Environmental Variation and Correlation of Seed Components in Nontransgenic Soybeans: Protein, Oil, Unsaturated Fatty Acids, Tocopherols, and Isoflavones

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ABSTRACT

The environment has a significant influence on the expression of traits contributing to soybean nutritional and/or industrial value. The aim of this study was to explore and describe the variability of nontransgenic soybean seed chemical components by investigating the environmental correlations among protein (Pr), oil (O), oleic (Ol), linoleic (La), and linolenic (Ln) acids, oleic to linolenic acid ratio (Ol:Ln) alpha- (AT), beta- (BT), gamma- (GT), delta- (DT), and total tocopherols (TT) and total isoflavones (TI) by means of principal component analysis. We analyzed seeds from multienvironment trials involving 23 field trials grown in Argentina (24 to 38° S latitude). A wide range of variability was observed for Ol, Ln, Ol:Ln, AT, BT, and TI. The strongest environment-induced relationships found were the negative correlation between DT and AT and the positive correlation between DT and Ln. Increased Ol:Ln was negatively correlated with Ln. High values of DT, Ln, and Pr were associated with cool environments, TI content was greater in temperate to cool environments, and AT, O, and Ol:Ln were associated with warm environments. Warm environments would be suitable for obtaining products with higher O concentration of low oxidation capacity and greater vitamin E content. In turn, temperate to cool environments would be suitable for the production of soybean with higher TI, La, Ln, and TT content; in addition, these environments would favor seeds of higher Pr concentration.

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Abbreviations: AT, alpha-tocopherol; BT, beta-tocopherol; DAD, diode array detector; DT, delta-tocopherol; GT, gamma-tocopherol; HPLC, high performance liquid chromatography; INTA, Instituto Nacional de Tecnología Agropecuaria; La, linoleic acid; Ln, linolenic acid; O, oil; Ol, oleic acid; Ol:Ln, oleic to linolenic acid ratio; Pr, protein; PCA, principal component analysis; pp-PET, precipitation minus potential evapotranspiration; Sr, mean solar radiation; TI, total isoflavones; Tm, average daily mean air temperature; TT, total tocopherols.

Soybean [*Glycine max* (L.) Merr.] is a key species for human nutrition and the food industry (Seguin et al., 2009) due to the high protein content of high quality and low cost, considerable oil content rich in essential polyunsaturated fatty acids and high vitamin and mineral content. Essential fatty acids, linoleic (La) and linolenic (Ln), must be supplied to organisms through food of plant origin (Lehninger et al., 1993) and are necessary for growth and reproduction. Essential fatty acid deficiency has direct effects on health and nutrition both in humans and animals (Valenzuela et al., 1999, 2000). Soybean seed is also beneficial to health due to its tocopherol and isoflavone content.

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Tocopherols are natural lipophilic antioxidants that have an important biological role as vitamin E and can improve immune system functionality (Shintani and DellaPenna, 1998). These compounds may interrupt the chain reaction of lipid peroxidation by scavenging peroxyl radicals; they may also inhibit triacylglycerol peroxidation at initiation by accepting free radicals (Kamal-Eldin and Appelqvist, 1996). Soybean oil typically contains four types of isomers; these are in decreasing order of antioxidant activity in seed: delta-tocopherol (DT), gamma-tocopherol (GT), beta-tocopherol (BT), and alpha-tocopherol (AT) (Sherwin, 1976), the latter exhibiting the greatest vitamin E activity in human body (Warner, 2003).

It has been suggested that isoflavones may play a positive potential role in preventing colon, prostate, and breast cancer, cardiovascular diseases, and bone resorption; they also have the ability to attenuate many menopausal symptoms (Messina, 1995; Anderson and Garner, 1997; Barnes et al., 2000) because they have antiestrogenic and antioxidant activities and inhibit tyrosin kinase proteins (McCue and Shetty, 2004).

The expression of traits that contribute to soybean nutritional and/or industrial value has a strong genetic control. Argentina is one of the principal soybean seed exporters and the main processor of byproducts. Ninety-nine percent of the soybean production area is cultivated with transgenic germplasm, the chemical composition of which is sensitive to environmental variations but shows little variation among genotypes (Carrera et al., 2009). Therefore, nontransgenic germplasm with differential chemical characteristics for some of the seed components has been developed (Soldini, 1998). Several works analyzing environmental variation effects on seed chemical composition have shown important changes in protein, oil, and unsaturated fatty acid concentrations of soybean seed associated with temperature during seed filling (Wolf et al., 1982; Dornbos and Mullen, 1992; Wilson, 2004). Some works argue that both isoflavone (Tsukamoto et al., 1995; Lozovaya et al., 2005) and tocopherol content (Almonor et al., 1998; Dolde et al., 1999; Britz et al., 2008) vary with temperature during seed filling. Besides temperature, other climatic variables seem to modify seed chemical composition. Water deficit during seed filling can affect oil and protein concentrations (Rose, 1988; Boydak et al., 2002; Kumar et al., 2006; Rotundo and Westgate, 2008; Carrera et al., 2009), fatty acids (Dornbos and Mullen, 1992; Lee et al., 2008, Bellaloui and Mengistu, 2008; Gao et al., 2009), tocopherols (Britz and Kremer, 2002; Carrao-Panizzi and Erhan, 2007), and isoflavonoids (Caldwell et al., 2005; Lozovaya et al., 2005; Rasolohery et al., 2008). Intercepted solar radiation has been found to affect some components of seed chemical quality such as oil concentration during seed filling (Aguirrezábal et al., 2003) and the relative proportion of each fatty acid in soybean oil (Izquierdo et al., 2009).

Although many studies identified relationships between environmental variables and variables of the chemical composition of soybean seed, the covariation or joint variation among the different components across environments has received less attention. Environmentally determined correlations among the main seed chemical components might provide useful information to implement management practices aimed at obtaining a specific nutraceutical quality. The aim of this study was to explore and describe the variability in nontransgenic soybean seed chemical components by investigating the environmental correlations among protein, oil, unsaturated fatty acids, tocopherols, and total isoflavones.

MATERIALS AND METHODS

Plant Material and Experimental Design of Field Trials

A multienvironment trial network was established at eight experimental stations of the Instituto Nacional de Tecnología Agropecuaria (INTA) distributed across the Argentine soybean region, covering a latitudinal range from 24° S to 38° S. Two sowing dates per site were determined during the 2006–2007 and 2007–2008 crop seasons. In each site, the first and second sowing dates were defined as early and late sowing date, respectively. With the aim of capturing high environmental variability, sowing dates were set as far away from each other as possible within each sowing period. The mean separation period between sowing dates was 33 d, with a minimum of 27 d (Paraná and Reconquista in the 2006–2007 crop season) and a maximum of 63 d (Manfredi in the 2007–2008 crop season). The trials were laid out as a randomized complete-block design, with two replications in each environment (defined as crop season, site, and sowing date combinations). Each environment included six nontransgenic genotypes with differential quality characteristics (maturity group and growth habit are given in parenthesis): (i) high oil types: AC 0730–3 (IV long), AC 0916–1 (IV long), and AC 0124–1 (V short); and (ii) high protein types: ALIM 3.14 (V indeterminate), ALIM 4.13 (V indeterminate), and ALIM 3.20 (V indeterminate). Germplasm registration information was generated by the Breeding Program developed by INTA Agricultural Experimental Station in Marcos Juarez (Córdoba, Argentina), but these genotypes are still not officially released. Of the 32 planned environments, nine were discarded due to different adverse abiotic conditions (floods, hail, and extreme drought). The environments used are listed in Table 1. Chemical and physical analyses of soil samples from each site did not indicate any physical or nutritional constraint for crop development. Crops were grown under rainfed conditions and following cultural practices recommended by INTA's National Soybean Network for testing cultivars. These practices include disease, insect, and weed control (with specific products for nontransgenic soybean crop) to prevent any biotic factor from reducing yields. Plant density in all trials was about 25 plants m^{-2} with rows 0.52 m apart.

