location affects the tocopherol concentrations in soybean seeds. Almonor et al. [10] observed that low temperatures decrease total tocopherol content, which was attributed to loss of γ-tocopherol. The same authors reported that low linolenic varieties also presented higher content of α-tocopherol. Dolde et al. [11] also showed that 12 soybean breeding lines grown in five locations presented significant correlations with planting location, breeding line, tocopherol concentration, and fatty acid composition. Higher concentrations of tocopherols are formed under warmer conditions [12].

In Brazil, soybeans are produced in regions that include environments from 32°S to 5°N latitude. Therefore, a study to determine tocopherol contents in different Brazilian soybean cultivars and growing locations is relevant. Eighty-nine different soybean cultivars and soybeans grown in different locations of South and Northeast regions of Brazil were analyzed.

Materials and Methods

Materials

Experiment 1. To determine genetic variations in tocopherols among soybean genotypes, 89 Brazilian soybean cultivars grown in Ponta Grossa, PR, (soybean season 2001), in fertile soil that was corrected with pH 5.0, Al = 0.0, K = 0.43, Ca = 2.97, Mg = 1.29, and H + Al = 10.45 cmolc/dm^3, C = 22.75 g/dm^3, and P = 11.96 mg/dm^3, were analyzed.

Experiment 2. To determine environmental effects on tocopherol composition, seed samples of soybean cultivars of the Embrapás uniform field test program, grown in two different locations of South and Northeast regions of Brazil (growing season 2002), were analyzed.

Experiment 3. Soybean cultivars "IAS 5", grown in 12 locations, and MGBR46 Conquista, grown in 16 different locations (2002), were also analyzed for tocopherol composition.

Tocopherol analyses were carried out at the USDA/ARS/NCAUR/FIO, in Peoria, IL, USA.

Sample Preparation

Soybean seed samples (10.0 g) were dried for 5 days at 50 °C in a ventilated oven and then ground in a coffee grinder. Oil was extracted from ground soybean samples using the Soxhlet technique in an Avanti 2050 Soxtec from Foss Tecator (Denmark). Samples were weighed (5.0 g) into Whatman #4 filter paper, which was then folded and placed into cellulose extraction thimbles. Hexane (60 mL) was placed in aluminum extraction cups and the cups placed in position. Hexane was boiled for 20 min with the thimble in the hexane. Then the thimble was raised and the hexane continued to boil for 40 min (called the rinse phase). After rinsing, the remaining hexane was removed by blowing nitrogen gas over the extracted samples. After extraction, the oil was transferred to 1 dram amber vials covered with Argon gas and placed in a freezer.

HPLC Analysis

Standards for each of the tocopherols (α-,β-,γ-, and δ-), were diluted in hexane from a stock solution of 50,000 ppm to various levels: alpha: 50, 250, 500 ppm; beta: 10, 20, 50 ppm; gamma: 250, 500, 1,000, 2,500 ppm; delta: 100, 250, 500, 1,000 ppm. The resulting peak areas were graphed and linear regression was calculated. These results were then used to calculate the parts per million of the tocopherols in the samples from the respective peak areas.

Conditions for the Analysis

The HPLC column used was a Metachem Inertsil 5 u Si 150A. The system was a Varian Pro Star pump, with auto sampler and fluorescence detector. The detector, which was equipped with a Xe bulb, was set at 290–330 nm. The system was run by Varian Pro Star Chromatography Workstation software 6.0. Samples (100 μL) were diluted in 900 μL hexane. The auto sampler had a 100-μL loop. Each sample was injected as one loop and was run in duplicate. The pump was run at 1 mL/min. The solvent (mobile phase) was 99.5% hexane and 0.5% isopropanol. Each run was set for 30 min.

Statistical Design and Analysis

A Completely Randomized Design (CRD) with two replications was used to evaluate tocopherol composition in the experiments by SISVAR System [13]. ANOVA and Scott-Knott [13] cluster analysis ($\alpha \leq 0.05$) was used for grouping the 89 cultivars (experiment 1). ANOVA and Duncan's multiple range test ($\alpha \leq 0.05$) were used for comparing data from cultivars, regions and locations. These analyses and the matrix of Pearson correlations of all tocopherols and fatty acids were done by SAS/STAT system [14].

