Amino acid composition of soybean seeds as affected by climatic variables

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Abstract – The objective of this work was to perform a quantitative analysis of the amino acid composition of soybean seeds as affected by climatic variables during seed filling. Amino acids were determined from seed samples taken at harvest in 31 multi-environment field trials carried out in Argentina. Total amino acids ranged from 31.69 to 49.14%, and total essential and nonessential amino acids varied from 12.83 to 19.02% and from 18.86 to 31.15%, respectively. Variance components expressed as the percentage of total variation showed that the environment was the most important source of variation for all traits, followed by the genotype x environment interaction. Significant explanatory linear regressions were detected for amino acid content regarding: average daily mean air temperature and cumulative solar radiation, during seed filling; precipitation minus potential evapotranspiration, during the whole reproductive period; and the combinations of these climatic variables. Each amino acid behaves differently according to environmental conditions, indicating compensatory effects among them.

Index terms: *Glycine max*, environmental variation, multiple linear regression, multi-environment trials, protein composition.

Conteúdo de aminoácidos em sementes de soja em função de variáveis climáticas

Resumo – O objetivo deste trabalho foi realizar uma análise quantitativa da composição de aminoácidos de sementes de soja em função de variáveis climáticas, durante o período de formação do grão. Foram analisados os aminoácidos de amostras de sementes colhidas no momento da colheita a partir de 31 ensaios multiambientais realizados a campo na Argentina. Os aminoácidos totais variaram de 31,69 a 49,14%, e o total de aminoácidos essenciais e não essenciais variou de 12,83 a 19,02% e de 18,86 a 31,15%, respectivamente. Os componentes de variância expressos como percentagem da variação total mostraram que o ambiente foi a fonte de variação mais importante, seguido da interação genótipo x ambiente. Regressões lineares significativas foram detectadas para o conteúdo de aminoácidos em relação a: temperatura média diária do ar e radiação solar acumulada, durante a formação dos grãos; precipitação menos evapotranspiração potencial, durante todo o período reprodutivo; e combinações dessas variáveis climáticas. Cada aminoácido comporta-se de forma diferente de acordo com as variações nas condições ambientais, o que indica efeitos compensatórios entre eles.

Termos para indexação: *Glycine max*, variação ambiental, regressão linear múltipla, ensaios multiambientais, composição de proteínas.

Introduction

Soybean [*Glycine max* (L.) Merr.] seed is a major source of protein worldwide. The average protein content of soybean reaches 40%, whereas, in most common beans, this value is of 20 to 25% (Mateos-Aparicio et al., 2008). Consequently, soybean meal is a valuable and desirable product for both human nutrition and livestock feeding, since it contains all essential amino acids and has low cost compared to other high-quality protein sources. Therefore, the study of soybean amino acid composition is important for nutritional purposes and for consumer acceptance of soy food products, as it contributes to the taste of foodstuffs (Gao et al., 2011).

Argentina is the leading soybean meal exporter and the third largest soybean seed producer. The soybean region in the country covers a wide ecological area between the 23 and 39º southern latitudes, with great variation in environmental conditions, such as rainfall,

radiation and temperature. Multi-environment trials are usually conducted to compare grain yield performance of a large number of genotypes, but could be useful to assess environmental effects on seed chemical composition.

It has been widely documented that soybean seed composition varies with environmental factors, especially during the seed filling period when accumulation of the seed chemical components occurs (Wolf et al., 1982; Wilson, 2004; Carrera et al., 2009, 2011). The relationship of protein contents (the sum of all amino acids) with temperature (Piper & Boote, 1999; Wilson, 2004) and water availability (Rose, 1988; Boydak et al., 2002; Kumar et al., 2006; Carrera et al., 2009) has also been studied, but results have shown dissimilar patterns. Therefore, it is interesting to analyze the response of each amino acid to different climatic variables, because their behavior could contribute to explain these discrepancies. Some researchers have reported the effect of temperature on soybean seed amino acid composition. For example, Wolf et al. (1982) found that higher deposition of sulfur amino acids (methionine and cystine) takes place at higher temperatures. Grieshop & Fahey (2001) and Karr-Lilienthal et al. (2005) reported that essential, nonessential, and total amino acid content were lower in northern zones of the United States, which are cooler than central and southern zones.

