

## Yield, proteins, bioactive compounds and minerals in food-type soybean grains in different environments

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ABSTRACT: Soybean is characterized as a nutritional and functional food by virtue of its high concentrations of minerals and bioactive compounds - the group of phenolic compounds, mainly. The yield and chemical composition of soybean grains are influenced by genetic and environmental factors and their interactions. This study proposes to examine the yield and chemical composition of grains of food-type soybean genotypes in different environments. Six food-type soybean genotypes sown in the first week of October and in the first week of November in the municipalities of Londrina and Guarapuava (Paraná State, Brazil) were evaluated. Grain yield and weight, protein percentages, phenolic compound and total flavonoid contents and Ca, Mg, Cu, Fe and Zn contents were evaluated. Combined analysis of variance revealed a genotype × environment interaction (GE) effect for the yield traits, 100-grain weight and for the mineral Ca. Higher yields and 100-grain weights were observed under milder maximum-temperature conditions. High maximum temperatures resulted in higher protein percentages and total flavonoid, Ca and Zn contents. Soybean lines UEL 110 and UEL 115 possess high grain yield capacity and higher Ca, Mg and Zn contents.

Key words: flavonoids; genotype × environment; Glycine max; minerals; phenolic compounds

# Produtividade, proteínas, compostos bioativos e minerais em grãos de soja tipo alimento em diferentes ambientes

**RESUMO:** A soja caracteriza-se como um alimento nutritivo e funcional pois possui altos teores de minerais e de compostos bioativos. A produtividade e a composição química de grãos são influenciadas por fatores genéticos, ambientais e suas interações. Com isso, o objetivo do estudo foi avaliar a produtividade e a composição química de grãos de genótipos de soja tipo alimento em diferentes ambientes. Foram avaliados seis genótipos de soja tipo alimento semeadas na primeira semana de outubro e primeira semana de novembro nos municípios de Londrina e Guarapuava, PR, Brasil. Avaliou-se a produtividade e massa de grãos, percentuais proteicos, teores de compostos fenólicos e flavonoides totais, e os minerais Ca, Mg, Fe, Zn e Cu. Pela análise de variância conjunta foi constatada interação genótipo x ambiente (GA) para as características produtividade, massa de cem grãos e para o mineral Ca. Maiores produtividades e massa de cem grãos foram observadas em condições de temperaturas máximas amenas. Temperaturas máximas elevadas provocaram maiores percentuais de proteínas, de flavonoides totais, e de Ca e Zn. As linhagens UEL 110 e UEL 115 agregam alta produtividade de grãos e uma composição química equilibrada.

Palavras-chave: flavonoides; genótipo x ambiente; Glycine max; minerais; compostos fenólicos

#### Introduction

According to data from the Food and Drug Administration (FDA), soy consumption offers benefits to human health due to its nutritional properties. Soy is characterized as one of the main protein sources, in addition to containing essential amino acids in an adequate proportion for our diet, constituting an alternative to animal protein (Seibel et al., 2013).

Soybean and its byproducts are classified as functional foods, as they have antiinflamatory, anticarcinogenic and antioxidant action. Such functionalities are attributed mainly to the phenolic compounds produced in response to the secondary plant metabolism, with the major benefits found in the group of flavonoids (Rigo et al., 2015).

Despite the nutritional composition of soybean grains, their use in the human diet is still low. This is related to their unpleasant taste and aroma, known as "beany flavor", which is attributed to the action of specific enzymes, the lipoxygenases ( $L_{ox}$ ). The genetic elimination of  $L_{ox}$  improves the tastes of soybean grains and derivatives by reducing the production of hexanal, and allows the development of specific cultivars for human consumption, classified as food-type (Seibel et al., 2013).

According to the Ministry of Agriculture, Livestock and Supply (MAPA), the number of food-type soybean cultivars registered in Brazil is limited to 15 versus more than 1,500 grain-type cultivars (MAPA, 2019). Thus, the expansion of the food-type soybean market depends on new breeding programs and on the development of new cultivars free of  $L_{ox}$  and including other favorable traits such as high grain-yield potential, protein and mineral contents and presence of bioactive compounds (Freiria et al., 2016; 2018a; 2018b).

