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Nutritional prediction of corn grains: integration of genotypes and meteorological variables for swine production

Predição Nutricional de Grãos de Milho: Integração de Genótipos e Dados Meteorológicos para Suinocultura

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Abstract: In this study, the aim was to evaluate whether there is variability in the protein nutritional composition of grains between maize genetic bases (simple hybrids, triple hybrids, double hybrids and varieties) and sowing dates, and predict digestible amino acids for pigs based on crude protein and meteorological variables. 773 grain samples from four maize genetic bases cultivated on ten sowing dates were evaluated using Near Infrared Reflectance Spectroscopy. Simple linear regressions were performed for protein nutritional traits in different genetic backgrounds and sowing dates. Principal component analysis was used to group data on the protein nutritional composition of grains and meteorological variables, by genetic bases and sowing dates. There is variation in the digestible levels of the eleven amino acids in grains between maize genetic bases and sowing dates. Maize varieties have the highest digestible levels of eleven amino acids in the grains, compared to simple hybrids, triple hybrids and double hybrids, regardless of the sowing date. Sowings carried out in October and November show higher digestible levels of the eleven amino acids in maize grains, in relation to sowings in the months of September, December, January and February, regardless of the genetic basis. The digestible contents of methionine, cystine, threonine, valine, isoleucine, leucine, phenylalanine, histidine and arginine in maize kernels can be predicted from crude protein with high accuracy, on all genetic bases.

Key-words: animal feed; digestible amino acids; nutritional profile; crude protein; *Zea mays*.

Resumo: Este estudo teve por objetivo avaliar se há variabilidade da composição nutricional proteica de grãos entre as bases genéticas de milho (híbridos simples, híbridos triplo, híbridos duplo e variedades de polinização aberta) e as datas de semeadura, e predizer os aminoácidos digestíveis para suínos com base na proteína bruta e nas variáveis meteorológicas. Foram avaliadas, por meio de Espectroscopia de Refletância no Infravermelho Próximo, 773 amostras de grãos provenientes de quatro bases genéticas de milho cultivadas em dez datas de semeadura. Foram realizadas regressões lineares simples para os caracteres nutricionais proteicos em diferentes bases genéticas e datas de semeadura. Foi utilizada a análise de componentes principais para agrupar dados da composição nutricional proteica de grãos e variáveis meteorológicas, por bases genéticas e por datas de semeadura. Há variação dos teores digestíveis dos onze aminoácidos nos grãos entre as bases genéticas de milho e as datas de semeadura. As variedades de polinização aberta de milho apresentam os maiores teores digestíveis dos onze aminoácidos nos grãos, em comparação aos híbridos simples, híbridos triplo e híbridos duplo, independentemente da data de semeadura. Semeaduras realizadas em outubro e novembro exibem maiores teores digestíveis dos onze aminoácidos nos grãos de milho, em relação às semeaduras nos meses de setembro, dezembro, janeiro e fevereiro, independentemente da base genética. Os teores digestíveis de metionina, cistina, treonina, valina, isoleucina, leucina, fenilalanina, histidina e arginina nos grãos de milho podem ser preditos a partir da proteína bruta com alta precisão, em todas as bases genéticas.

Palavras-chave: alimentação animal; aminoácidos digestíveis; perfil nutricional; proteína bruta; *Zea mays*.

1. Introduction

Maize is one of the main ingredients used in swine feed due to its high starch content, which provides easily digestible energy, and low levels of anti-nutritional factors (1,2). Although it has low contents of crude protein and amino acids compared to vegetable protein sources, such as soybean (3), its inclusion in large quantities in swine diets allows small increments in these components to result in significant nutritional contributions. Adequate protein and amino acid supply in swine diets promotes efficient growth, and excess can intensify environmental contamination through nitrogen leaching due to increased excretion by the animal, water contamination and emission of volatile nitrogen (4, 5).

The protein and digestible amino acid contents in maize grains are lower than the total amino acid contents, so it is necessary to determine them to meet the daily requirements of pigs in a balanced way. Predictive methods such as linear regression analysis based on protein content have been used to estimate the contents of these components in the ingredients. These predictive models were developed to predict the total amino acids contents in wheat (6), maize (3, 6), and soybean (3), the amino acid digestibility coefficient in maize (7), and the digestible amino acid contents in maize (8) and sorghum (9). The results of these studies highlight the importance of expanding the representativeness of the samples, considering different combinations of genetic bases and sowing dates.