Results and Discussion

A large range of variability was observed for tocopherol content. Tocopherol compositions of 89 soybean cultivars were distributed according to the Scott–Knott test [15], and by determining the quarter where each average was located in relation to the 89 cultivars (Table 1). A matrix of Pearson correlation coefficients among the variables α-, β-, δ-, γ-, and total tocopherols (sum of 04 former variables) was also calculated. Significant correlations ($\alpha \leq 0.001$) were observed among total and α-tocopherol ($r = 0.51$), total and γ-tocopherol ($r = 0.96$), total and δ- ($r = 0.84$), α- and β-tocopherol ($r = 0.72$), as well as between γ- and δ-tocopherol ($r = 0.70$). Weak correlations, although significant ($\alpha < 0.004$, 0.001, and 0.002, respectively) were observed between total and β-tocopherols ($r = 0.38$), between β-tocopherols and δ-tocopherol ($r = 0.48$), and between α- and γ-tocopherol ($r = 0.41$), and non-significant correlation was observed between α- and γ-tocopherol. These correlations confirmed what was observed for the grouping test, where, with few exceptions (α- and β-tocopherol), the highest values of total tocopherols were associated with high values for all tocopherol forms. Among tocopherols and fatty acids, weak significant correlations were observed for α-tocopherol and linolenic acid ($r = 0.23$, $\alpha = 0.0245$) and for δ-tocopherol with linoleic, oleic and stearic acids ($r = -0.33$, $\alpha = 0.0021$; $r = 0.34$, $\alpha = 0.0017$; and $r = -0.24$, $\alpha = 0.0193$, respectively). Among fatty acids, correlations were highly significant ($\alpha \leq 0.001$) between oleic and linoleic ($r = -0.95$), between oleic and linolenic ($r = -0.62$), and between linoleic and linolenic ($r = 0.40$).

Using the Scott–Knott test grouping as a reference for total tocopherol, it was observed that cultivars of the group 1 belong to the first quarter of total tocopherol as well as almost all other tocopherol forms. Twenty five percent of the cultivars had lower tocopherol concentrations for all forms, except α- and β-tocopherol for cv. BRS156, β-tocopherol for cv. Embrapa 25, and α-tocopherol for cv. Dourados. The aforementioned cultivars were in the fourth quarter. The cultivar averages of groups 3, 4, and 5, obtained by the Scott–Knott test [15], were in the fourth quarter for total tocopherol as for the other forms, except for OCEPAR-9, FT-9 and FT-14 that were in group 3 of the Scott–Knott and third quarter of total tocopherol (Table 1).

The observed range was 561.2–1982.7 ppm for total tocopherols (BRS 62 Carla and BR4-RC, respectively) (Table 1), which is within the values listed at the official standards for vegetable oils of the Codex Alimentariys (660–3,370 mg/kg) [16]. Cultivars BR-4 RC, Bienville, OCEPAR 16, SPS 1, IAS 5 and FT-6, presented the highest values for total tocopherols and were located within the fourth quarter. Cv. Bienville, however, presented α- and β-tocopherols in the second quarter and cv. IAS-5 had α-tocopherol in the third quarter.

Among the 89 soybean cultivars grouping in the fourth quarter, those which presented the highest concentrations of total and all forms of tocopherols were cvs. BR 14, KI-S 702, FT 6, SPS-1, OCEPAR 16, and BR 4RC (Table 1). The same tendency was observed for cvs. BRS 134, BR 4, CEP 10, and FT- Jatobá, except for β-tocopherol, and cv. IAS 5 except for α-tocopherol.