The environment contributes significantly to the expression of those traits, with temperature and precipitation—during the flowering and grain-filling stages, mainly—being key climatic variables in the definition of yield, protein percentage and bioactive compounds (Freiria et al., 2016). However, those may interact with genetic factors [genotype × environment interaction (GE)], thereby requiring a discrimination of the responses of each genotype to environmental changes.

Soy grains are also an important food source to reduce deficiencies of Ca, Mg, Cu, Fe and Zn. In this respect, breeding targeted for human nutrition is the key to obtaining new biofortified cultivars (White & Broadley, 2005). Genotypic differences are known to influence mineral accumulation (Vieira et al. 1999; Yamada et al., 2003), but the interaction between this factor and the environment is little investigated.

The study of the GE interaction is essential for breeding programs, as it allows the selection of new, high-yielding cultivars of superior nutritional and functional value. The aim of this study was to examine the yield and chemical composition of grains of food-type soybean genotypes in different environments.

#### **Materials and Methods**

Soybean lines UEL 110, UEL 114, UEL 115, UEL 122 and UEL 123, of the Soy Breeding Program for Human Consumption

of the State University of Londrina (PMSAH/UEL) and cultivar BRS 257, all free of lipoxygenase enzymes in the grains and classified as food-type, were evaluated in the municipalities of Londrina (23°21' S, 51°09' W and 576 m above sea level) and Guarapuava (25°23' S, 52°27' W and 1120 m above sea level), Paraná State, Brazil, in the 2016/2017 crop.

According to the Köppen-Geiger classification, the climate in the municipality of Londrina is a Cfa type, whereas Guarapuava has a Cfb-type climate. Local precipitation and mean temperature data recorded during the trials are given in Figure 1.

The experiments were carried out at two sowing times: the first week of October (sowing time 1) and the second week of November (sowing time 2) of 2016. The soil of Londrina was classified as a *Latossolo Vermelho eutroférrico* (Eutrophic Red Oxisol), and, in Guarapuava, as a *Latossolo Bruno distrófico* (Typic Hapludox), according to the Brazilian Soil Classification System (SiBCS) (Embrapa, 2014). Soybean was sown by a mechanical plot seeder with four rows, in a randomized complete block design with four replicates.

Base fertilization consisted of 250 kg ha<sup>-1</sup> of 00-20-20 N-P<sub>2</sub>0<sub>5</sub>-K<sub>2</sub>0. The seeds were treated with carboxanilide and dimethyldithiocarbamate (Vitavax-Thiram<sup>\*</sup>) at the concentration of 250 mL to 100 kg of seeds and inoculated at the time of sowing with the *Bradyhizobium japonicum* strains SEMIA 5079 and 5080 with  $5.0 \times 10^9$  colony-forming units per milliliter of the commercial product at the dose of 100 mL to 50 kg of seeds. The adopted management system was direct seeding. Plots were formed by four 5-m rows spaced 45 cm apart, with a plant density of 15 plants m<sup>-1</sup>. Phytosanitary control was achieved following the recommendations indicated by Embrapa (2011).

The grains were harvested after the R8 development stage. The two outermost rows as well as 50 cm of each extremity of the central rows (bordering) were disregarded, resulting in a usable area of 3.6 m<sup>2</sup>. Results for grain yield (GY) were expressed in kg ha<sup>-1</sup>, with correction for 13% moisture.

Hundred-grain weight (HGW) was estimated by weighing two subsamples of 100 grains for each field replicate. Results were corrected for 13% moisture and expressed in grams.

Once harvested, the grains were kept in a cold chamber (10 °C) with hygroscopic balance achieved at approximately 8% moisture. Subsequently, the grains were ground through an analytical mill (IKA\* A11) and the protein percentage was determined by the Kjedahl method. The nitrogen percentage was converted to protein by applying the factor 6.25, in accordance with method 920.152 of AOAC (2012). The data were expressed as wet basis (wb).

Mineral composition [calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe) and zinc (Zn) was determined using an atomic absorption spectrophotometer (GBC 932AA<sup>\*</sup>). The data were also expressed as wb.