Solar radiation and temperature data were recorded daily for each crop season and site at INTA meteorological stations located up to 10 km away from each trial. Rainfall records were obtained

Table 1. Crop seasons, sites, sowing dates, average daily mean air temperature (Tm), mean solar radiation (Sr), and precipitation minus potential evapotranspiration (pp-PET) involved in the multienvironment trials conducted across the soybean Argentine crop area.

Crop season	Site†	Latitude (S), longitude (W)	Sowing date				Climatic variable		
			October	November	December	January	Tm‡ (°C)	Sr‡ (MJ m^2)	pp-PET§ (mm)
			————Day of month————						
2006–2007	BW	38° 19', 60° 14'		6			18.8	18.9	2.8
					15		17.3	16.5	–39.9
	BO	37° 49', 63° 02'		29			18.6	15.8	39.3
	CE	24° 54', 65° 29'		14			20.5	15.1	35.6
					22		20.4	13.3	17.5
	CA	27° 38', 55° 30'	27				25.2	21.5	179.9
				27			24.9	20.0	107.2
	FA	27° 04', 65° 25'				19	22.5	11.2	8.2
	MA	31° 49', 63° 46'	20				22.1	18.9	6.8
					21		20.5	15.0	132.2
	PA	31° 44', 60° 32'			1		22.0	16.3	433.6
					28		21.3	13.3	497.7
	RQ	29° 40', 59° 12'			6		24.9	20.2	190.0
						3	24.6	16.6	159.2
2007–2008	BO	37° 49', 63° 02'				7	17.8	15.9	–39.5
	CE	24° 54', 65° 29'		13			19.2	14.5	224.3
					17		18.4	13.8	90.1
	CA	27° 38', 55° 30'		15			24.3	19.7	37.1
	FA	27° 04', 65° 25'		26			24.3	16.7	141.0
						8	22.8	13.4	260.4
	MA	31° 49', 63° 46'	30				22.0	18.9	24.0
						2	19.6	14.8	46.8
	PA	31° 44', 60° 32'				15	21.1	17.0	–97.0

†In Argentina: BW, Barrow; BO, Bordenave; CE, Cerrillos; CA, Cerro Azul; FA, Famaillá; MA, Manfredi; PA, Paraná; RQ, Reconquista.

‡The period considered to generate Tm and Sr was between the date when the earliest cultivar at each sowing date reached R5 (beginning seed) phenological stage and the date when the latest cultivar reached R7 (physiological maturity).

§The period considered to generate pp-PET was between the date when the earliest cultivar at each sowing date reached R1 (beginning flowering) phenological stage and the date when the latest cultivar reached R7.

from rain gauges placed close to each plot (less than 500 m away). The potential evapotranspiration was calculated by means of the Penman (1948) equation. Dates of occurrence of R1 (beginning flowering), R5 (beginning seed), and R7 (physiological maturity) were recorded using the scale of Fehr and Caviness (1977). Using the daily climatic data, the following environmental factors were generated for each environment: (i) average daily mean air temperature (Tm), (ii) mean solar radiation (Sr), and (iii) precipitation minus potential evapotranspiration (pp-PET) (Table 1). The period considered to generate Tm and Sr was between the date when the earliest cultivar at each sowing date reached the R5 stage and the date when the latest cultivar reached the R7 stage. Meanwhile the period considered to generate pp-PET was between the date when the earliest cultivar at each sowing date reached the R1 stage and the date when the latest cultivar reached the R7 stage. The variable pp-PET was included because water balance from the whole reproductive period considers moisture storage in the soil profile which could influence water availability during grain filling (Carrera et al., 2009). The variable pp-PET was used as a simple indicator of water stress.

Chemical Analyses

Protein concentration (expressed as percentage of dry matter) was determined by the Kjeldahl method, using: (i) a digestion unit Tecator Auto 1001 3844/Rev 1 (Foss Tecator, Höganäs, Sweden), (ii) a scrubber unit Tecator 1001 4329/Rev 1 (Foss Tecator, Höganäs, Sweden), and (iii) a distillation unit K-350 (Büchi, Flawil, Switzerland) and using the conversion factor 6.25 (AOCS, 1998; Ac 4-91). Oil concentration (expressed as percentage of dry matter) was extracted using Twisselmann equipment, as specified by the Official Methods of American Oil Chemists' Society (AOCS, 1998; Ac3-44), with n-hexane solvent. Methyl esters of unsaturated fatty acids: oleic acid (Ol), La, and Ln (expressed as percentage of dry matter) were prepared following AOCS Ce 1-62 (AOCS, 1998). Unsaturated fatty acids were analyzed with a Gas Chromatograph (Hewlett-Packard 6890, Wilmington, DE) equipped with a flame ionization detector and using a capillary column HP-INNOWAX (Cross-linked Polyethylene Glycol), with a film 0.32 mm × 30 m and 0.5 μm in thickness. Standard fatty acid mixtures (FAME Mix Rapeseed, AOCS) purchased from Sigma-Aldrich (St. Louis, MO) were used as calibration standards. The variable defined as oleic to linolenic acid ratio (Ol:Ln) was constructed because this relationship is considered a general indicator of oil quality. Tocopherol determination (expressed as mg kg^{-1} oil) was performed from previously extracted oil according to methods and practices recommended by AOCS Ce 8-89 (AOCS, 1998) standards with a Liquid Chromatograph (Agilent 1100 high performance resolution; Wilmington, DE) equipped with a diode array detector (DAD). Separation was achieved with a Zorbax