Values for α-tocopherol ranged from 11.7 ppm (cv. Davis) to 191.2 ppm (cv. IPB-T), which is also within the values listed at the official standards for vegetable oils of the Codex Alimentarius (9–352 mg/kg) [16]. Cultivars BRS 156, Viçoja, BRS 138, BRS 132, BR 14, IPB-T, KI-S 702, FT-6, OCEPAR 16 e BR-4 RC were in the fourth and up groups according to Scott–Knott grouping, which means higher concentrations of α-tocopherol. Cvs. BRS 156, BR-16, MG/BR 58, Cobb, BRS 138 and IPB-T presented higher concentrations of both α- and β-tocopherols. Range values for β-tocopherol were 5.9 ppm (cv. IAC 1) to 64.3 ppm (cv. IPB-T) (Table 1).

For γ-tocopherol the range values were 304.4 ppm for cv. BRS 62 Carla to 1333.4 ppm for cv. Bienville; while for δ-tocopherol the range values were 174.1 ppm (cv. UFV 15) to 579.8 ppm (cv. IAS 5). Cultivars São Luis, BR-4, BR-14 BRS 135, CEP 10, Ivaí, FT-Jatobá, BR-8 and Bienville had higher concentration of γ- and δ-tocopherols and were also located in the fourth quarter (Table 1). For γ-tocopherol the official standards values for vegetable oils at Codex Alimentarius are 89–2,307 mg/kg, while for δ-tocopherol the values are 154–932 mg/kg [16].

Soybean cultivar "Davis" had a low concentration of total tocopherols (706 ppm). Davis also generally has low seed quality [17], which could be a response to the low antioxidant activity. As lipophilic antioxidants, tocopherols have been reported to increase in the embryonic axis of germinating soybeans exposed to various forms of environmental stress [18, 19]. Cultivar IAS 5 presented high content of total tocopherols (Table 1). This cultivar also presents high seed quality characteristics [17]. As an antioxidant compound, tocopherol may improve seed quality characteristics, which is highly important for good seed germination at tropical regions. Antioxidant compounds may have positive effects when seed coats are mechanically damaged. Considering food processing

Table 1 Mean values, group classification by Scott–Knott cluster analysis ($P = 0.05$) and quarter criteria of tocopherol content (ppm) in 89 soybean cultivars, grown in Ponta Grossa, PR, Brazil, 2001