However, there are no records of research evaluating the levels of protein and digestible amino acids in maize grains from genetic bases (single, triple, double hybrids and open-pollinated varieties) under different meteorological conditions. Although the influence of factors such as oil content (10), endosperm type (2), geographic origin (11, 8), genotype (12) and industrial processes, such as extrusion (13), on the synthesis of these compounds is already known, there is still a gap regarding the interaction between genetic bases and meteorological conditions.

Research indicates that low rainfall volumes promote increased protein content in maize grains (14-16), while positive relationships have been observed between air temperature and protein content in grains (17, 14). Predictive models corroborate this relationship, showing positive associations between crude protein and cumulative temperature before anthesis ($R^2 = 0.31$) and average daily temperature after anthesis ($R^2 = 0.83$) (18), as well as between total amino acid contents and minimum and maximum temperature and global solar radiation (19). These results reinforce the importance of aligning the development stage of maize with environmental conditions, in order to optimize the nutritional quality of grains.

Detailed knowledge of the nutritional composition of maize, considering its genetic origin and cultivation environment, can maximize its use in feed formulation. This contributes to adequate and environmentally sustainable feed, reducing nutrient excretion in the pig production system. In this study, the aim was to evaluate whether there is variability in the protein nutritional composition of grains between maize genetic bases (single hybrids, triple hybrids, double hybrids and varieties) and sowing dates, and predict digestible amino acids for pigs based on crude protein and meteorological variables.

2. Material and methods

The experiments were carried out in the area of the Plant Science Department of the Federal University of Santa Maria, located at 29º42'S, 53º49'W, at 95 m altitude. According to Köppen's classification, the climate of the region is classified as humid subtropical Cfa, with hot summers and no defined dry season (20). The soil of the region is classified as Argissolo vermelho distrófico arênico (Ultisol) (21).

In the 2021/2022 season, maize genotypes were evaluated considering five sowing dates: September 21, 2021, October 20, 2021, November 20, 2021, December 20, 2021 and January 30, 2022. Except for sowing on September 21, 2021, which was composed of 71 genotypes, on all other dates, 78 maize genotypes of different genetic bases (single hybrids, triple hybrids, double hybrids and varieties) were sown. In the 2022/2023 season, the same 78 maize genotypes were evaluated considering five sowing dates: September 6, 2022, October 14, 2022, November 24, 2022, December 30, 2022 and February 6, 2023. In this study, maize genotypes were replicated on ten sowing dates. Descriptive statistics, principal component analysis, correlation, and simple and multiple linear regression were performed based on these replicates. The experiments were conducted without the use of supplemental irrigation.

On each sowing date the maize genotypes were sown in one-row plots, side by side. Each plot consisted of one row of 5 m in length at spacing of 0.80 m between rows and 0.20 m between plants in the row, totaling 4 m^2. Density was adjusted by thinning to 62,500 plants ha^{-1} (25 plants per plot). Borders were made on the sides and ends of the blocks, with maize plants. The area was harrowed and fertilized with 415 kg ha^{-1} of chemical fertilizer with the formula (NPK) 05-20-20. Nitrogen fertilization with urea (N - 46%) was split, with the first application of 250 kg ha^{-1} at the V4 stage and the second application of 150 kg ha^{-1} at the V6 stage of the crop. The other crop management practices, such as weed, pest and disease control, were carried out according to the technical indications for the maize crop, homogeneously for all genotypes (22).

At the point of harvesting the maize plants, when the husk and leaf at the base of the ear were 100% senescent, all ears were harvested individually for each genotype and on each sowing date. Then, the ears were threshed, and a 100 g grain sample was taken from each genotype × sowing date combination, totaling 773 samples. The samples were placed in paper bags and taken to the greenhouse with forced air circulation until they reached 13% moisture content. Subsequently, the grains were ground in a Retsch Mill (model ZM 200) coupled with a 1-mm-mesh sieve to obtain samples. Then, ground samples were scanned using Near Infrared Reflectance Spectroscopy in a FOSS NIRSTM DS2500 analyzer belonging to the company Adisseo Brasil Nutrição Animal. With the absorbance spectra generated for each sample, the predictions of nutritional contents were performed using multivariate calibration for ground maize on the Precision Nutrition Evaluation (PNE) platform. Thus, for each sample, the crude protein (CP, g/100 g) content and the contents of the following digestible amino acids for pigs were estimated: lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g).