RX-Sil column (4.6 mm i.d. × 250 mm long and 5 μm particle diam.), maintained at 25.5°C during the run. The mobile phase was n-Hexane 99.5% in 2-Propanol, both of high performance liquid chromatography (HPLC) purity. UV absorbance was monitored at 292 nm. Injection volume was 20 μL and flow rate was 1 mL min^{-1}. Calibration curves were obtained using commercial AT and DT standards purchased from Sigma-Aldrich (St. Louis, MO, USA). Response factors of BT and GT were calculated from AT and DT, respectively, and were corrected for their molar extinction coefficient and molecular mass. Total tocopherol (TT) was calculated by summing of individual isomers´ content. Isoflavones (expressed as mg g^{-1} of dry defatted flour) were analyzed using the method developed by Murphy et al. (1999) and modified by Hubert et al. (2005) from defatted flour, using a Liquid Chromatograph (Agilent 1100 high performance resolution; Wilmington, DE) equipped with a DAD. Separation was achieved with a ZORBAX Eclipse XDB-C18 column (4.6 mm i.d. × 250 mm long and 5 μm particle diam.) maintained at 40°C during the run. The mobile phase was glacial acetic acid 1% (v/v) in water and glacial acetic acid 1% (v/v) in acetonitrile; all solvents were of HPLC purity. Ultraviolet absorbance was monitored at 254 nm. Injection volume was 5 μL and flow rate was 1 mL min^{-1}. Calibration curves were determined using the following commercial standards: glycitin, acetyl daidzin, malonyl genistin, daidzein, acetyl genistin, and genistein, which were purchased from LC Laboratories (Woburn, MA). Response factors of the isoflavones daidzin, malonyl daidzin, and genistin were calculated from their corresponding aglycones, whereas response factors of the isoflavones glycitein, acetyl glycitin, and malonyl glycitin were calculated from glycitin and were corrected in a molecular mass ratio. Total isoflavones were calculated as the sum of the 12 forms (genistein, daidzein, glycitein, genistin, daidzin, glycitin, acetyl genistin, acetyl daidzin, acetyl glycitin, malonyl genistin, malonyl daidzin, malonyl glycitin). All determinations were made at the Grain Quality Laboratory, located at INTA Agricultural Experimental Station in Manfredi, Córdoba, Argentina.

Statistical Analysis

At each environment, mean values (across genotypes) of protein (Pr), oil (O), Ol, La, Ln, Ol:Ln, AT, BT, GT, DT, TT, and total isoflavones (TI) were analyzed using a biplot (Gabriel, 1971) constructed by plotting the symmetrically scaled principal component 1 (PC1) scores against the principal component 2 (PC2) scores obtained via principal component analysis (PCA) of an environment × trait matrix containing standardized trait data (Fig. 1). Therefore, the input matrix to the PCA contained environmental plus environmental × trait effects (Yan and Rajcan, 2002). Biplots can be multidimensional, but two-dimensional biplots, using only the first and the second principal components, are most common, both for biological reasons as well as for easy comprehension (Yan et al., 2000). The biplot is an ideal tool for multienvironment trials data analysis and represents an optimum space for analyzing the underlying variability and correlation between environmentally induced chemical variables. In the biplot, a vector is drawn from the origin to each trait marker to better visualize the relationships between chemical traits. The cosine of the angle between two trait vectors approximates the environmental correlation between the traits. An acute angle indicates a positive correlation and an obtuse angle indicates a negative correlation. In the biplot the environments were divided into three groups as a temperature function: cool (17.5 to 20.5°C), temperate (20.6 to 22.4°C), and warm (22.5 to 25.0°C); the biplot was generated using Info-Gen software (Balzarini and Di Rienzo, 2009). Data point classification in the biplot was suggested by previous linear regression of seed chemical variables on Tm, Sr, and pp-PET as well as combinations of these variables (Tm with pp-PET; Sr with pp-PET). Mallows' Cp statistic was used to identify the most important explanatory variable (at the 0.05 probability level).

RESULTS AND DISCUSSION

Most of the chemical components exhibited a high coefficient of variation, which indicates the environmental impact on seed composition (Table 2). Other studies involving multienvironment trials have reported a marked variability in seed chemical composition of transgenic cultivars, mainly in Ol, Ln, Ol:Ln (Maestri et al., 1998; Kumar et al., 2006), AT, BT, GT, DT, TT (Dolde et al., 1999; Carrao-Panizzi and Erhan, 2007), and TI (Hoeck et al., 2000; Morrison et al., 2008; Murphy et al., 2009). In the present work, the ranges in nontransgenic genotypes obtained for the aforementioned components were wider, except for GT, DT, and thus TT. Protein and oil concentrations exhibited lower variability (Table 2), and these ranges were analogous to those reported in previous works in a similar area involving a high number of transgenic cultivars (Dardanelli et al., 2006; Carrera et al., 2009). Linoleic acid also exhibited low variability, with ranges similar to those reported by Maestri et al. (1998) and Kumar et al. (2006).

Linear regressions showed that the average daily mean air temperature from R5 to R7 was the best predictor for O ($p < 0.0001$), Ol ($p < 0.0001$), Ln ($p < 0.0001$), Ol:Ln ($p < 0.0001$), AT ($p < 0.0001$), DT ($p < 0.0001$), and TI ($p < 0.0002$) (Fig. 2). Although Sr was a significant predictor for all these variables except TI, the Mallows' Cp indicated that the Tm effect was 10 times stronger than Sr. The environmental factor pp-PET was a significant explanatory variable but only for the unsaturated fatty acids and the Ol:Ln and also showed lower Mallows' Cp than Tm. Thus, we clustered the 23 environments by their Tm from R5 to R7 into three groups with higher heterogeneity between than within groups, cool (17.5 to 20.5°C), temperate (20.6 to 22.4°C), and warm (22.5 to 25.0°C), presented in Table 3. Also in this table are given the environmental values, that is, the average of different soybean seed component values (Pr, O, Ol, La, Ln, Ol:Ln, AT, BT, GT, DT, TT, and TI) across all nontransgenic genotypes at each environment. Information contained in this table contributes to clarify the interpretation of the environment × trait biplot shown in Fig. 1. In the biplot the first axis (PC1) explains 50% and the first two axes explain 70.1% of the total variation of the trait-standardized data. The third axis (PC3) was also examined and it explained 17.4% of total variation. One