Cultivar	Total (Σ)	α	β	γ	δ	Scott–Knott					Quarter				
						Σ	α	β	γ	δ	Σ	α	β	γ	δ
BRS 62	561.21	16.67	12.53	304.40	227.61	1	1	1	1	1	1	1	1	1	1
Davis	706.14	11.68	7.47	462.00	224.99	1	1	1	2	1	1	1	1	1	1
MS/BRS 168	724.56	31.19	25.98	442.14	225.26	1	1	2	2	1	1	1	3	1	1
BR 12	742.92	48.94	14.45	468.51	211.03	1	2	1	2	1	1	2	1	1	1
RS 5	775.74	24.50	22.63	454.09	274.53	1	1	2	2	2	1	1	3	1	2
IAC 100	810.86	29.45	10.83	564.61	205.97	1	1	1	3	1	1	1	1	1	1
UFV 15	830.19	83.76	21.06	551.31	174.07	1	3	2	3	1	1	3	2	1	1
IAC 2	830.95	31.69	15.77	521.11	262.38	1	1	1	3	1	1	1	1	1	1
BRS 158	837.41	14.99	8.31	532.08	282.04	1	1	1	3	2	1	1	1	1	2
Embrapa 19	844.26	27.85	13.66	553.91	248.83	1	1	1	3	1	1	1	1	1	1
DM Rainha	850.07	35.03	11.09	569.52	234.43	1	1	1	3	1	1	1	1	1	1
Emgopa 311	856.16	61.57	22.00	533.03	239.56	1	2	2	3	1	1	2	3	1	1
Liderança	905.15	78.87	22.15	565.19	238.94	1	3	2	3	1	1	3	3	1	1
BRS 156	911.30	106.95	32.89	564.48	206.97	1	4	3	3	1	1	4	4	1	1
Embrapa 25	925.99	80.19	31.95	515.56	298.29	1	3	3	3	2	1	3	4	1	2
MG/BR 22	929.85	52.26	12.43	625.48	239.67	1	2	1	4	1	1	2	1	2	1
MG/BR 46	935.01	40.22	25.59	555.58	313.61	1	1	2	3	2	1	1	3	1	3
Dourados	942.44	87.00	19.86	633.54	202.05	1	3	2	4	1	1	4	2	2	1
FT 19	947.99	35.24	18.47	607.15	287.13	1	1	2	4	2	1	1	2	2	2
IAS 4	949.17	54.38	21.50	594.31	278.99	1	2	2	4	2	1	2	2	1	2
IAS 3	952.31	48.87	11.76	670.31	221.36	1	2	1	4	1	1	1	1	2	1
Embrapa 1	975.29	47.01	23.46	566.97	337.85	2	2	2	3	2	1	1	3	1	3
Confiança	982.98	60.66	18.38	627.37	276.57	2	2	2	4	2	2	2	2	2	2
FT Cometa	991.82	60.92	25.21	585.94	319.75	2	2	2	3	2	2	2	3	1	3
DM Soberana	993.76	41.56	11.45	674.24	266.52	2	1	1	4	1	2	1	1	3	2
Santa Rosa	999.53	51.51	18.12	632.74	297.17	2	2	2	4	2	2	2	2	2	2
OCEPAR 2	1004.25	60.96	14.38	681.03	247.87	2	2	1	4	1	2	2	1	3	1
BRS 153	1005.13	59.41	23.93	608.99	312.79	2	2	2	4	2	2	2	3	2	3
Viçoja	1008.12	133.42	26.14	642.56	206.00	2	5	2	4	1	2	4	3	2	1
Campos Gerais	1010.58	78.36	27.34	617.25	287.63	2	3	2	4	2	2	3	3	2	2
Embrapa 48	1015.32	83.83	37.20	599.38	294.90	2	3	4	4	2	2	3	4	1	2
IAC 20	1016.32	79.72	20.93	654.89	260.79	2	3	2	4	1	2	3	2	2	1
BR 16	1022.60	97.45	41.88	572.20	311.07	2	3	4	3	2	2	4	4	1	3
Emgopa 310	1027.02	86.87	20.28	667.96	251.91	2	3	2	4	1	2	4	2	2	1
OCEPAR 19	1031.86	47.84	12.40	694.00	277.62	2	2	1	4	2	2	1	1	3	2
MS/BR 20	1034.91	75.55	27.84	609.60	321.93	2	3	2	4	2	2	3	4	2	3
Mineira	1036.43	54.01	30.78	617.74	333.90	2	2	3	4	2	2	2	4	2	3
IAC 16	1039.76	33.72	11.52	714.60	279.92	2	1	1	4	2	2	1	1	3	2
OCEPAR 15	1051.06	62.57	14.33	714.30	259.87	2	2	1	4	1	2	2	1	3	1
Stwart 1	1053.36	92.90	27.16	628.32	304.98	2	3	2	4	2	2	4	3	2	2
União	1,053.39	42.02	41.38	534.41	435.58	2	1	4	3	4	2	1	4	1	4
MG/BR 54	1069.16	58.96	20.99	655.99	333.22	2	2	2	4	2	2	2	2	2	3
Santa Maria	1070.46	79.90	20.80	667.21	302.55	2	3	2	4	2	2	3	2	2	2
MG/BR 58	1074.38	96.54	29.89	653.44	294.51	2	3	3	4	2	2	4	4	2	2
MS/BRS 170	1075.47	84.21	27.72	670.73	292.81	2	3	2	4	2	3	3	3	2	2
Vila Rica	1098.92	61.74	23.44	719.92	293.82	2	2	2	4	2	3	2	3	3	2