Meteorological variables were obtained at the National Institute of Meteorology (INMET) station, located 100 meters away from the experimental area. For each day, the hourly global solar radiation, in MJ m^{-2} $hour^{-1}$, the hourly maximum and minimum temperatures, in ºC, and precipitation in mm, were obtained for the period between sowing and the point of harvest of the genotypes, referring to each sowing date. For each day, the daily global solar radiation, in MJ m^{-2} day^{-1}, was calculated by adding the hourly global solar radiation, in MJ m^{-2} $hour^{-1}$. Then, for each genotype, considering each sowing date, the daily global solar radiation was used to calculate the cumulative global solar radiation in the subperiods: sowing to female flowering (RSFF, MJ m^{-2}) and female flowering to harvest (RFFH, MJ m^{-2}).

For each day, the 24 values of hourly average temperature were calculated from the hourly maximum (Tmax) and minimum (Tmin) temperatures. Next, the average daily air temperature (Tavg), in °C, was calculated based on the average of the 24 values of average hourly temperature. Afterwards, the degree days (DD, in °C) were calculated using the method proposed by Arnold(23) by the expression: DD = Tavg - Tb, where: Tavg = average daily air temperature, in ºC, and Tb = lower base temperature (10 ºC). Tb is the temperature below which plant development does not occur and, if any development does occur, it will occur at a significantly reduced rate. When Tavg ≤ Tb, DD was considered to be equal to zero. For each genotype, considering each sowing date, the thermal sum was obtained by adding the DD values of each of the following subperiods: sowing to female flowering (SSFF, °C day) and female flowering to harvest (SFFH, °C day). For each genotype, considering each sowing date, the cumulative precipitation was calculated in each of the following subperiods: sowing to female flowering (PSFF, mm) and female flowering to harvest (PFFH, mm).

Density and bar graphs were created for the meteorological variables considering each sowing date, in order to characterize the meteorological conditions in each environment. For each protein nutritional trait, considering each sowing date, the following statistics were calculated: minimum, mean, maximum and coefficient of variation. Then, for each trait, considering each sowing date and genetic base, the mean and coefficient of variation were calculated and a Boxplot was constructed to represent the distribution of protein nutritional traits. Means of the traits between the sowing dates, regardless of the genetic bases, and between the genetic bases considering each sowing date were compared using Student's t-test at 5% significance level. Principal component analysis was applied to group the data by genetic bases and by sowing dates as a function of protein nutritional traits and meteorological variables. For each maize genetic base, Pearson's linear correlation coefficients were calculated between the pairs of traits, with the significance of the coefficients verified by Student's t-test at 5% significance level. For each maize genetic base, simple linear regressions ($y = a + bx$) of Lys (y), Met (y), Cys (y), Thr (y), Trp (y), Val (y), Ile (y), Leu (y), Phe (y), His (y) and Arg (y) were performed as a function of CP (x).

For each genetic base, the parameters of the stepwise multiple linear regression model with backward selection and the adjusted coefficient of determination (R^2) were estimated, considering CP and digestible amino acids as the dependent variable and the other variables (RSFF, RFFH, SSFF, SFFH, PSFF, PFFH) as independent. The coefficient of determination of the leave-one-out cross-validation (R^2 cv) was calculated, which generates the model with n-1 observations and evaluates it using the remaining observation. This procedure was repeated n times, excluding a different observation at each moment. All statistical analyses were performed using R software (24).

3. Results

3.1 Variability of protein nutritional traits

There was a greater occurrence of high values of global solar radiation, average air temperature and degree days in the vegetative and reproductive stages for the sowings in September, October and November of the two crop seasons (Figure 1). In general, there was a greater cumulative precipitation for the sowings of the 2021/2022 season compared to the sowings of the 2022/2023 season. Sowings carried out in December, January and February of the two harvests had a lower frequency of high daily values of these variables. Sowings in September, December and January 2021/2022 and in December 2022/2023 had cumulative precipitation greater than 200 mm in the vegetative and reproductive stages. The lowest accumulations (< 200 mm) occurred for sowings in September and November of the 2022/2023 season.