In spite of these findings, there are no known quantitative studies on the effect of temperature, solar radiation and water deficit during seed filling on the nutritional composition and quality of soybean seed protein. About 90% of the world's soybean production occurs under rainfed conditions, which are characterized by high temperatures and low or erratic rainfall (Thuzar et al., 2010). Given the current trend of rising temperatures, droughts, floods and extreme weather events (Intergovernmental Panel on Climate Change, 2009), the exposure of the crop to thermal and water stresses will increase. For an initial understanding of the direct effects of climatic variables on amino acid composition, multi-environment field trials are highly valuable because they capture the environmental effects between and within years and locations.

The objective of this work was to analyze amino acid composition of soybean seeds as affected by climatic variables during seed filling.

Materials and Methods

A data set was constructed from 31 soybean multi-environment field trials conducted during two crop years (2001/2002 and 2002/2003) at the agricultural experimental stations of Instituto Nacional de Tecnología Agropecuaria (Inta), across the Argentine soybean growing regions. The trials were set in a randomized complete block design with three replicates, in each environment, defined as crop year, location, and sowing date combinations. The trial network involved commercial cultivars from several maturity groups sown at different locations and sowing dates per growing season (Table 1). The following cultivars were evaluated (maturity group is given between parentheses): DM 3100 RR (III), DM 3700 RR (III), DM 4400 RR (IV), DM 4600 RR (IV), DM 4800 RR (IV), A 5520 RG (V), A 6445 RG (VI), A 7636 RG (VII), and A 8000 RG (VIII). The database was constructed so as to capture the highest possible environmental variability without intending to have all cultivars in all environments. Soil analyses did not indicate any physical or nutritional constraint for crop development. Crops were grown under rainfed conditions and followed cultural practices recommended by Inta (Baigorri, 1997). Final plant density in all trials was about 35–40 plants per square meter, with 0.52 m between rows.

Table 1. Crop seasons, locations, and sowing dates of 31 multi-environment field trials conducted in the Argentine soybean crop area.

Location	Coordinates	Sowing date (day of the month)					
		Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
		2001/2002 crop season					
Concepción del Uruguay	32º29', 58º14'	-	-	22	-	-	5
General Pico	35º40', 63º44'	-	5	-	-	3	-
Manfredi	31º49', 63º46'	-	12	27	-	-	-
Marcos Juárez	32º41', 62º06'	12	-	-	5	-	-
Paraná	31º44', 60º32'	23	-	12	-	12	-
		2002/2003 crop season					
Balcarce	37º52', 58º15'	-	25	15	23	-	-
Bellocq	38º20', 60º13'	-	9	-	-	3	-
Barrow	38º19', 60º14'	-	-	5	10	-	-
Concepción del Uruguay	32º29', 58º14'	23	-	-	26	-	-
Manfredi	31º49', 63º46'	-	-	1	27	-	-
Marcos Juárez	32º41', 62º06'	23	2	5	-	5	-
Paraná	31º44', 60º32'	23	-	12	-	13	-
Reconquista	29º40', 59º12'	26	-	-	-	9	-

Temperature and solar radiation data were recorded daily for each crop season and site at Inta meteorological stations, up to 10 km away from each experimental field. Rainfall records were obtained from rain gauges placed in each experimental plot site, and potential evapotranspiration was calculated using the Penman (1948) equation. Dates of occurrence of R1 (beginning of flowering), R5 (beginning of seed filling), and R7 (physiological maturity) were assessed in the field, at every trial, using the scale of Fehr & Caviness (1977). From the daily climatic data, the following variables were generated for each environment: average daily mean air temperature during seed filling (Tm_{R5R7}); cumulative solar radiation during seed filling (Sr_{R5R7}), calculated as the sum of daily solar radiation in that period; and precipitation minus potential evapotranspiration during the whole reproductive period (pp-PET_{R1R7}). The variable pp-PET_{R1R7} was used as a simple indicator of water availability in the evaluated period, since water balance from the whole reproductive period considers water storage in the soil profile, which could influence water availability during grain filling (Carrera et al., 2009).