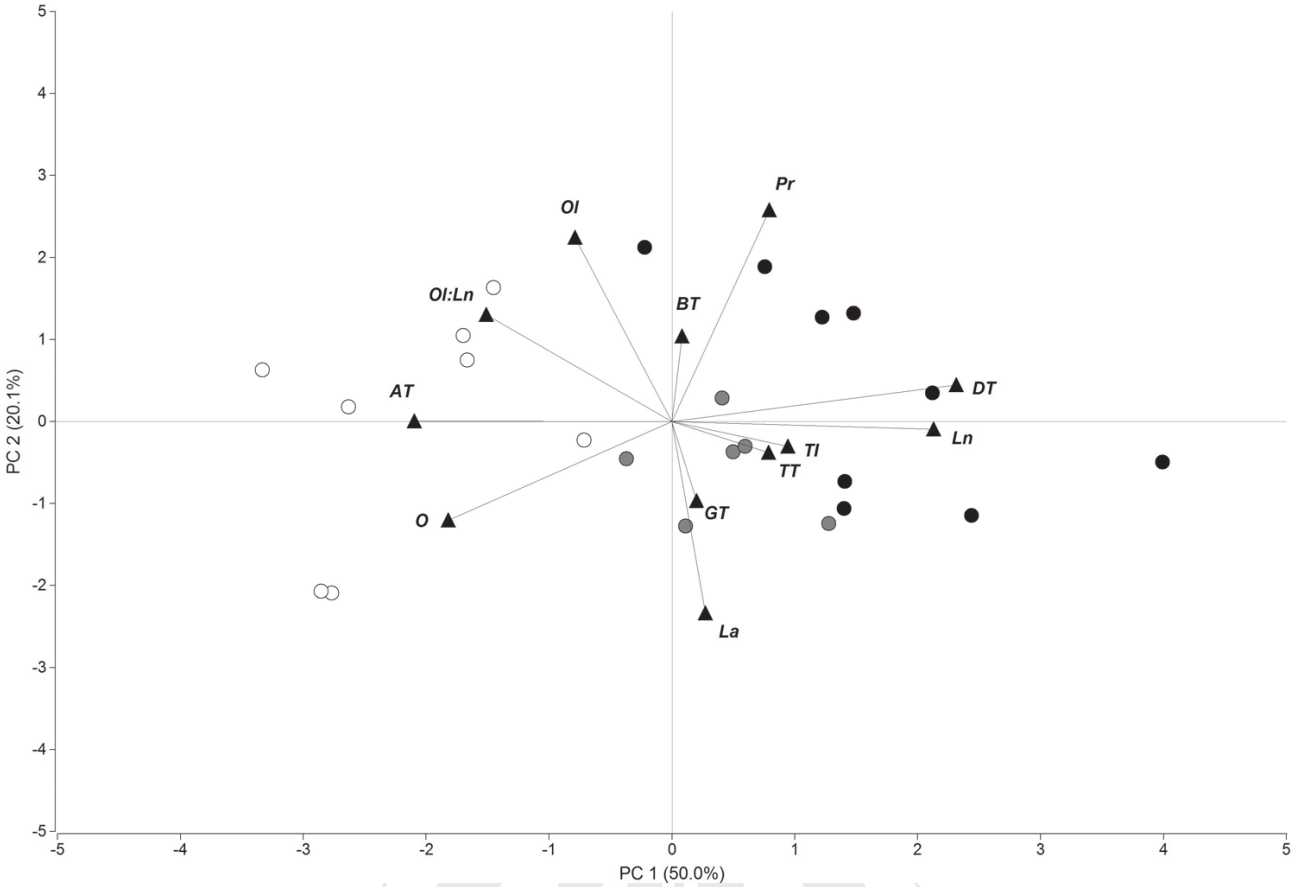

Figure 1. Environmentally determined correlations between nontransgenic soybean seed components (triangles) from twenty-three environments (circles). Environment × trait type biplot. See Table 2 for seed component denomination. Warm, temperate, and cool environments are represented by white, gray, and black circles, respectively.

of the highest environment-induced relationships between components revealed in the plane determined by the two first axes of the biplot is that DT is negatively correlated with AT and positively with Ln. The negative correlation found between DT and AT agrees with results reported by Seguin et al. (2009) and could be reflecting the competition for 2-methyl-6-phytyl-1,4-benzoquinone, the common precursor from which DT and AT are synthesized (Sattler et al., 2003). Dolde et al. (1999) found increases in DT together with increases in Ln. It has been suggested that DT has the greatest relative antioxidant efficacy of the four isomers (Sherwin, 1976) and that a very important function of tocopherols is to protect polyunsaturated fatty acid chains from lipid peroxidation (Girotti, 1998). Therefore, increases in DT would be expected, resulting in the positive correlation between Ln and DT. Figure 1 also shows that the trait vectors corresponding to DT and Ln were oriented toward cool environments, whereas the AT trait vector was oriented toward warm environments. This is consistent with studies conducted under controlled conditions (Britz and Kremer, 2002), which showed that under warm temperatures during seed filling, AT increases were achieved at the expense of compensatory DT decreases. In field studies involving several soybean lines, Britz et al. (2008) found that AT:TT ratio was the highest (which indirectly implied higher AT proportion) at warm temperatures for all the lines at all the environments considered.

Table 2. Mean, coefficient of variation (CV) and ranges for seed protein (Pr), oil (O), oleic (Ol), linoleic (La) and linolenic (Ln) acids, oleic to linolenic acid ratio (Ol:Ln), alpha- (AT), beta- (BT), gamma- (GT), delta- (DT), and total tocopherol (TT), and total isoflavone (TI) values in nontransgenic genotypes across the Argentine soybean crop area.

Chemical variable	Mean	CV	Min.	Max.
Pr†	40.8	4.5	36.0	44.7
O†	22.0	7.9	17.4	25.3
Ol†	22.7	10.4	18.1	29.0
La†	52.7	4.1	47.3	57.1
Ln†	7.8	15.3	4.7	10.3
Ol:Ln†	3.0	26.3	1.8	6.1
AT‡	128.6	38.1	45.7	246.0
BT‡	44.2	41.4	15.4	110.7
GT‡	989.4	14.0	481.1	1441.6
DT‡	347.9	27.5	154.6	572.3
TT‡	1510.1	11.0	975.0	2004.3
TI§	3.8	36.5	1.0	7.5

†Percentage of dry matter.

‡mg kg^{-1} oil.

§mg g^{-1} of dry defatted flour.