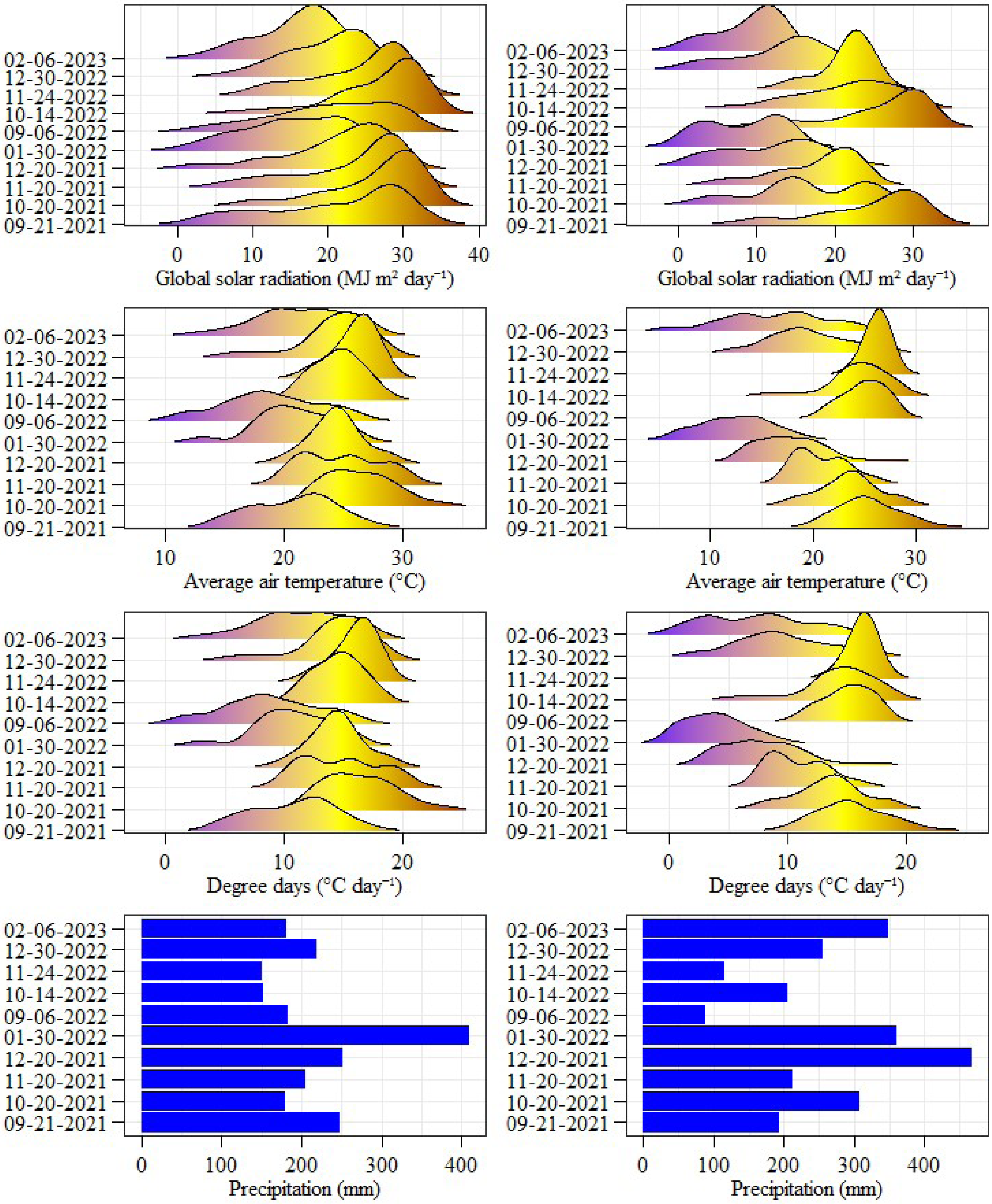

Figure 1. Meteorological conditions from sowing to female flowering and from female flowering to harvest of maize genotypes grown with ten sowing dates.

The contents of CP and digestible amino acids showed variations, with the greatest variability observed in Leu and Phe, whose coefficients of variation were higher than 13.055% (Table 1). The decreasing sequence of variability was: Leu, Phe, Ile, Thr, Val, Trp, CP, His, Cys, Arg, Lys and Met, with CV values ranging from 15.446% to 8.560%. Sowings carried out on September 21, 2021, January 30, 2021, and February 6, 2023 were associated with the highest variations in CP and digestible amino acid contents, highlighting the influence of cultivation conditions on these nutritional traits.