For all determinations, a randomized 300-g grain sample was made using the collected samples at harvest from the three replicates of each cultivar, at each location and sowing date. Amino acid content (expressed as percentage, in dry matter basis) was analyzed in triplicate, using oxidation and acid and alkaline hydrolysis methods for sulfur amino acids. Acid hydrolysis, alkaline hydrolysis, and oxidation of sulfur amino acids were made according to the procedures described in AOAC official method 982.30, 988.15, and 994.12, respectively (Horwitz, 2005), with the following modifications: after acid hydrolysis, the sample was transferred quantitatively to a 10-mL flask with buffer pH 9.5 (0.4 N sodium borate); after alkaline hydrolysis, the sample was transferred quantitatively to a 50-mL flask, with pH adjusted using a 2 N HCl solution, completed with water; after oxidation of sulfur amino acids, the open flask was heated with 5 mL of 6 N HCl in the Pierce thermo bath during 1 hour at 113±1ºC. The next steps were the same as those of acid hydrolysis. Then, 500 µL of each extract was mixed with 500 µL of the internal standard (sarcosine 20 µg mL^{-1} plus dl-norvaline 20 µg mL^{-1}, in 0.01 mol L^{-1} HCl). Amino acid concentrations were determined using a liquid chromatograph Agilent 1100, high performance resolution, (Agilent Technologies, Wilmington, DE, USA) with a fluorescence detector, quaternary pump and a degasser. Separation was achieved using a Adsorbosphere OPA HS column (100x4.6 mm, 5 µm) for primary amino acids, and a Adsorbosphere OPA HR column (150x4.6 mm, 5 µm) for proline (pro), hydroxyproline (HO-pro) and tryptophan (trp). Both columns had a BDS-Hypersil-C18 pre-column (10x4 mm, 5 µm). Flow rate was 1 mL min^{-1}, and column temperature was maintained at 40ºC during the run. Amino acids were analyzed using different pre-column derivatization methods and mobile phases. For primary amino acids and trp, a derivatization method with o-phthaldialdehyde-2-mercaptoethanol was used. The mobile phase was: A, tetrahydrofuran (20% in MeOH); B, MeOH; and C, sodium acetate buffer 60 mmol L^{-1}, pH 5.5. For pro and HO-pro, the derivatization method was performed with o-phthaldialdehyde-3-mercaptopropionicacid and 9-fluorenylmethyl-chloroformate. The mobile phase was: B, MeOH; and C, sodium acetate buffer 60 mmol L^{-1}, pH 5.5; all solvents were of high performance liquid chromatography purity. Primary amino acids and trp were detected at 230 and 450 nm, and pro and HO-pro at 266 and 313 nm, respectively. Calibration curves were obtained using standard amino acid solution purchased from Pierce (Rockford, IL, USA), which contained all the amino acids [cysteine (cys), aspartate (asp), glutamate (glu), serine (ser), histidine (his), glycine (gly), threonine (thr), methionine (met), alanine (ala), arginine (arg), tyrosine (tyr), valine (val), phenylalanine (phe), isoleucine (ileu), leucine (leu), lysine (lys), and proline (pro)], except trp and HO-pro, purchased from Sigma-Aldrich (St. Louis, MO, USA) and from Fluka Chemie (Steinheim, Germany), respectively. The standard amino acid working solution was prepared in 0.1 N HCl. Ten concentration levels from the amino acid solution and 12 levels for pro and HO-pro were used for calibration curves. Methionine and cystine were quantified as methionine sulfone and cysteic acid and calculated as sulfur amino acids. For quantification, peak areas were correlated with the concentrations according to calibration curves (R^2>0.99). The relative standard deviation (RSD) was less than 3%, except for met, cys, tyr, and trp, in which it was lower than 10%. Total amino acids (TAA) were calculated by summing individual amino acid concentrations (cys, asp, glu,

ser, his, gly, thr, met, ala, arg, tyr, trp, val, phe, ileu, leu, lys, HO-pro, and pro). The variable total essential amino acids (TEAA) was determined by summing the contents of arg, his, ileu, leu, lys, met, phe, thr, trp, and val; whereas total nonessential amino acids (TNEAA) were generated by summing ala, asp, cys, glu, gly, pro, ser, tyr, and HO-pro concentrations. All determinations were made at the Departamento de Química Orgánica of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