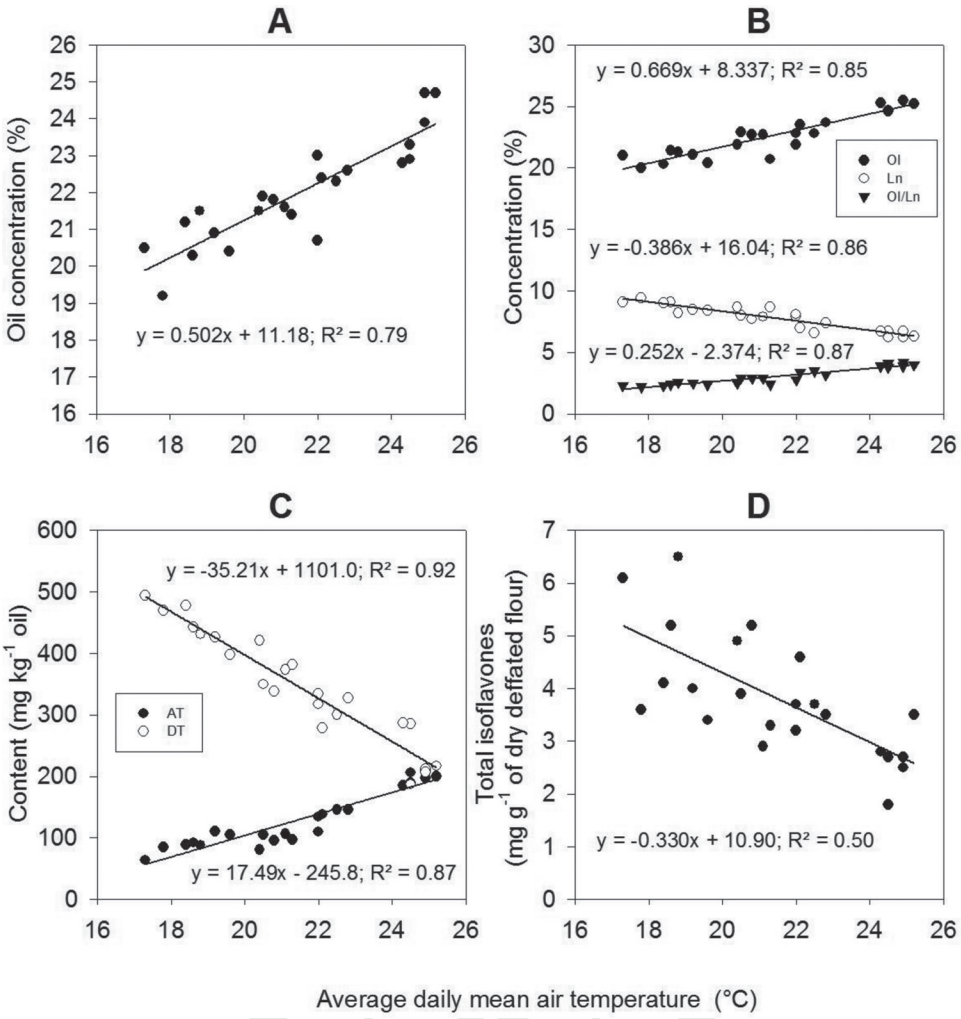

Figure 2. Regression between (A) oil concentration (percentage of dry matter); (B) oleic (Ol) and linolenic (Ln) acids and oleic to linolenic acid ratio (Ol:Ln) concentration (percentage of dry matter); (C) alpha-tocopherol (AT) and delta-tocopherol (DT) content (mg kg^{-1} oil); (D) total isoflavones (TI) content (mg g^{-1} of dry defatted flour) and the average daily mean air temperature (Tm). See Table 1 for the period considered to estimate Tm.

Dolde et al. (1999) found increases in TT content and concentration of unsaturated fatty acids, which includes Ln, with decreasing temperatures. Decreasing temperature during seed filling increases Ln levels (Howell and Cartter, 1958; Gibson and Mullen, 1996). Under these conditions, higher DT levels would be expected, resulting in the positive correlation between Ln and TT (Fig. 1); therefore, a higher amount of polyunsaturated fatty acids would be protected from peroxidation. Although the biochemical basis for the positive environmentally determined correlation between Ln and DT is unknown to our knowledge, Wilson (2004) suggested that the ω-6 desaturase (that catalyze the conversion of oleic to linoleic acid) activity not only imposed a considerable influence on tocopherol content but also on tocopherol composition. In general, this author found that low linolenic soybeans oils exhibited elevated levels of alpha-tocopherol or vitamin E, especially when grown under warmer commercial production environments. Our study suggested a strong negative correlation between Ln and AT that is environmentally determined. It would be interesting to study the mechanisms through which the enzymes involved in the consecutive desaturation of the fatty acids (Ol, La, and Ln) regulate the tocopherol content and its composition.

A higher Ol:Ln was negatively correlated with Ln, in agreement with previous works (Kumar et al., 2006; Carrao-Panizzi and Erhan, 2007; Gao et al., 2009). The Ol:Ln trait vector was oriented toward warm environments, whereas the La and Ln trait vectors pointed toward temperate and cool environments, respectively. Howell and Collins (1957), Carver et al. (1986), and Dornbos and Mullen (1992) found that under high air temperatures during soybean seed filling, La and Ln concentrations were proportionally reduced and Ol increased. Likewise, in multienvironment trials involving numerous genotypes, Oliva et al. (2006) observed increases in Ol and decreases in Ln with increasing temperatures. The increase in Ol:Ln in warm environments (which implies increases in Ol and decreases in Ln with increasing temperatures) might be due to the temperature effect on the key enzymes (oleate and linoleate desaturases), which

Table 3. Environment classification (E Class) by the average daily mean air temperature (Tm) and environmental values (across nontransgenic genotypes) of protein (Pr), oil (O), oleic (Ol), linoleic (La), linolenic (Ln), oleic to linolenic acid ratio (Ol:Ln), alpha- (AT), beta- (BT), gamma- (GT), delta- (DT), and total tocopherols (TT), and total isoflavones (TI) in soybean grains at 23 environments from the Argentine crop region.

Environment[†]	E Class[‡]	Pr[§]	O[§]	Ol[§]	La[§]	Ln[§]	Ol:Ln[§]	AT[¶]	BT[¶]	GT[¶]	DT[¶]	TT[¶]	TI[#]
1_E_BO	Cool	41.9	20.3	21.4	53.0	9.1	2.4	92.3	57.5	932.0	442.5	1524.3	5.2
1_E_BW	Cool	38.9	21.5	21.3	54.6	8.2	2.6	88.1	45.4	898.0	431.0	1462.5	6.5
1_E_CA	Warm	40.7	24.7	25.2	54.1	6.3	4.0	199.8	37.8	1095.1	217.1	1549.8	3.5
1_E_CE	Cool	42.7	21.9	22.9	49.7	8.0	2.9	104.8	46.0	860.5	349.5	1360.8	3.9
1_E_MA	Temperate	39.5	22.4	23.5	52.6	7.0	3.4	138.4	49.8	958.5	278.4	1425.0	4.6
1_E_PA	Temperate	39.1	23.0	21.9	54.6	7.9	2.8	110.0	32.3	953.8	317.3	1413.5	3.7
1_E_RQ	Warm	40.1	23.9	25.5	52.5	6.2	4.2	209.7	54.7	1024.0	212.8	1501.2	2.5
1_L_BW	Cool	39.7	20.5	21.0	54.4	9.1	2.3	63.7	37.6	991.2	494.5	1586.9	6.1
1_L_CA	Warm	40.7	24.7	25.5	53.2	6.7	3.9	196.7	29.1	1043.3	206.4	1475.5	2.7
1_L_CE	Cool	41.6	21.5	21.9	50.7	8.7	2.5	80.3	44.5	865.4	420.7	1410.9	4.9
1_L_FA	Warm	41.3	22.3	22.8	51.8	6.6	3.5	145.8	40.2	936.6	299.9	1422.5	3.7
1_L_MA	Temperate	40.5	21.8	22.7	53.3	7.7	2.9	95.1	32.5	891.2	338.6	1357.3	5.2
1_L_PA	Temperate	39.3	21.4	20.7	54.4	8.7	2.4	97.5	37.1	1041.3	381.2	1557.1	3.3
1_L_RQ	Warm	41.1	22.9	24.7	52.0	6.7	3.8	188.9	54.4	1021.0	285.7	1549.9	2.7
2_E_CE	Cool	42.8	20.9	21.1	50.9	8.5	2.5	110.6	49.9	971.1	426.2	1557.7	4.0
2_E_FA	Warm	40.7	22.8	25.3	51.7	6.7	3.9	185.4	71.1	1026.4	287.2	1570.1	2.8
2_E_MA	Temperate	42.2	20.7	22.8	52.8	8.1	2.8	134.2	34.1	891.8	334.3	1394.4	3.2
2_L_BO	Cool	40.9	19.2	20.0	52.8	9.4	2.2	85.3	66.2	1218.7	469.6	1839.7	3.6
2_L_CA	Warm	40.5	23.3	24.6	51.6	6.2	4.1	206.0	47.0	988.3	187.1	1428.5	1.8
2_L_CE	Cool	41.8	21.2	20.3	51.0	9.0	2.3	89.1	45.5	1005.7	478.2	1618.5	4.1
2_L_FA	Warm	40.5	22.6	23.7	52.9	7.4	3.2	145.8	33.2	980.3	327.4	1486.7	3.5
2_L_MA	Cool	41.1	20.4	20.4	54.6	8.4	2.4	104.9	35.2	1064.1	397.8	1602.0	3.4
2_L_PA	Temperate	40.6	21.6	22.7	52.1	7.9	2.9	106.1	34.9	1107.3	373.9	1622.2	2.9
Mean		40.8	22.0	22.7	52.7	7.8	3.0	129.5	44.2	989.8	346.0	1509.4	3.8
Max		42.8	24.7	25.5	54.6	9.4	4.2	209.7	71.1	1218.7	494.5	1839.7	6.5
Min		38.9	19.2	20.0	49.7	6.2	2.2	63.7	29.1	860.5	187.1	1357.3	1.8

[†]Crop season, sowing date, and site combinations. Crop season: 1 = 2006–2007, 2 = 2007–2008; sowing date: E = early, L = late; site (in Argentina): BW = Barrow; BO = Bordenave; CE = Cerrillos; CA = Cerro Azul; FA = Famaillá; MA = Manfredi; PA = Paraná; RQ = Reconquista.