There was a significant difference in the average contents of protein nutritional traits between the sowing dates (Table 1 and Figure 2). Sowing carried out on November 24, 2022 led to the highest means of CP and digestible amino acid contents, with a gradual reduction towards the first (September 6) and fifth (February 6) sowing dates. In the 2021/2022 season, the sowing carried out on October 20 stood out with the highest means of CP and digestible amino acid contents, surpassing the overall mean of the ten sowing dates. In the 2022/2023 season, exception for the sowing on February 6, all others led to average CP and digestible amino acid contents higher than the overall mean.

In the 2021/2022 season, single hybrids, triple hybrids, double hybrids and open pollinated varieties (OPV) showed higher average contents of all traits when sown on October 20, with a performance above the overall mean (Table 2 and 3 and Figures 3 and 4). On average, single hybrids, triple hybrids, double hybrids and open pollinated varieties (OPV) showed higher contents of all traits in the 2022/2023 season. In this season, all genetic bases showed the highest means of the contents of the twelve traits with sowing carried out on November 24, with a gradual reduction towards the sowing carried out on September 6 and February 6.

Table 1. Minimum (Min), mean (Mean) and maximum (Max) contents and coefficient of variation (CV, %) of protein nutritional traits: crude protein (CP), lysine (Lys), methionine (Met), cystine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg), of single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons.

Dates*	CP (g/100 g)				Lys (g/100 g)				Met (g/100 g)			
	Min	Mean	Max	CV	Min	Mean	Max	CV	Min	Mean	Max	CV
09/21/2021	6.810	8.244e	10.290	10.076	0.170	0.191ef	0.230	6.800	0.120	0.139d	0.160	6.616
10/20/2021	7.550	9.572b	11.570	8.285	0.190	0.214b	0.240	4.789	0.130	0.153b	0.170	6.063
11/20/2021	7.200	8.705d	10.760	7.966	0.170	0.200d	0.220	5.439	0.130	0.142c	0.170	5.678
12/20/2021	7.190	8.858d	10.660	7.861	0.170	0.194e	0.220	6.292	0.120	0.143c	0.160	5.743
01/30/2022	7.370	8.762d	10.650	8.987	0.160	0.191ef	0.230	7.565	0.130	0.139d	0.160	5.529
09/06/2022	7.800	9.584b	11.870	7.692	0.160	0.202d	0.230	8.244	0.140	0.159a	0.190	8.052
10/14/2022	7.710	9.616b	11.930	7.785	0.200	0.227a	0.260	5.305	0.130	0.153b	0.180	5.750
11/24/2022	8.340	9.975a	11.670	7.320	0.200	0.226a	0.250	4.981	0.140	0.156a	0.170	5.005
12/30/2022	7.300	9.149c	11.440	8.027	0.190	0.211c	0.250	6.072	0.130	0.145c	0.160	5.187
02/06/2023	6.340	7.967f	10.650	9.563	0.140	0.188f	0.230	7.531	0.110	0.131e	0.190	8.953
Overall	6.340	9.051	11.930	10.695	0.140	0.205	0.260	9.152	0.110	0.146	0.190	8.560

Dates	Cys (g/100 g)				Thr (g/100 g)				Trp (g/100 g)			
	Min	Mean	Max	CV	Min	Mean	Max	CV	Min	Mean	Max	CV
09/21/2021	0.130	0.157e	0.190	8.569	0.170	0.218e	0.280	11.951	0.050	0.055d	0.070	9.644
10/20/2021	0.150	0.179c	0.210	7.064	0.190	0.253bc	0.300	8.550	0.050	0.060bc	0.070	7.812
11/20/2021	0.140	0.165d	0.200	6.615	0.190	0.230d	0.280	8.192	0.050	0.056d	0.070	9.687
12/20/2021	0.140	0.164d	0.190	6.612	0.180	0.231d	0.290	8.663	0.050	0.056d	0.070	9.180
01/30/2022	0.140	0.163d	0.200	8.060	0.170	0.220e	0.270	10.138	0.050	0.056d	0.070	10.626
09/06/2022	0.150	0.175c	0.200	6.796	0.190	0.248c	0.290	8.434	0.050	0.062b	0.080	7.443
10/14/2022	0.150	0.187b	0.220	6.902	0.210	0.269a	0.330	7.609	0.050	0.062bc	0.080	9.471
11/24/2022	0.160	0.192a	0.220	6.562	0.230	0.275a	0.320	7.068	0.050	0.063a	0.070	8.356
12/30/2022	0.150	0.176c	0.210	6.649	0.210	0.255b	0.300	7.346	0.050	0.060c	0.090	9.515
02/06/2023	0.130	0.152f	0.190	8.645	0.150	0.210f	0.270	11.180	0.040	0.052e	0.070	9.572
Overall	0.130	0.171	0.220	10.173	0.150	0.241	0.330	12.429	0.040	0.058	0.090	10.907