A multiple linear regression procedure was performed to model all the analyzed amino acids (cys, asp, glu, ser, his, gly, thr, met, ala, arg, tyr, trp, val, phe, ileu, leu, lys, HO-pro, and pro) as a function of Tm_{R5R7}, Sr_{R5R7} and pp-PET_{R1R7}, and as a function of the combinations of these climatic variables (Tm_{R5R7} with pp-PET_{R1R7}; Sr_{R5R7} with pp-PET_{R1R7}). The t test, at 5% probability, was used on regression parameter estimates. Model selection was based on Mallows' Cp coefficient and residual analyses. All calculations were made with the statistical software InfoStat (Di Rienzo et al., 2009).

Results and Discussion

Mean values for individual amino acids (Table 2) were close to those reported by Grieshop & Fahey (2001) and Karr-Lilienthal et al. (2004). Although these authors observed higher TEAA values, in the present study mean TNEAA values were higher. Interestingly, even though TNEAA are less important for human and livestock nutrition than TEAA, they markedly contributed to TAA (Table 3).

A high variability (coefficient of variation) was observed in all individual amino acid compositions (Table 3). Likewise, a large variation of temperature (14.1 to 25.7ºC), solar radiation (240 to 1,676.7 MJ m^{-2}) and water availability (-262 to 410.5 mm) was obtained in the multi-environmental field trials. This was reflected in the high yield variability, which had approximately 6-fold variation, from 885 to 5,571 kg ha^{-1}. Differences between extreme values across the data points (cultivar x environment combinations) during seed filling were 11.6ºC for temperature and 1,436 MJ m^{-2} for solar radiation, whereas the extreme values of precipitation minus potential evapotranspiration during the whole reproductive period differed in 673.2 mm.

Significant explanatory regressions were obtained for all amino acids on Tm_{R5R7}, Sr_{R5R7} and pp-PET_{R1R7}, as well as on the combinations of these climatic variables (Tm_{R5R7} with pp-PET_{R1R7}, and Sr_{R5R7} with pp-PET_{R1R7}). The only exception was valine, whose variations were not explained by any of these environmental factors. A quadratic regression including Tm_{R5R7} was the best explanatory model for this amino acid, although temperature was not a significant predictor (Table 4).

Polynomial-quadratic functions were fitted for ileu, met, phe, ala, glu, pro vs. Tm_{R5R7}, and a linear model for aspartate vs. Tm_{R5R7} (Tables 4 and 5). The regression fitted for these amino acids indicated that all of them increased with increasing values of Tm_{R5R7} until a threshold was achieved, after which they decreased. However, the temperature above which each amino acid started to decrease varied among them (pro, 22ºC; phe, 21ºC; glu, 20ºC; met, 19ºC; and ala and ileu, 16ºC). By contrast, lysine concentration showed an inverse relationship with temperature (Table 4), decreasing with increasing temperature of 14.1 to 22ºC, after when it began to increase. Aspartate increased linearly with temperature (Table 5). Regression equations for leucine and cystine showed a linear positive contribution for pp-PET_{R1R7} and a linear and quadratic one for Tm_{R5R7}. In addition, pp-PET_{R1R7} had the highest contribution (Mallows' Cp) in the fitted regression model. For both amino acids, the regression coefficients for pp-PET_{R1R7} and Tm_{R5R7} indicated that leucine and cystine increased with higher water availability (increasing pp-PET_{R1R7} values) and temperature. However, temperature had less effect at higher Tm_{R5R7} values because of its negative quadratic effect on amino acid contents.