Table 1 continued

Cultivar	Total (Σ)	α	β	γ	δ	Scott–Knott					Quarter				
						Σ	α	β	γ	δ	Σ	α	β	γ	δ
BRS 155	1103.53	45.95	15.50	718.53	323.55	2	2	1	4	2	3	1	1	3	3
IAC 1	1106.55	25.02	5.88	781.45	294.20	2	1	1	5	2	3	1	1	3	2
Cobb	1106.67	93.34	39.61	648.63	325.09	2	3	4	4	2	3	4	4	2	3
BRS 137	1117.11	48.76	10.90	755.31	302.14	2	2	1	5	2	3	1	1	3	2
J 200	1118.92	70.96	20.80	763.69	263.47	2	2	2	5	1	3	3	2	3	1
BRS 138	1129.36	112.02	47.76	638.08	331.50	2	4	5	4	2	3	4	4	2	3
Ivorá	1133.29	63.06	27.18	671.43	371.62	2	2	2	4	3	3	2	3	3	4
CD 202	1133.46	64.35	21.46	712.91	334.74	2	2	2	4	2	3	2	2	3	3
Nova IAC 7	1138.21	47.59	11.33	789.99	289.30	2	2	1	5	2	3	1	1	3	2
BR 6	1141.27	66.74	28.61	668.11	377.81	2	2	3	4	3	3	3	4	2	4
BR 38	1141.85	74.28	35.00	631.12	401.45	2	3	3	4	4	3	3	4	2	4
FT líder	1145.60	92.93	23.40	708.73	320.54	2	3	2	4	2	3	4	3	3	3
BR 36	1153.80	78.70	24.58	743.08	307.45	2	3	2	4	2	3	3	3	3	3
BR 23	1165.06	85.68	16.25	812.03	251.11	2	3	1	5	1	3	4	2	4	1
BRS 133	1166.47	75.42	18.13	793.05	279.86	2	3	2	5	2	3	3	2	4	2
Embrapa 26	1166.78	79.24	23.38	757.13	307.04	2	3	2	5	2	3	3	3	3	3
IAC/PL1	1201.86	67.01	21.53	758.92	354.41	2	2	2	5	3	3	3	2	3	3
OCEPAR 9	1237.88	83.46	23.05	799.18	332.18	3	3	2	5	2	3	3	3	4	3
FT 9	1244.40	61.56	21.59	785.41	375.83	3	2	2	5	3	3	2	3	3	4
FT 14	1250.52	65.70	20.34	834.50	329.99	3	2	2	5	2	3	3	2	4	3
São Luiz	1281.51	49.55	15.67	804.73	411.56	3	2	1	5	4	4	2	1	4	4
MS/BRS 169	1295.33	80.53	34.83	793.04	386.94	3	3	3	5	3	4	3	4	3	4
OCEPAR 14	1297.25	24.19	24.31	791.46	457.28	3	1	2	5	5	4	1	3	3	4
BRS 132	1299.97	127.63	33.51	837.72	301.11	3	5	3	5	2	4	4	4	4	2
BRS 134	1305.00	84.28	20.02	876.26	324.44	3	3	2	6	2	4	4	2	4	3
CEP 26	1312.72	65.69	30.35	788.64	428.04	3	2	3	5	4	4	3	4	3	4
BR 4	1313.97	86.01	25.41	829.47	373.08	3	3	2	5	3	4	4	3	4	4
BR 14	1349.21	112.36	35.97	833.06	367.83	3	4	4	5	3	4	4	4	4	4
FT 2	1351.82	75.57	20.52	891.06	364.66	3	3	2	6	3	4	3	2	4	3
OCEPAR 6	1357.60	49.68	12.58	937.38	357.96	3	2	1	6	3	4	2	1	4	3
BRS 135	1359.80	62.50	19.38	876.11	401.82	3	2	2	6	4	4	2	2	4	4
IPB-T	1386.29	191.20	64.31	772.34	358.44	3	6	6	5	3	4	4	4	3	3
CEP 10	1410.19	90.34	25.46	928.49	365.89	3	3	2	6	3	4	4	3	4	4
Ivaí	1451.02	65.20	15.30	961.38	409.15	3	2	1	6	4	4	2	1	4	4
FT Jatobá	1511.54	91.24	20.52	1034.11	365.68	3	3	2	7	3	4	4	2	4	4
BR 8	1513.80	65.61	16.52	1067.00	364.68	3	2	1	7	3	4	2	2	4	4
KI-S 702	1527.87	130.47	36.89	943.78	416.73	3	5	4	6	4	4	4	4	4	4
FT 6	1609.11	108.90	39.60	1038.39	422.22	4	4	4	7	4	4	4	4	4	4
IAS 5	1618.86	76.51	48.68	913.87	579.79	4	3	5	6	6	4	3	4	4	4
SPS 1	1649.00	103.09	37.85	1070.31	437.76	4	3	4	7	4	4	4	4	4	4
OCEPAR 16	1678.91	112.99	30.25	1086.68	449.00	4	4	3	7	5	4	4	4	4	4
Bienville	1888.85	59.16	16.96	1333.47	479.26	5	2	1	8	5	4	2	2	4	4
BR4 RC	1982.69	125.31	39.55	1293.59	524.24	5	5	4	8	6	4	4	4	4	4