[‡]Cool = 17.5 to 20.5°C, temperate = 20.6 to 22.4°C, and warm = 22.5 to 25.0°C.

[§]Percentage of dry matter.

[¶]mg kg^{-1} oil.

[#]mg g^{-1} of dry defatted flour.

subsequently desaturate Ol into La and Ln in developing soybean seeds and which, as it is well known, reduce their activity with increasing temperature (Cheesbrough, 1989).

It should be noted that the positive association of GT and DT with TT has been frequently reported in soybean (Scherder et al., 2006; Carrao-Panizzi and Erhan, 2007; Seguin et al., 2009) and in other species such as canola (*Brassica napus* L.) (Marwede et al., 2004). This relationship is expected considering that these make up the majority of TT (60–66 and 24–29%, respectively, Eskin et al., 1996).

Although previous works observed a negative association between total isoflavone and protein (Seguin et al., 2004; Primomo et al., 2005b; Morrison et al., 2008), this relationship was not confirmed in our study even considering the third axis (PC3). The plane determined by PC1 and PC2 of the biplot showed that the TI trait vector was oriented toward temperate to cool environments; this was also observed when PC3 was analyzed. Several studies have indicated temperature influence on isoflavone content; for example, it has been demonstrated that isoflavone accumulation decreases in warm (Kitamura et al., 1991; Tsukamoto et al., 1995; Carrao-Panizzi et al., 1999; Caldwell et al., 2005; Primomo et al., 2005a; Murphy et al., 2009) and dry environments (Lozovaya et al., 2005). The increased TI content in temperate to cool environments could be due to the temperature effect on the key enzymes of the first common phenylpropanoid pathway steps, phenyl ammonialyase, chalcone synthase and chalcone reductase, which are strongly overexpressed with decreasing temperatures (Dixon and Paiva, 1995; Janas et al., 2002; Posmyk et al., 2005). By contrast, high temperatures decrease isoflavones synthesis (Mori et al., 2005). More detailed research measuring Tm in each genotype–environment combination might provide an accurate magnitude of the effect of this variable on TI content.

Oil concentration increased in warm environments (Fig. 1). Mean air temperature during crop reproductive growth plays a significant role in determining O concentration variability, as reported in several works (Piper and Boote, 1999; Thomas et al., 2003; Carrera et al., 2009).

Rotundo and Westgate (2008), integrating information from diverse manipulative studies and conditions to evaluate temperature effects on soybean seed composition, found that an increase in temperature in a temperature range lower to 26°C increased oil concentration. In the same way, Iyer et al. (2008) working with soybean embryos in vitro exposed to increased temperature in a range lower to 26°C observed an increase in sucrose partitioning to oil. According to Rotundo and Westgate (2008), high temperatures during seed filling (>26°C) reduce the accumulation of oil due to the dependence of oil synthesis on current photoassimilate production. Furthermore, high temperatures accelerate development, thus reducing the duration of seed filling (Triboi and Triboi-Blondel, 2002). Since our work involved environments with Tm between 17.3 and 25.2°C, oil concentration was higher in those environments classified as warm for our cropped region. Thus, our results add that in such environments it would be possible to achieve not only higher oil concentration but also higher Ol:Ln (which confer higher stability to the oil) and higher AT content, since these seed quality components are environmentally correlated. Future works focused on oil content and not only on final oil concentration would provide an important insight to understand the physiological factors regulating soybean oil or other components accumulation that determine the final concentration of these components. Protein trait vector was oriented toward those cool environments which were also characterized by lower radiation than the remaining cool environments (14.4 versus 16.5 MJ m^{-2}) (Fig. 1) and therefore had reduced photothermal quotient (Fischer, 1985). Under these conditions, potential yield decreases (Magrin et al., 1993), and because of the negative correlation between yield and Pr concentration (Wilcox and Guodong, 1997), we inferred that environments with lower potential yield would attain higher Pr concentrations. Analysis of the effects of the environmental factors (e.g., solar radiation, water deficits) on grain composition during the seed filling period will be useful to complement the present study that focuses on the environmental correlations among different seed chemical components. Moreover, further research based on manipulative experiments will be necessary to study the underlying physiological mechanisms behind the environmentally determined correlations among the whole set of seed chemical components described in this work. Nevertheless, our findings are encouraging for breeders because they suggest that environments that promote high soybean protein are compatible with increased levels of chemical components with nutraceutical properties and thus could be advantageous for use of soy in food products. Meanwhile, environments enhancing high oil concentrations are linked with those components demanded by oil and biodiesel industries. Thus, the analysis of the environmental variability and the interrelationships among chemical compounds in the seed provided useful information for planning breeding of soybean grain for specific uses to meet the many facets of the markets demand. Genetic improvement of a given component and environment selection in which the expression of such component correlates with that of other components would permit a grower to obtain desired profiles at a lower cost than the cost of analyzing components individually.

CONCLUSIONS

Our results showed high variability in soybean seed chemical components that correlates with variations in temperatures of the crop environments during the seed filling. Management practices that modify the thermal environment during this period (e.g., selection of sowing date and sites) might contribute to obtain grains of desired chemical quality. Warm environments would be suitable for obtaining products with higher O concentration of low oxidation capacity (higher Ol:Ln ratio), which is demanded by oil and biodiesel industries, as well as products of higher vitamin E content (mainly AT). On the other hand, temperate to cool environments promote higher TI, essential fatty acids (La and Ln), and TT and therefore would be suitable for soybean production intended for nutraceutical product development. These environments would also favor a higher Pr concentration, which is important for the meal industry.

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References

Aguirrezábal, L.A.N., Y. Lavaud, G.A.A. Dosio, N.G. Izquierdo, F.H. Andrade, and L.M. González. 2003. Intercepted solar radiation during the seed filling period determines sunflower weight per seed and oil concentration. Crop Sci. 43:152–161.

Almonor, G.O., G.P. Fenner, and R.F. Wilson. 1998. Temperature effects on tocopherol composition in soybeans with genetically improved oil quality. J. Am. Oil Chem. Soc. 75:591–596.

Anderson, J.J.B., and S.C. Garner. 1997. Phytoestrogens and human function. Nutr. Today 32:232–239.

AOCS. 1998. Official methods and recommended practices of the American oil chemists' society, 5th ed. American Oil Chemists' Society, Champaign, IL.