These results do not agree with previous studies. Wolf et al. (1982), for example, found that only methionine and cystine (sulfur amino acids) increased with higher temperatures in the beginning of soybean seed development. Grieshop & Fahey (2001) and Karr-Lilienthal et al. (2005) reported that TEAA, TNEAA, and TAA concentrations were lower for soybean meal from northern zones of the United States compared to central and southern zones. These authors attributed their findings to differences in temperature among regions, even when no climatic characterization of the regions was provided in the studies, which might play an important role in protein concentrations found in the soybean grains, and, therefore, in the resultant soybean meal. However, all of these studies only

considered the effect of temperature on amino acid composition without exploring other environmental factors. Disagreements among the findings of the present study and of previous reports could be explained, in part, by the inclusion of other environmental factors in regression analyses. Significant explanatory models showed different behaviors for each amino acid against temperature and, in some cases, water availability combined with temperature. Another important point is that previous analyses were made in a qualitative manner, which makes the interpretation of the effect of environment on amino acid composition more difficult.

The results obtained indicate compensatory effects among amino acids, which could explain to some extent the lack of relation between TAA and the climatic variables considered in the present study. Piper & Boote (1999) and Carrera et al. (2009) reported, in field studies, that a small fraction of the variation (R^2) accounted for protein concentration, which considers the sum of all amino acids versus temperature during the seed filling period. However, this could be also attributed to the compensatory responses. Carrera et al. (2009) found that the response of protein to Tm_{R5R7} was explained through a quadratic model, which indicated that protein percentage decreased, with increasing

Table 2. Content (% of dry matter) of total amino acids (TAA), and total essential (TEAA) and nonessential amino acids (TNEAA), and climatic variables of the 31 multi-environment field trials evaluated.

Location	Sowing date	Amino acid content			Climatic variables[(1)]		
		TEAA	TNEAA	TAA	Tm_{R5R7}	Sr_{R5R7}	pp-PET_{R1R7}
		---------- (%) ----------			(ºC)	(MJ m^{-2})	(mm)
2001/2002 crop season							
Concepción del Uruguay	11/22	15.8	23.2	39.0	21.8	604.1	252.5
	2/5	16.5	24.9	41.4	16.6	395.2	400.5
General Pico	10/5	15.1	23.1	38.3	20.6	920.2	-96.6
	1/3	12.8	18.9	31.7	15.1	494.5	0.6
Manfredi	10/12	15.7	28.0	43.7	22.0	750.1	-56.0
	11/27	15.6	28.1	43.7	21.2	714.7	60.9
Marcos Juárez	9/12	16.1	28.1	44.2	23.7	963.2	-152.6
	12/5	16.5	28.0	44.5	22.8	686.4	95.4
	9/23	18.7	26.6	45.3	23.5	1,213.8	-92.9
Paraná	11/12	13.9	23.2	37.1	23.2	632.1	-5.9
	1/12	16.7	25.2	42.0	18.9	404.7	134.4
2002/2003 crop season							
Balcarce	10/25	17.0	28.4	45.4	20. 7	634.5	-70.3
	11/15	14.8	25.3	40.1	19.9	473.2	57.5
	12/23	16.2	25.9	42.2	15.9	357.3	93.1
Bellocq	10/9	13.7	21.6	35.3	21.9	1,168.8	-179.9
	1/3	14.3	25.3	39.6	15.9	697.7	180.6
Barrow	11/5	13.1	21.5	34.6	20.7	805.6	-262.7
	12/10	14.0	23.6	37.6	18.1	680.6	-140.1
Concepción del Uruguay	9/23	17.4	25.1	42.5	23.5	1,349.1	111.4
	12/26	16.1	26.7	42.8	18.7	522.1	91.1
Manfredi	11/1	16.1	27.0	43.1	21.4	753.9	-147.4
	12/27	16.5	28.4	44.9	20.0	472.9	-74.6
	9/23	14.9	25.3	40.2	23.7	784.4	-65.9
Marcos Juárez	10/2	15.7	25.0	40.7	24.1	1,072.0	-43.7
	11/5	16.4	26.9	43.3	23.7	996.6	4.0
Paraná	11/12	16.9	28.5	45.5	22.1	592.0	105.5
	1/13	15.7	24.3	40.0	18.5	424.6	106.1
Reconquista	9/26	15.5	26.2	41.7	25.4	1,120.8	239.0
	1/9	15.0	24.4	39.4	21.3	526.1	335.9