Total phenolic compounds and flavonoids were extracted by following the methodology of Vázquez et al. (2008). One milliliter of the supernatant was used to determine the phenolic compounds. Gallic acid was used as standard at the



**Figure 1.** Rainfall and temperatures observed from October to March in the 2016/2017 crop in Londrina (A) and Guarapuava (B), Paraná, Brazil. Data obtained from the climatic stations of the Agronomic Institute of Paraná (IAPAR).

concentrations of 0, 10, 20, 30, 40 and 50 mg L<sup>-1</sup>, as described by Swain & Hills (1959). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of samples, in wb.

Flavonoid determination was carried out following the methodology of Woisky & Salatino (1998), with modifications. Two milliliters of the supernatant, 1 mL 10% aluminum chloride (w/v) in methanol and 2 mL methanol were added to the test tubes. For the reaction, the samples were left at rest for 15 min in the dark at 25 °C and then read in a spectrophotometer at 425 nm (Agilent Technologies<sup>®</sup> 8453). Quercetin was used as standard at the concentrations of 0, 50, 100, 200, 300, 400 and 500 mg L<sup>-1</sup>. Results were expressed in mg of quercetin equivalents (QE) per 100 g of sample, in wb.

Individual analyses of variance were performed for each location and sowing time. After homogeneity of variance was verified by the Hartley test, combined analysis of variance was performed and the sources of variation (genotype, location and sowing time) were considered fixed. The genotypes were compared by Tukey's test at the 5% probability level.

In the case of significance for the genotype × environment interaction, the adaptability and stability method proposed by Lin & Binns (1988) was applied, according to the Equation 1.

$$P_{i} = \sum_{i=1}^{n} \frac{\left(X_{ij} - M_{j}\right)^{2}}{2n}$$
(1)

where:  $P_i$  is the estimate of the stability parameter of cultivar *i*;  $X_{ij}$  is the yield of cultivar *i* in environment *j*;  $M_j$  is the maximum response observed among all cultivars in environment *j*; and *n* is the number of environments.

The analyses were performed using Genes statistical software (Cruz, 2016).

#### **Results and Discussion**

Combined analysis of variance revealed a genotype × environment (GE) interaction for grain yield (GY), 100-grain weight (HGW) and Ca content. By decomposing the GE interaction into genotype × location (GL), genotype × time (GT) and genotype × location × time (GLT), a triple interaction was observed for GY and HGW (Table 1).

Zinc showed significant differences for all sources of variation separately (genotypes, location and sowing time).

The total flavonoid, protein and Fe contents were influenced only by cultivation site. Magnesium showed significance only for genotype, whereas total phenolic compounds and Cu were not influenced by the studied sources of variation (Table 1).

In many studies, grain yield, weight and composition were influenced by genetic and/or environmental factors (Freiria et al., 2016; 2018a; 2018b) and the interaction between those factors resulted in differences in the performance of each genotype according to climatic conditions such as temperature, precipitation and photoperiod as well as factors related to type of soil.

The average GY in the state of Paraná in the 2016/17 crop was 3,731.0 kg ha<sup>-1</sup> (CONAB, 2017), which is lower than the 4,104.2 kg ha<sup>-1</sup> obtained in the present study. Overall, GY was higher in Guarapuava than in Londrina, with some exceptions occurring at the first sowing time (early October) such as higher GY in cultivar BRS 257 in Londrina and a lack of significant differences for lines UEL 115 and UEL 123 between the studied municipalities (Table 2).

The cultivation sites were conflicting with regard to type of soil and climate; in the latter case, with changes in precipitation and temperature (Figure 1). With average temperatures of 20.0 and 21.0 °C and precipitation rates of 798.3 and 732.7 mm in the first and second sowing times, respectively, the municipality of Guarapuava had a milder climate than Londrina, whose respective average temperature and precipitation were 23.9 and 24.3 °C and 725.1 and 620.9 mm.

Both locations showed average temperature and precipitation values within the range deemed ideal for the cultivation of soybean. However, Londrina had a higher number of days with maximum temperatures exceeding 30 °C, especially in the R<sub>1</sub> (start of flowering) to R<sub>6</sub> (end of grain-filling) development stages, considered auspicious for greater flower and pod abortion, which are unfavorable for GY (Schauberger et al., 2017). These climatic conditions might have been decisive for the lower yields obtained in that municipality.