Balzarini, M., and J. Di Rienzo. 2009. Info-Gen: versión 2009.

(In Spanish.) Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Barnes, S., H. Kim, V. Darley-Usmar, R. Patel, J. Xu, B. Boersma, and M. Luo. 2000. Beyond ER alpha and ER beta: Estrogen receptor binding is only part of the isoflavone story. J. Nutr. 130:656S–657S.

Bellaloui, N., and A. Mengistu. 2008. Seed composition is influenced by irrigation regimes and cultivar differences in soybean. Irrig. Sci. 26:261–268.

Boydak, E., M. Alpaslan, M. Hayta, S. Gercek, and M. Simsek. 2002. Seed composition of soybeans grown in the Harran region of Turkey as affected by row spacing and irrigation. J. Agric. Food Chem. 50:4718–4720.

Britz, S.J., and D.F. Kremer. 2002. Warm temperatures or drought during seed maturation increase free α-tocopherol in seeds of soybean. J. Agric. Food Chem. 50:6058–6063.

Britz, S.J., D.F. Kremer, and W.J. Kenworthy. 2008. Tocopherols in soybean seeds: Genetic variation and environmental effects in field-grown crops. J. Am. Oil Chem. Soc. 85:931–936.

Caldwell, C.R., S.J. Britz, and R.M. Mirecki. 2005. Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [*Glycine max* (L.) Merrill] grown in controlled environments. J. Agric. Food Chem. 53:1125–1129.

Carrao-Panizzi, M.C., A.D.P. Beleia, K. Kitamura, and M.C.N. Oliveira. 1999. Effects of genetics and environment on isoflavone content of soybean from different regions of Brazil. Pesquisa Agropecu. Bras. 34:1787–1795.

Carrao-Panizzi, M.C., and S.Z. Erhan. 2007. Environmental and genetic variation of soybean tocopherol content under Brazilian growing conditions. J. Am. Oil Chem. Soc. 84:921–928.

Carrera, C., M.J. Martínez, J. Dardanelli, and M. Balzarini. 2009. Water deficit effect on the relationship between temperature during the seed filling period and soybean seed oil and protein concentrations. Crop Sci. 49:990–998.

Carver, B.F., J.W. Burton, T.E. Carter, Jr., and R.F. Wilson. 1986. Response to environmental variation of soybean lines selected for altered unsaturated fatty acid composition. Crop Sci. 26:1176–1191.

Cheesbrough, T.M. 1989. Changes in the enzymes for fatty acid synthesis and desaturation during acclimation of developing soybean seeds to altered growth temperature. Plant Physiol. 90:760–764.

Dardanelli, J.L., M. Balzarini, M.J. Martinez, M. Cuniberti, S. Resnik, S.F. Ramunda, R. Herrero, and H. Baigorri. 2006. Soybean maturity groups, environments, and their interaction define mega-environments for seed composition in Argentina. Crop Sci. 46:1939–1947.

Dixon, R.A., and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.

Dolde, D., C. Vlahakis, and J. Hazebrock. 1999. Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development. J. Am. Oil Chem. Soc. 76:349–355.

Dornbos, D.L., Jr., and R.E. Mullen. 1992. Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. J. Am. Oil Chem. Soc. 69:228–231.

Eskin, N.A.M., B.E. McDonald, R. Przybylski, L.J. Malcomson, R. Scarth, T. Mag, K. Ward, and D. Adolph. 1996. Canola oil. p. 53–77. *In* H.I. Hui (ed.) Bailey's industrial oil and fat products. 5th ed, Vol. 2. John Wiley & Sons, Hoboken, NJ.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Spec. Rep. 80, Iowa State University, Ames, Iowa.

Fischer, R.A. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. J. Agric. Sci. 105:447–461.

Gabriel, K.R. 1971. Bi-plot display of multivariate matrices with application to principal component analysis. Biometrika 58:453–467.

Gao, J., X. Hao, K.D. Thelen, and G.P. Robertson. 2009. Agronomic management system and precipitation effects on soybean oil and fatty acid profiles. Crop Sci. 49:1049–1057.

Gibson, L.R., and R.E. Mullen. 1996. Soybean seed composition under high day and night growth temperatures. J. Am. Oil Chem. Soc. 73:733–737.

Girotti, A.W. 1998. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J. Lipid Res. 39:1529–1542.

Hoeck, J.A., W.R. Fehr, P.A. Murphy, and G.A. Welke. 2000. Influence of genotype and environment on isoflavone contents of soybean. Crop Sci. 40:48–51.

Howell, R.W., and F.I. Collins. 1957. Factors affecting linolenic and linoleic acid content of soybean oil. Agron. J. 49:593–597.

Howell, R.W., and J.L. Cartter. 1958. Physiological factors affecting composition of soybeans: II. response of oil and other constituents of soybeans to temperature under controlled conditions. Agron. J. 50:664–667.

Hubert, J., M. Berger, and J. Dayde. 2005. Use of a simplified HPLC-UV analysis for soyasaponin B determination: Study of saponin and isoflavone variability in soybean cultivars and soy-based health food products. J. Agric. Food Chem. 53:3923–3930.

Iyer, V.V., G. Sriram, D.B. Fulton, R. Zhou, M.E. Westgate, and J.V. Shanks. 2008. Metabolic flux maps comparing the effect of temperature on protein and oil biosynthesis in developing soybean cotyledons. Plant Cell Environ. 31:506–517.

Izquierdo, N.G., L.A.N. Aguirrezábal, F.H. Andrade, C. Geroudet, O. Valentinuz, and M. Pereyra Iraola. 2009. Intercepted solar radiation affects oil fatty acid composition in crop species. Field Crops Res. 114:66–74.

Janas, K.M., M. Cvikrova, A. Palagiewicz, K. Szafranska, and M.M. Posmyk. 2002. Constitutive elevated accumulation of phenylpropanoids in soybean roots at low temperature. Plant Sci. 163:369–373.

Kamal-Eldin, A., and L.A. Appelqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701.

Kitamura, K., K. Igita, A. Kikuchi, S. Kudoi, and K. Okubo. 1991. Low isoflavone content in some early maturing cultivars, so-called "summer-type soybeans". Jpn. J. Breed. 41:651–654.

Kumar, V., A. Rani, S. Solanki, and S.M. Hussain. 2006. Influence of growing environment on the biochemical composition and physical characteristics of soybean seed. J. Food Compost. Anal. 19:188–195.

Lee, J.D., M.L. Oliva, D.A. Sleper, and J.G. Shannon. 2008. Irrigation has little effect on unsaturated fatty acid content in soya bean seed oil within genotypes differing in fatty acid profile. J. Agron. Crop Sci. 194:320–324.

Lehninger, A., D. Nelson, and M. Cox. 1993. Principles of biochemistry. 2nd ed. Worth Publishers, New York, NY.

Lozovaya, V.V., A.V. Lygin, A.V. Ulanov, R.L. Nelson, J. Dayde, and J.M. Widholm. 2005. Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. Crop Sci. 45:1934–1940.

Maestri, D., D.O. Labuckas, J.M. Meriles, A.L. Lamarque, J.A.