[(1)]Tm_{R5R7}, average daily mean air temperature during seed filling; Sr_{R5R7}, cumulative solar radiation during seed filling; pp-PET_{R1R7}, precipitation minus potential evapotranspiration during the whole reproductive period.

temperature of 14 to 19.3ºC, and then increased, in accordance with Piper & Boote (1999), but in sharp contrast with the results obtained in the present study regarding the response of some amino acids to temperature. In previous studies, Tm_{R5R7} accounted for a small fraction of the variation in total protein concentration: adjusted $R^2 = 0.019$ in Piper & Boote (1999) and adjusted $R^2 = 0.045$ in Carrera et al. (2009). However, Dornbos & Mullen (1992) observed that protein concentration decreased above 21ºC, whereas Kumar et al. (2006) found that protein concentration was positively associated with the mean temperature during soybean development under field experiments. As to water availability, Carrera et al. (2009) stated that, under water stress conditions in the field, protein had a linear positive association with Tm_{R5R7}, indicating increasing protein concentration with rising temperatures and a linear negative correlation with increasing water deficit. By contrast, Kumar et al. (2006) observed that rainfall during soybean development showed a significantly negative linear correlation with protein concentration, in agreement with Dornbos & Mullen (1992), who reported increases in protein concentration under severe drought stress. Consequently, different patterns for the relationship of protein with temperature and water availability were

Table 3. Content (% of dry matter) of seed amino acids, coefficient of variation and ranges observed in soybean multi-trials across the Argentine soybean crop.

Amino acid	Mean	CV (%)	Min.	Max.
Cystine	0.80	33.32	0.28	1.35
Aspartate	4.44	18.72	2.40	5.77
Glutamate	8.14	13.32	5.37	10.19
Serine	2.46	16.00	1.36	3.48
Histidine	1.05	21.34	0.55	1.49
Glycine	1.92	22.26	1.04	2.98
Threonine	1.63	17.23	0.87	2.19
Methionine	0.59	21.42	0.31	0.85
Alanine	1.78	14.70	1.06	2.51
Arginine	2.91	21.57	1.53	4.01
Tyrosine	1.35	14.81	0.66	1.90
Tryptophan	0.57	20.80	0.30	0.80
Valine	1.94	12.50	1.32	2.59
Phenylalanine	2.25	10.54	1.86	2.79
Isoleucine	1.97	12.09	1.41	2.47
Leucine	3.47	9.81	2.94	4.24
Lysine	2.37	33.42	0.88	3.92
Hydroxyproline	0.05	46.90	0.01	0.10
Proline	2.23	10.26	1.22	2.80
TEAA	15.85	8.76	12.83	19.02
TNEAA	26.00	9.54	18.86	31.15
TAA	41.84	8.11	31.69	49.14

TEAA, total essential amino acids; TNEAA, total nonessential amino acids; TAA, total amino acids.

Table 4. Regression models for essential amino acid contents (% of dry matter), on climatic variables[(1)], for all crop year, loation souring date, and cultivar combinations.