Climatic variations between sowing times within the same cultivation site were less disparate. In Londrina, the difference in average temperature between the sowing times did not exceed 1 °C. Precipitation differed by 100 mm, with a higher volume occurring in the sowing performed in early October (time 1). This was also true for Guarapuava, where the difference in water volume was approximately 60 mm less. However, this volume was sufficient for genotypes BRS 257, UEL 115 and UEL 123, which had lower yields when sown in early November (time 2) in Londrina, and line UEL 122, at the same time, when cultivated in Guarapuava (Table 2).

According to Cruz et al. (2012), the magnitude of the GE interaction is specific to each genotype, which is possibly due to a greater or lesser tolerance to environmental changes. For Meotti et al. (2012), differences between sowing times may also be related to the sensitivity of each genotype to the photoperiod.

Cultivar BRS 257, which produced 4,988.7 kg ha<sup>-1</sup> of grains, obtained the highest GY at the first sowing time in the municipality of Londrina, not differing from lines UEL 115 and UEL 123. Having produced 5,127.5 and 5,447.7 kg ha<sup>-1</sup>, lines UEL 110 and UEL 122, respectively, showed to be more productive when sown in early October in Guarapuava, not differing from lines UEL 114 and UEL 115. Line UEL 110 remained in the group of highest-yielding genotypes with the change in sowing time in Guarapuava (Table 2).

Hundred-grain weight values are described in Table 2. The greatest differentiation between cultivation sites occurred when the genotypes were sown in early November, with the highest means being found in Guarapuava for all studied genotypes. At the first sowing time, only cultivar BRS 257 and line UEL 122 exhibited significant differences with the variation in cultivation site, with higher weights obtained also in Guarapuava.

Freiria et al. (2016) found a higher HGW in genotypes UEL 110, UEL 115, UEL 123 and BRS 257 when grown in milder temperature conditions, which is corroborated by the present results shown in Table 2. As stated by Farias et al. (2007),

Variation course	D.F.	F									
		GY	HGW	PC	FLAV	PROT	Са	Mg	Fe	Zn	Cu
(Block/time) /location	12	1.05	0.50	1.30	1.02	0.18	0.60	2.00	0.68	0.54	1.30
Genotype (G)	5	3.10*	14.75**	1.42	0.88	0.12	0.57	4.98**	0.66	3.92**	1.60
Environment	3	37.65**	397.32**	1.75	4.90*	5.03*	45.48**	0.61	4.31*	9.28**	2.72
Location (L)	1	77.39**	902.87**	4.56	11.56**	13.25**	105.74**	0.07	12.82**	5.70*	5.26
Sowing time (T)	1	21.21**	131.91**	0.59	2.33	1.19	21.44**	1.37	0.07	16.99**	0.40
L×T	1	14.34**	157.17**	0.08	0.81	0.65	9.66*	0.33	0.06	5.15	2.51
G × Environment	15	4.32**	23.14**	0.57	0.71	0.24	4.12**	1.36	1.37	1.89	1.35
G×L	5	5.54**	38.34**	0.72	0.34	0.38	5.68**	1.87	1.89	1.38	1.45
G×T	5	3.05*	19.82**	0.80	0.79	0.18	4.76**	1.96	1.32	1.88	0.63
G×L×T	5	4.35**	11.25**	0.21	1.00	0.15	1.86	0.29	0.92	2.40	1.98
Average		4104.15	16.76	125.97	77.59	36.37	2.01	2.20	142.83	29.09	26.31
C.V.(%)		12.05	2.29	20.18	4.08	17.90	15.48	6.59	29.44	13.60	26.09

**Table 1.** F values obtained by the combined variance analysis for 10 characters of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil.

GY: grain yield; HGW: hundred-grain weight; PC: phenolic compounds; FLAV: flavonoids; PROT: protein; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinco; Cu: copper. \*\* and \* Significant at 1 and 5%, respectively, by the F test. **Table 2.** Grain yield e hundred-grain weight of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil.