Table 2 Tocopherol composition (ppm) of soybean cultivar IAS five grown in 12 different locations of Paraná (PR) and São Paulo (SP) States, in South Region (ca. 23° latitude S) of Brazil, 2002

Locations	Temperature[a] (°C)	Precipitation[a] (mm)	Altitude (m)	Alpha	Beta	Gamma	Delta	Total
Londrina (PR)	24	121	585	141.0 a	29.1 bc	911.0 a	334.0 b	1415.0 a
Cascavel (PR)	25	126	781	93.6 b	31.0 ab	569.1 ef	286.0 ed	979.7 cd
Pedrinhas Paul (SP)	26	179	330	92.8 b	32.5 a	930.4 a	351.9a	1407.6 a
Pirassununga (SP)	24	86	627	73.7 c	31.5 a	609.4 de	319.2 bc	1033.8 c
Ibirarema (SP)	26	344	483	73.6 c	26.3 d	638.5 cd	295.3 d	1033.8 c
Ponta Grossa (PR)	23	271	969	62.3 d	25.4 d	724.5 b	315.6 bc	1127.7 b
Luiziana (PR)	24	89	752	48.2 e	20.8 f	530.0 fg	256.2 gh	855.2 fg
Guaíra (SP)	25	206	–	41.0 f	21.7 ef	608.1 de	275.8 ef	946.6 de
Mandaguacu (PR)	26	195	580	37.5 fg	28.6 c	527.2 fg	316.1 bc	909.5 ef
Cambará (PR)	26	177	545	37.3 g	18.1 g	602.9 de	247.9 h	906.2 ef
Morro Agudo (SP)	25	387	546	34.3 g	23.2 e	501.5 g	266.2 fg	825.2 g
Nuporanga (SP)	25	455	776	27.1 h	22.8 e	661.7 c	313.7 c	1025.4 c

Mean values followed by the same letters in the columns are not statistically different by Duncan test ($P \leq 0.05$)

[a] Average temperatures (°C) and total precipitation (mm), at the pod filling period (February, March and April) (http://www.agritempo.org.br, September 2006)

Table 3 Tocopherol composition of soybean cultivar MG/BR46 Conquista grown in different locations of Minas Gerais State (MG) and Goiás State (GO), in the Central region (ca. 15° latitude S) of Brazil, 2002