Explanatory variable	Regression coefficient	Standard error	p	Mallows' Cp
		Arginine		
Constant	3.00	0.07	<0.0001	
pp-PET_{R1R7} (linear)	-0.0015	0.00041	0.0003	14.81
		Histidine		
Constant	1.53	0.16	<0.0001	
pp-PET_{R1R7} (linear)	-0.00061	0.00016	0.0004	16.61
Sr_{R5R7} (linear)	-0.0011	0.00038	0.0049	11.27
Sr_{R5R7} (quadratic)	5.7E 07	2.1E 07	0.0076	10.42
		Isoleucine		
Constant	-1.42	1.28	0.2698	
Tm_{R5R7} (linear)	0.32	0.13	0.0142	8.22
Tm_{R5R7} (quadratic)	-0.01	0.0031	0.0205	7.53
		Leucine		
Constant	-2.02	1.62	0.2161	
pp-PET_{R1R7} (linear)	0.0011	0.0002	<0.0001	30.74
Tm_{R5R7} (linear)	0.52	0.16	0.0020	13.13
Tm_{R5R7} (quadratic)	-0.01	0.004	0.0031	12.18
		Lysine		
Constant	19.25	4.07	<0.0001	
Tm_{R5R7} (linear)	-1.72	0.41	0.0001	19.79
Tm_{R5R7} (quadratic)	0.04	0.01	<0.0001	20.28
		Methionine		
Constant	-0.65	0.70	0.3541	
Tm_{R5R7} (linear)	0.13	0.07	0.0500	5.69
Tm_{R5R7} (quadratic)	-0.0035	0.0017	0.0432	6.18
		Phenylalanine		
Constant	-1.97	1.27	0.1230	
Tm_{R5R7} (linear)	0.42	0.13	0.0012	13.10
Tm_{R5R7} (quadratic)	-0.01	0.0031	0.0013	13.05
		Threonine		
Constant	2.08	0.19	<0.0001	
pp-PET_{R1R7} (linear)	-0.00098	0.0002	<0.0001	27.44
Sr_{R5R7} (linear)	-0.00096	0.00046	0.0398	7.32
Sr_{R5R7} (quadratic)	4.9E-07	2.5E-07	0.0506	6.90
		Tryptophan		
Constant	0.80	0.08	<0.0001	
pp-PET_{R1R7} (linear)	-0.00032	8.8E-05	0.0006	15.80
Sr_{R5R7} (linear)	-0.00048	0.0002	0.0217	8.43
Sr_{R5R7} (quadratic)	2.2E-07	1.1E-07	0.0492	6.95
		Valine		
Constant	-0.08	1.36	0.9520	
Tm_{R5R7} (linear)	0.19	0.14	0.1539	4.06
Tm_{R5R7} (quadratic)	-0.0046	0.0033	0.1688	3.92

[(1)]Tm_{R5R7}, average daily mean air temperature during seed filling; Sr_{R5R7}, cumulative solar radiation during seed filling; pp-PET_{R1R7}, precipitation minus potential evapotranspiration during the whole reproductive period.

observed, probably due to the fact that amino acids were considered all together in protein variation analyses, in response to different environmental factors, unlike in the present study, in which each amino acid responded differently to Tm_{R5R7} and pp-PET_{R1R7}, when analyzed independently. Therefore, further studies based on manipulative experiments are required to fully elucidate the physiological mechanisms regulating the differential amino acid responses to environmental conditions during seed filling.

Table 5. Regression models for nonessential amino acid content (% of dry matter), on climatic variables[(1)], for all crop year, loation souring date, and cultivar combinations.