	Location					
Genotypes	Lond	rina	Guarapuava			
	S. time 1	S. time 2	S. time 1	S. time 2		
	Grain yield (kg ha <sup>-1</sup> )					
BRS 257	4988.7 AaA	2741.8 BbA	3965.1 Ba <i>B</i>	3718.3 AaB		
UEL 110	3811.2 Ba <i>B</i>	3661.8 BaA	5127.5 AaA	5017.5 AaA		
UEL 114	3714.0 Ba <i>B</i>	3513.2 BaA	4491.2 AaAB	4541.3 AaAB		
UEL 115	4377.3 AaAB	3313.6 BbA	4594.4 Aa <i>AB</i>	4869.5 AaA		
UEL 122	3471.4 Ba <i>B</i>	3107.5 BaA	5447.7 AaA	4483.7 AbAB		
UEL 123	4136.9 Aa <i>AB</i>	2957.4 BbA	3980.0 Aa <i>B</i>	4468.6 Aa <i>AB</i>		
	Hundred-grain weight (g)					
BRS 257	16.8 BaA	14.2 BbC	17.8 Aa <i>B</i>	17.2 Ab <i>CD</i>		
UEL 110	16.8 AaA	15.6 Bb <i>B</i>	16.8 Aa <i>CD</i>	16.5 Aa <i>D</i>		
UEL 114	17.3 AaA	15.1 Bb <i>B</i>	17.6 Aa <i>B</i>	17.8 Aa <i>BC</i>		
UEL 115	15.7 Ab <i>B</i>	16.5 BaA	16.2 Ab <i>D</i>	17.2 Aa <i>CD</i>		
UEL 122	15.9 Ba <i>B</i>	14.9 Bb <i>BC</i>	19.8 AaA	18.5 AbAB		
UEL 123	17.1 AaA	15.3 Bb <i>B</i>	17.3 Ab <i>BC</i>	18.6 AaA		

Means followed by the same letter (capital letters for sowing time comparison between locations, small for sowing time comparison within each location and italics for genotype comparison within each sowing time and location) do not differ at 5% significance by the F, F and Tukey test, respectively.

average temperatures lower than 22 <sup>o</sup>C, as observed in the municipality of Guarapuava, tend to allow for better grain development.

With the variation in sowing time within the same cultivation site, in the presence of significant differences, the highest HGW were obtained for the first sowing time, except for line UEL 115, which, regardless of the location, exhibited higher values in the second sowing. The environment greatly contributed to HGW, which prevented the identification of genotypes with higher values, irrespective of location and sowing time.

Total phenolic compounds had an overall mean of 126.0 mg GAE 100 g<sup>-1</sup>. With an average of 78.9 mg QE 100 g<sup>-1</sup> for total flavonoids and 37.6% for proteins, Londrina produced grains with higher concentrations of those bio-compounds, compared to Guarapuava (Figures 2a and 2b, respectively). A caveat should be mentioned, however: when the protein percentages were transformed into kilograms per hectare, by multiplying the values by GY, an accumulation per area of 1,604.2 kg of protein ha<sup>-1</sup> was obtained in Guarapuava, exceeding that obtained in Londrina by 233.4 kg ha<sup>-1</sup>.

The phenolic compound and total flavonoid contents and the protein percentages are within the range reported by Vernetti & Vernetti Junior (2017) for soybean. In a breeding program for food-type grains, high percentages of proteins and bioactive compounds, such as flavonoids, are desirable and provide functional characteristics to their products and byproducts, e.g. antioxidant activity and prevention of vascular disease and some types of cancer.

The main responses to differences in the flavonoid content in the grain, with the change in cultivation site, are associated with the temperature. According to Wu et al. (2016), higher temperatures increased the activity of phenylalanine



**Figure 2.** Total flavonoids (A) and protein (B) of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil. Means followed by the same letter do not differ at 5% significance by the F test.

ammonia-lyase, a key enzyme in the production of phenolic compounds and flavonoids, which resulted in an increase in its contents in different sorghum genotypes. Ziegler et al. (2016) found higher flavonoid contents in soybean grains when these were exposed to higher temperatures. High incidence of ultraviolet light (Karppinen et al., 2016) and water deficit (Wu et al., 2016) were additional environmental factors that provided a greater accumulation of this bioactive compound.

Temperature is also the climatic variable most highly correlated with the protein percentage in soybean grains, especially during the grain-fill period. Freiria et al. (2016; 2018b) found higher protein percentages in higher temperature conditions when these were accompanied by lower precipitation rates.