Locations	Temperature[a] (°C)	Precipitation[a] (mm)	Alpha	Beta	Gamma	Delta	Total
Conquista (MG)	25	452	100.0 a	27.2 cb	837.7 a	406.5 b	1371.5 a
Chapadão do Céu (GO)	25	–	82.8 b	28.7 b	743.9 b	330.2 de	1185.7 cb
Alvorada (GO)	25	–	67.6 c	19.9 ef	579.9def	200.8 g	868.2 hg
Uberaba (MG)	25	450	65.1 c	24.6 cd	729.6 b	316.0 ef	1135.3 cd
Rio Verde (GO)	25	368	58.3 d	37.8 a	500.2 g	458.4 a	1054.6 ed
Luiziânia (GO)	–	–	53.5 e	16.8 f	610.2 cd	295.0 f	975.5 ef
Senador Canhedo (GO)	–	–	51.5 e	22.1 ed	568.8 def	317.0 ef	959.4 efg
Sacramento (MG)	25	430	41.5 f	22.5 ed	664.6 c	373.1 c	1101.6 cd
Anápolis (GO)	23	577	40.1 f	30.7 b	564.7 def	329.3 de	964.8 efg
Iraí (MG)	–	–	37.9 f	10.8 g	525.2 gf	213.0 g	786.9 hi
Cerrado (GO)	–	–	29.6 g	20.3 ef	822.9 a	353.9 cd	1226.6 b
São Miguel Passa Quatro (GO)	–	–	26.1 hg	19.2 ef	602.6 cd	311.9 ef	959.8 efg
Buritis (MG)	22	243	22.8 h	28.9 b	541.5 efg	365.5 c	958.8 efg
Uberlândia (MG)	25	114	13.5 I	19.8 ef	416.1 h	322.7 ef	772.1 I
Cristalina (GO)	23	500	13.0 I	19.2 ef	547.0 efg	316.2 ef	895.5 fg

Mean values followed by the same letters in the columns are not statistically different by Duncan test ($P \leq 0.05$)

[a] Average temperatures (°C) and total precipitation (mm), at the pod filling period (February and March) (http://www.agritempo.org.br, September 2006)

issues, cv. BR 4 RC with high tocopherol content should also be appropriate for food purposes, mainly because of the highest content of α-tocopherol (125.63 ppm), which is the precursor of vitamin E.

It has been observed that seed tocopherols are highly affected by environmental conditions, weather or climate. Britz and Kremer [12, 20] showed that α-tocopherol increases several fold as a result of relatively mild increases in temperature or extreme drought during seed maturation. In Brazil, soybeans are grown from latitude 32° South up to the Equator (5° North), which means both temperate and tropical climate, as well as microclimates due to different altitudes and local conditions. Effects of this wide range of environments are complex. The early cultivar "IAS 5", grown in 12 different locations of Paraná and São Paulo states had higher concentrations of all

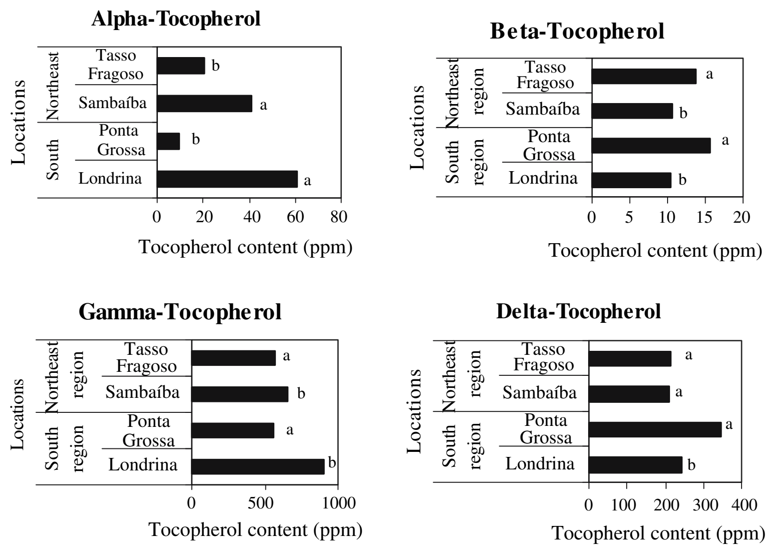

Fig. 1 Tocopherol content (ppm) in cv. BRS 134 grown in different locations of the South Region and cv. BRS Seridó grown in Northeast Region, Brazil, 2002. Mean values followed by the same letters are not statistically different by Tukey test ($P \leq 0.05$)