Zygadlo, and C.A. Guzman. 1998. Seed composition of soybean cultivars evaluated in different environmental conditions. J. Sci. Food Agric. 77:494–498.

Magrin, G.O., A.J. Hall, C. Baldy, and M.O. Grondona. 1993. Spatial and interannual variations in the phototermal quotient: Implications for the potential kernel number of wheat crops in Argentina. Agric. For. Meteorol. 67:29–41.

Marwede, V., A. Schierholt, C. Mölers, and H.C. Becker. 2004. Genotype x environment interactions and heritability of tocopherol contents in canola. Crop Sci. 44:728–731.

McCue, P., and K. Shetty. 2004. Health benefits of soy isoflavonoids and strategies for enhancement: A review. Crit. Rev. Food Sci. Nutr. 44:361–367.

Messina, M. 1995. Modern applications for an ancient bean: Soybeans and the prevention and treatment of chronic disease. J. Nutr. 125:S567–S569.

Mori, K., S. Sugaya, and H. Gemma. 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Sci. Hortic. (Amsterdam) 105:319–330.

Morrison, M.J., E.R. Cober, M.F. Saleem, N.B. McLaughlin, J. Frégeau-Reid, B.L. Ma, and L. Woodrow. 2008. Changes in isoflavone concentration with 58 years of genetic improvement of short-season soybean cultivars in Canada. Crop Sci. 48:2201–2208.

Murphy, P.A., T. Song, G. Buseman, K. Barua, G.R. Beecher, D. Trainer, and J. Holden. 1999. Isoflavones in retail and institutional soy foods. J. Agric. Food Chem. 47:2697–2704.

Murphy, S.E., E.A. Lee, L. Woodrow, P. Seguin, J. Kumar, I. Rajcan, and G.R. Ablett. 2009. Genotype × environment interaction and stability for isoflavone content in soybean. Crop Sci. 49:1313–1321.

Oliva, M.L., J.G. Shannon, D.A. Sleper, M.R. Ellersieck, A.J. Cardinal, R.L. Paris, and J.D. Lee. 2006. Stability of fatty acid profile in soybean genotypes with modified seed oil composition. Crop Sci. 46:2069–2075.

Penman, H.L. 1948. Natural evaporation from open water, bare soil, and grass. Proc. R. Soc. Lond. A 193:120–146.

Piper, E.L., and K.J. Boote. 1999. Temperature and cultivar effects on soybean seed oil and protein concentrations. J. Am. Oil Chem. Soc. 76:1233–1241.

Posmyk, M.M., C. Bailly, K. Szafranska, K.M. Janas, and F. Corbineau. 2005. Antioxidant enzymes and isoflavonoids in chilled soybean [*Glycine max* (L.) Merrill] seedlings. J. Plant Physiol. 162:403–412.

Primomo, V.S., V. Poysa, G.R. Ablett, C.J. Jackson, M. Gijzen, and I. Rajcan. 2005b. Mapping QTL for individual and total isoflavone content in soybean seeds. Crop Sci. 45:2454–2464.

Primomo, V.S., V. Poysa, G.R. Ablett, C.J. Jackson, and I. Rajcan. 2005a. Agronomic performance of recombinant inbred line populations segregating for isoflavone content in soybean seeds. Crop Sci. 45:2203–2211.

Rasolohery, C.A., M. Berger, A.V. Lygin, V.V. Lozovaya, R.L. Nelson, and J. Daydé. 2008. Effect of temperature and water availability during late maturation of the soybean seed on germ and cotyledon isoflavone content and composition. J. Sci. Food Agric. 88:218–228.

Rose, I.A. 1988. Effects of moisture stress on the oil and protein components of soybean seeds. Aust. J. Agric. Res. 39:163–170.

Rotundo, J.L., and M.E. Westgate. 2008. Meta-analysis of environmental effects on soybean seed composition. Field Crops Res. 110:147–156.

Sattler, S.E., E.B. Cahoom, S.J. Coughlan, and D. Dellapenna. 2003. Characterization of tocopherol cyclases from higher plants and cyanobacteria. Evolutionary implications for tocopherol synthesis and function. Plant Physiol. 132:2184–2195.

Scherder, C.W., W.R. Fehr, G.A. Welke, and T. Wang. 2006. Tocopherol content and agronomic performance of soybean lines with reduced palmitate. Crop Sci. 46:1286–1290.

Seguin, P., P. Turcotte, G. Tremblay, D. Pageau, and W. Liu. 2009. Tocopherols concentration and stability in early maturing soybean genotypes. Agron. J. 101:1153–1159.

Seguin, P., W. Zheng, D.L. Smith, and W. Deng. 2004. Isoflavone content of soybean cultivars grown in eastern Canada. J. Sci. Food Agric. 84:1327–1332.

Sherwin, E.R. 1976. Antioxidants for vegetable oils. J. Am. Oil Chem. Soc. 53:430–436.

Shintani, D., and D. DellaPenna. 1998. Elevating the vitamin E content of plants through metabolic engineering. Science 282:2098–2100.

Soldini, D.O. 1998. Control genético de la composición del aceite de soja: ácidos grasos y antioxidantes. Monograph. (In Portuguese.) Biblioteca de la Escola Superior de Agricultura "Luis de Queiroz" (ESALQ) de la Universidade de San Pablo (USP), Piracicaba, Brazil.

Thomas, J.M.G., K.J. Boote, L.H. Allen, Jr., M. Gallo-Meagher, and J.M. Davis. 2003. Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance. Crop Sci. 43:1548–1557.

Triboi, E., and A.M. Triboi-Blondel. 2002. Productivity and grain or seed composition: A new approach to an old problem—invited paper. Eur. J. Agron. 16:163–186.

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura. 1995. Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J. Agric. Food Chem. 43:1184–1192.

Valenzuela, A., S. Nieto, and A. Garrido. 1999. Long chain polyunsaturated fatty acids: When and why is necessary the supplementation with these fatty acids. Aceites y Grasas 9:294–299.

Valenzuela, A., J. Sanhueza, and S. Nieto. 2000. Long-chain omega-3 fatty acids in human and animal health and nutrition: A model for development of functional foods. Aceites y Grasas 10:526–533.

Warner, K.A. 2003. Effects of antioxidants in frying oils. p. 210–227. *In* M. Gupta, K. Warner, and P. White (ed.) Chemistry of frying oils. AOCS, Champaign, IL.

Wilcox, J.R., and Z. Guodong. 1997. Relationships between seed yield and seed protein in determinate and indeterminate soybean populations. Crop Sci. 37:361–364.

Wilson, R. 2004. Seed composition. p. 621–677. *In* B.A. Stewart and D.R. Nielsen (ed.) Soybeans: Improvement, production and uses, 3rd ed. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.

Wolf, R.B., J.F. Canvins, R. Kleiman, and L.T. Black. 1982. Effect of temperature on soybean seed constituents; oil, protein, moisture, fatty acids, amino acids and sugars. J. Am. Oil Chem. Soc. 59:230–232.

Yan, W., L.A. Hunt, Q. Sheng, and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci. 40:597–605.

Yan, W., and I. Rajcan. 2002. Biplot analysis of test sites and trait relations of soybean in Ontario. Crop Sci. 42:11–20.