High maximum temperatures—around 35 °C—may favor the production of heat-stress defense proteins, to allow an adequate cell function, and consequently increase their percentages (Taiz & Zeiger, 2009). Other factors, such as N uptake, are essential for protein synthesis in soybean. However, inoculation with selected strains of *Bradyhizobium* spp. showed to be efficient in supplying the N required by the crop (Embrapa, 2011).

Additionally, with a Pearson's correlation coefficient of -53% with GY, when the environmental factors are not sufficient to explain the lower GY percentages, these may be related to a higher GY per area, as reported by Albrecht et al. (2008).

Calcium concentrations in the grain ranged from 1.4 to 2.6 g kg<sup>-1</sup> (Table 3). These values are close to the 1.7 to 3.1 and 1.5 to 4.5 g kg<sup>-1</sup> obtained by Vieira et al. (1999) and Moreira et al. (2016), respectively, with a significant participation of genotype. Higher Ca concentrations were obtained in Londrina, with the exception of lines UEL 114 and UEL 122, which showed no significant differences between the cultivation sites.

The genetic factor greatly contributed to the concentration of Mg in the grains. With an average concentration of 2.3 g kg<sup>-1</sup>, the group formed by cultivar BRS 257 and lines UEL 122 and UEL 123 had the higher accumulations of Mg, differing from the UEL 114 line (Figure 3). The Mg concentration was within the range reported by Magalhães et al. (2015) and Moreira et al. (2016).

The average Fe content of 157.5 mg kg<sup>-1</sup> in the grains from Guarapuava exceeded that obtained in Londrina by 29.2 mg kg<sup>-1</sup> (Figure 4a). Yamada et al. (2003) and Moreira et al. (2016) studied the Fe concentration in different soybean genotypes and found values ranging from 84.5 to 110.3 and 48.6 to 85.8 mg kg<sup>-1</sup>, respectively, which is a lower range than that found in the present study. Yamada et al. (2003) reported a higher bioavailability of this mineral when compared to other plant products such as wheat, maize, common bean and rice.

**Table 3.** Calcium mineral content (g kg<sup>-1</sup>) in grains of six foodtype soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil.

Construnce	Lo	cation	Sowing times		
Genotypes	Londrina Guarapuava		Time 1	Time 2	
BRS 257	2.6 Aab	1.4 Bb	2.0 Aab	2.0 Aa	
UEL 110	2.6 Aa	1.6 Bab	2.2 Aab	2.0 Aa	
UEL 114	2.1 Ab	1.8 Aab	1.8 Ab	2.0 Aa	
UEL 115	2.3 Aab	1.7 Bab	2.3 Aab	1.7 Ba	
UEL 122	2.1 Ab	2.0 Aa	2.5 Aa	1.6 Ba	
UEL 123	2.3 Aab	1.8 Bab	2.1 Aab	1.9 Aa	

Means followed by the same letter (capital letters on the line and small on the column) do not differ at 5% significance by the F and Tukey test, respectively.



**Figure 3.** Magnesium mineral content (g kg<sup>-1</sup>) in grains of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil. Means followed by the same letter do not differ at 5% significance by the Tukey test.



А

**Figure 4.** Iron (A) and Zinc (B,C e D) mineral content (g kg<sup>-1</sup>) in grains of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil. Means followed by the same letter do not differ at 5% significance by the F test (figures A, B e C) and Tukey (Figure D).

For Zn, all sources of variation played an important role in its accumulation in the grain, but did not show interactions with each other. Between the locations, the highest Zn concentrations were found in Londrina, with better performance observed at the first sowing time. The highest Zn contents were found in lines UEL 110 and UEL 114 (average: 31.4 mg kg<sup>-1</sup>). However, they differed significantly only from cultivar BRS 257 (25.6 mg kg<sup>-1</sup> of Zn) (Figure 4b, c and d). Magalhães et al. (2015) reported that the Zn concentration in the grain is influenced by the genotype, with contents varying between 24.0 and 30.9 mg kg<sup>-1</sup>, which is corroborated by the current results.