Explanatory variable	Regression coefficient	Standard error	p	Mallows' Cp
		Alanine		
Constant	-1.15	1.46	0.4311	
Tm_{R5R7} (linear)	0.29	0.15	0.0470	6.03
Tm_{R5R7} (quadratic)	-0.01	0.0036	0.0479	6.00
		Aspartate		
Constant	2.28	0.69	0.0014	
Tm_{R5R7} (linear)	0.10	0.03	0.0022	10.90
		Cystine		
Constant	-2.5	1.42	0.0823	
pp-PET_{R1R7} (linear)	0.00053	0.00018	0.0039	11.73
Tm_{R5R7} (linear)	0.32	0.14	0.0246	8.20
Tm_{R5R7} (quadratic)	-0.01	0.0035	0.0260	8.10
		Glutamate		
Constant	-16.09	5.56	0.0049	
Tm_{R5R7} (linear)	2.42	0.55	<0.0001	20.88
Tm_{R5R7} (quadratic)	-0.06	0.01	<0.0001	20.76
		Glycine		
Constant	2.74	0.29	<0.0001	
pp-PET_{R1R7} (linear)	-0.0014	0.00031	<0.0001	24.08
Sr_{R5R7} (linear)	-0.0017	0.00071	0.0194	8.63
Sr_{R5R7} (quadratic)	7.9E-07	3.8E 07	0.0419	7.23
		Proline		
Constant	-1.84	2.16	0.3977	
TmR5R7 (linear)	0.44	0.22	0.0457	6.08
TmR5R7 (quadratic)	-0.01	0.01	0.0315	6.74
		Serine		
Constant	2.81	0.1	<0.0001	
pp-PET_{R1R7} (linear)	-0.00094	0.00026	0.0005	14.83
Sr_{R5R7} (linear)	-0.00041	0.00013	0.0022	11.89
		Tyrosine		
Constant	1.57	0.13	<0.0001	
Sr_{R5R7} (linear)	-0.00062	0.00033	0.0508	5.61
Sr_{R5R7} (quadratic)	3.7E-07	1.8E-07	0.0422	6.22
		Hydroxyproline		
Constant	0.09	0.02	<0.0001	
pp-PET_{R1R7} (linear)	-4.1E-05	1.8E-05	0.0217	8.43
Sr_{R5R7} (linear)	-0.00012	4.1E-05	0.0056	11.03
Sr_{R5R7} (quadratic)	7.1E-08	2.2E-08	0.0019	13.19

[(1)]Tm_{R5R7}, average daily mean air temperature during seed filling; Sr_{R5R7}, cumulative solar radiation during seed filling; pp-PET_{R1R7}, precipitation minus potential evapotranspiration during the whole reproductive period.

Polynomial quadratic functions were fitted for his, thr, trp, gly, and HO-pro with Sr_{R5R7} combined with linear functions for pp-PET_{R1R7}, indicating that decreases of all these amino acids occurred under conditions of higher solar radiation and water availability (Tables 4 and 5). Likewise, tyrosine was negatively related to Sr_{R5R7} through a polynomial quadratic function (Table 5), serine displayed negative linear relationships both with pp-PET_{R1R7} and Sr_{R5R7}, and arginine showed a linear negative association with pp-PET_{R1R7} (Table 4).

The negative response of some amino acids to more favorable environmental conditions, such as higher solar radiation and water availability, could be attributed to a dilution effect, contributing to the decline of protein concentration, which is a result of the accumulation of relatively higher oil content in seeds. This is supported by the highly negative correlation between oil and protein concentration, often reported in the literature, which is frequently related to yield increase, since there is also a negative correlation between protein and yield (Wilcox & Shibles, 2001; Proulx & Naeve, 2009). In fact, cumulative solar radiation during the seed filling period was positively related to yield ($p = 0.009$), probably due to an increase in seed weight (Aguirrezábal et al., 2003). This relationship supports the negative correlation between seed yield and seed protein concentration found in other studies on soybean (Wilcox & Shibles, 2001). Although these inferences are valid for a group of only eight amino acids, which represent 42% of the total, they should be considered in future studies along with the inferences for ileu, met, phe, ala, glu, pro, asp, lys, leu, and cys, since they provide background information, indicating that each amino acid behaves differently under different environmental conditions.

In this context, the present study is the first step in what could be a more comprehensive investigation to improve and provide insights for soybean management strategies in order to maximize levels of protein and its quality. An in-depth research is required to complement the present study and to draw a clear conclusion on how environment conditions during the seed filling period may influence the quality of soybean seed protein. Moreover, it would be interesting to assess how climatic factors regulate soybean seed

amino acid accumulation through their effect on seed metabolism and development, which determines the final concentration of these components.

Conclusions

1. Amino acid composition of soybean grains is strongly affected by environmental factors during the seed filling period.

2. Average daily mean air temperature and cumulative solar radiation during seed filling, precipitation minus potential evapotranspiration during the whole reproductive period, as well as combinations of these climatic variables, are significant explanatory variables for all amino acids, except valine.

3. Each amino acid behaves differently according to environmental conditions, indicating compensatory effects among them.

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