tocopherols in Londrina and Pedrinhas Paulista, while lower concentrations were observed in Luiziana and Morro Agudo (Table 2). Average temperatures at the seed filling periods (February and March) among locations ranged from 23 to 26 °C, and precipitation was also variable among locations (Table 2). Londrina (24 °C), Cascavel (25 °C) and Pedrinhas Paulista (26 °C) had low precipitation at the filling period which, according to Britz and Kremer [20], can favor formation of α-tocopherols, as well as total tocopherols. Low precipitation in Pirassununga also increased α- and total tocopherol in this location, which did not happen in Luiziânia (Table 2). Ibirarema, however, had high temperatures (26 °C) and high precipitation, which produced seeds having high α- and total tocopherol contents. Mandaguaçu and Cambará, which had better precipitation conditions produced seeds with lower α-tocopherol and differences in total tocopherol. Since a full observation of daily mean temperatures with exact days of seed filling for each location was not made, it is impossible to define which environmental factors affected tocopherol formation in these locations.

In Minas Gerais and Goiás states, at the central region of Brazil, cultivar MG/BR 46 Conquista, grown in 16 locations, had higher contents of α-tocopherol in Conquista, Chapadão do Céu, Alvorada, Uberaba than in other locations. The average temperatures in Conquista in February, March and April was about 25 °C, with a precipitation of about 450 mm (Table 3). These results suggest, as observed by Britz and Kremer [20], that high temperatures increase α- and total tocopherol (Table 3). At other locations with similar temperatures and precipitation amounts, other unidentified environmental factors could affect α-tocopherol content. Buritis and Cristalina had lower average temperatures of about 22 °C, and precipitations of 243 and 500 mm, respectively. Uberlândia had a lower precipitation (114 mm) and an average temperature of 24 °C. The lower temperatures decreased α-tocopherol in Buritis and Cristalina, but in Uberlândia, the lower precipitation could have caused a reduction in α- and total tocopherol contents. Higher γ- and δ-tocopherols were observed in Conquista than elsewhere, while lower γ-tocopherol content was observed in Rio Verde, and lower δ-tocopherol in Alvorada.

Different soybean cultivars, (BRS Seridó and BRS 134) grown in two different locations of the Northeast region (<10° latitude) and of the South Region (ca. 23° latitude S) showed differences in total and individual tocopherols. In the South, Londrina was the location that had a marked higher difference for α-tocopherol (Fig. 1), as well as for total tocopherols, 1222.98 and 928.15 ppm, for Londrina and Ponta Grossa, respectively, in cv. BRS 134. Average local temperatures were higher (25 °C) in Londrina than in Ponta Grossa (22 °C) (Table 4), which, combined with

Table 4 Environmental data of the locations of the South and Northeast regions of Brazil, 2002

Regions/ locations	Latitude	Altitude (m)	Average temperature[a] (°C)	Precipitation[b] (mm)
South				
Londrina	23°18′	585	25.0	144.6
Ponta Grossa	25°05′	969	22.4	313.4
Northeast				
Sambaíba	07°08′	212	26.5	541
Tasso Fragoso	08°28′	242	26.0	346

[a] Average of daily minimal and maxima temperatures of February, March and April, 2002

[b] Total precipitation in the same months (http://www.agritempo.org.br, September 2006)

lower precipitation in 2002 (Table 4), favored formation of α-tocopherol, and total tocopherol as observed by Britz and Kremer [20]. In the Northeast region, cv. "BRS Seridó" showed higher concentrations of α- and γ-tocopherols grown in Sambaíba (Fig. 1), while concentrations of total and δ-tocopherol were the same for both locations.

Genetic variability and the environment were shown to cause variations in total individual tocopherols in Brazilian soybean cultivars. However, more studies for more definitive descriptions of environmental effects on soy tocopherols in the tropics should be conducted. Detailed observations of temperatures, precipitation amounts, as well as light incidence should be measured at specific stages of soybean seed development, at the different locations are among the parameters that need to be tested. For the current work, critical warm days or drought conditions, that could occur during period of tocopherol formation, were not specifically measured, which may explain some of the differences noted in the various locations.

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