Overall, the highest Ca and Zn concentrations in the grains of food-type soybean genotypes were found in the municipality of Londrina. Climatic factors such as temperature, solar radiation and precipitation can influence the accumulation of those nutrients in the plant. In a study led by Samarah et al. (2004), water-restricted environments provided higher Ca and Zn concentrations in soybean grains. However, in the present study, despite having a lower water volume than Guarapuava, the amount and distribution of rainfall in Londrina was not restrictive.

Nobile et al. (2016) studied the effect of climatic factors on the mineral concentration of soybean grains in different regions of Argentina and reported that air temperature was the factor of highest correlation with the Ca contents in higher maximum temperature conditions (above 30.0 °C overall). When this climatic variable was not sufficient to explain the mineral concentrations, the soil pH was the most indicative attribute, with lower values resulting in lower Ca concentrations.

Ribas (2010) reported that the Typic Hapludox soil in the municipality of Guarapuava is naturally acidic and chemically poor, as a consequence of the sum of weathering and climatic factors such as higher precipitation and lower temperature, which can further contribute to the lower concentration of those minerals in the grain. However, adequate Fe availability was noted due to the presence of Fe oxides at concentrations higher than those observed.

An aspect not yet fully elucidated in the literature is the correlation between yield and mineral concentrations, in soybean. The negative Pearson's correlation coefficient of medium magnitude with Ca (both -47%) indicated, as already mentioned for the protein percentages, that when the environmental factors (climate and soil type) are not sufficient to explain the differences between locations, these may be related to a higher or lower GY per area, due to a possible dilution effect.

The presence of a GE interaction for GY, HGW and Ca showed to be a complicating factor for the selection of superior genotypes, in the set of test environments. The GE interaction may result in a lack of correlation between measurements of the same cultivar in distinct environments, which results in an inconsistency of its superiority with the environmental variations. Under such conditions, biometric tools must be used that control or lessen its effects, such as the study of adaptability and stability (Cruz et al., 2012).

The estimate of the  $P_i$  parameter suggested by Lin & Binns (1988) (Table 4) revealed that the lowest GY values were obtained by lines UEL 115 and UEL 110. Lower  $P_i$  values indicate genotypes of greater adaptability and with a high overall mean for the trait of interest, as mentioned by Freiria et al. (2018a). Lines UEL 110 and UEL 115 were among the

**Table 4.** Percentage estimate of the Lin & Binns Pi stability variable (1988) for the characteristics grain yield (GY), hyndred-grain weight (HGW) and calcium (Ca) of six food-type soybean genotypes in two sowing times in the 2016/2017 crop in the municipalities of Londrina and Guarapuava, State of Paraná, Brazil.

Ganaturas	GY	HGW	Са			
Genotypes	(%)					
BRS 257	28.2 (6)	18.1 (4)	24.7 (6)			
UEL 110	8.9 (2)	23.4 (5)	8.3 (1)			
UEL 114	16.6 (3)	11.8 (2)	21.6 (4)			
UEL 115	7.4 (1)	27.4 (6)	10.1 (2)			
UEL 122	17.2 (4)	6.9 (1)	24.5 (5)			
UEL 123	21.9 (5)	12.5 (3)	10.8 (3)			

Value in parentheses indicates the ranking of genotypes from the smallest to the highest Pi value.

genotypes with the lowest  $P_i$  for Ca accumulation. Cultivar BRS 257 showed instability for GY (lower P<sub>i</sub> value), HGW and Ca.

In view of the results attained with the adaptability and stability methodology proposed by Lin & Binns (1988) and those above-described, lines UEL 110 and UEL 115 possess high grain yield capacity and a balanced chemical composition, which are essential traits for a new cultivar of food-type soybean. However, key traits such as grain yield should be evaluated in a higher number of environments to increase the accuracy of the obtained information.

## Conclusions

Grain yield and 100-grain weight are influenced by cultivation site and sowing time, with the best responses achieved in mild maximum temperature conditions during the flowering and grain-filling stages.

High maximum temperatures allow, direct or indirectly, for increased grain yield and greater accumulation of proteins, flavonoids, Ca and Zn in the grains of food-type soybean genotypes.

The significance of the genotype × environment interaction for grain yield, 100-grain weight and calcium results in genotypic superiority inconsistent with environmental variation. Lines UEL 110 and UEL 115 possess high grain yield capacity and higher Ca, Mg and Zn contents.

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