Dates	Val (g/100 g)				Ile (g/100 g)				Leu (g/100 g)			
	Min	Mean	Max	CV	Min	Mean	Max	CV	Min	Mean	Max	CV
09/21/2021	0.280	0.345e	0.430	10.340	0.190	0.237e	0.310	12.484	0.600	0.841e	1.180	15.814
10/20/2021	0.310	0.403c	0.490	8.702	0.210	0.281c	0.360	10.130	0.700	1.030bc	1.360	12.109
11/20/2021	0.310	0.367d	0.460	7.747	0.200	0.251d	0.320	9.328	0.690	0.908d	1.250	12.121
12/20/2021	0.300	0.370d	0.450	7.919	0.200	0.257d	0.320	9.626	0.680	0.940d	1.250	11.918
01/30/2022	0.310	0.365d	0.450	8.917	0.210	0.257d	0.330	10.951	0.720	0.932d	1.210	12.585
09/06/2022	0.320	0.396c	0.470	7.318	0.220	0.280c	0.350	8.531	0.760	1.046b	1.390	11.153
10/14/2022	0.320	0.421b	0.520	8.269	0.220	0.291b	0.380	9.375	0.770	1.066b	1.430	10.746
11/24/2022	0.350	0.435a	0.520	7.732	0.230	0.303a	0.370	9.124	0.820	1.121a	1.420	10.348
12/30/2022	0.320	0.397c	0.500	7.986	0.210	0.274c	0.360	9.370	0.740	1.007c	1.230	10.707
02/06/2023	0.270	0.335e	0.440	10.111	0.180	0.232e	0.330	11.814	0.540	0.816e	1.190	14.962
Overall	0.270	0.384	0.520	11.600	0.180	0.267	0.380	12.918	0.540	0.972	1.430	15.446

Dates	Phe (g/100 g)				His (g/100 g)				Arg (g/100 g)			
	Min	Mean	Max	CV	Min	Mean	Max	CV	Min	Mean	Max	CV
09/21/2021	0.280	0.355e	0.470	12.812	0.170	0.201e	0.240	8.796	0.310	0.350g	0.410	6.981
10/20/2021	0.320	0.427c	0.540	10.325	0.180	0.230b	0.270	7.579	0.330	0.397b	0.440	5.938
11/20/2021	0.300	0.380d	0.500	9.902	0.180	0.212d	0.250	6.535	0.310	0.367e	0.420	5.859
12/20/2021	0.290	0.388d	0.490	10.002	0.180	0.213d	0.250	6.816	0.300	0.361f	0.410	6.095
01/30/2022	0.320	0.388d	0.490	10.954	0.180	0.206e	0.240	7.467	0.290	0.346g	0.410	7.634
09/06/2022	0.330	0.427c	0.540	9.066	0.190	0.226b	0.260	6.237	0.340	0.386c	0.430	5.125
10/14/2022	0.320	0.443b	0.580	9.915	0.190	0.236a	0.280	7.212	0.340	0.408a	0.480	6.411
11/24/2022	0.360	0.462a	0.570	9.252	0.200	0.241a	0.270	6.199	0.370	0.413a	0.460	5.496
12/30/2022	0.320	0.418c	0.530	9.424	0.190	0.221c	0.260	6.943	0.320	0.378d	0.430	6.431
02/06/2023	0.270	0.349e	0.490	12.686	0.150	0.187f	0.230	9.026	0.270	0.328h	0.400	7.467
Overall	0.270	0.404	0.580	13.505	0.150	0.217	0.280	10.224	0.270	0.374	0.480	9.525

*71 samples (09/21/2021); 78 samples (10/20/2021, 11/20/2021, 12/20/2021, 01/30/2022, 09/06/2022, 10/14/2022, 11/24/2022, 12/30/2022 and 02/06/2023); 773 samples (Overall). Means not followed by the same lowercase letter in the column differ by the Student's t-test at 5% significance level. Protein traits: crude protein (CP), lysine (Lys), methionine (Met), cystine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg).

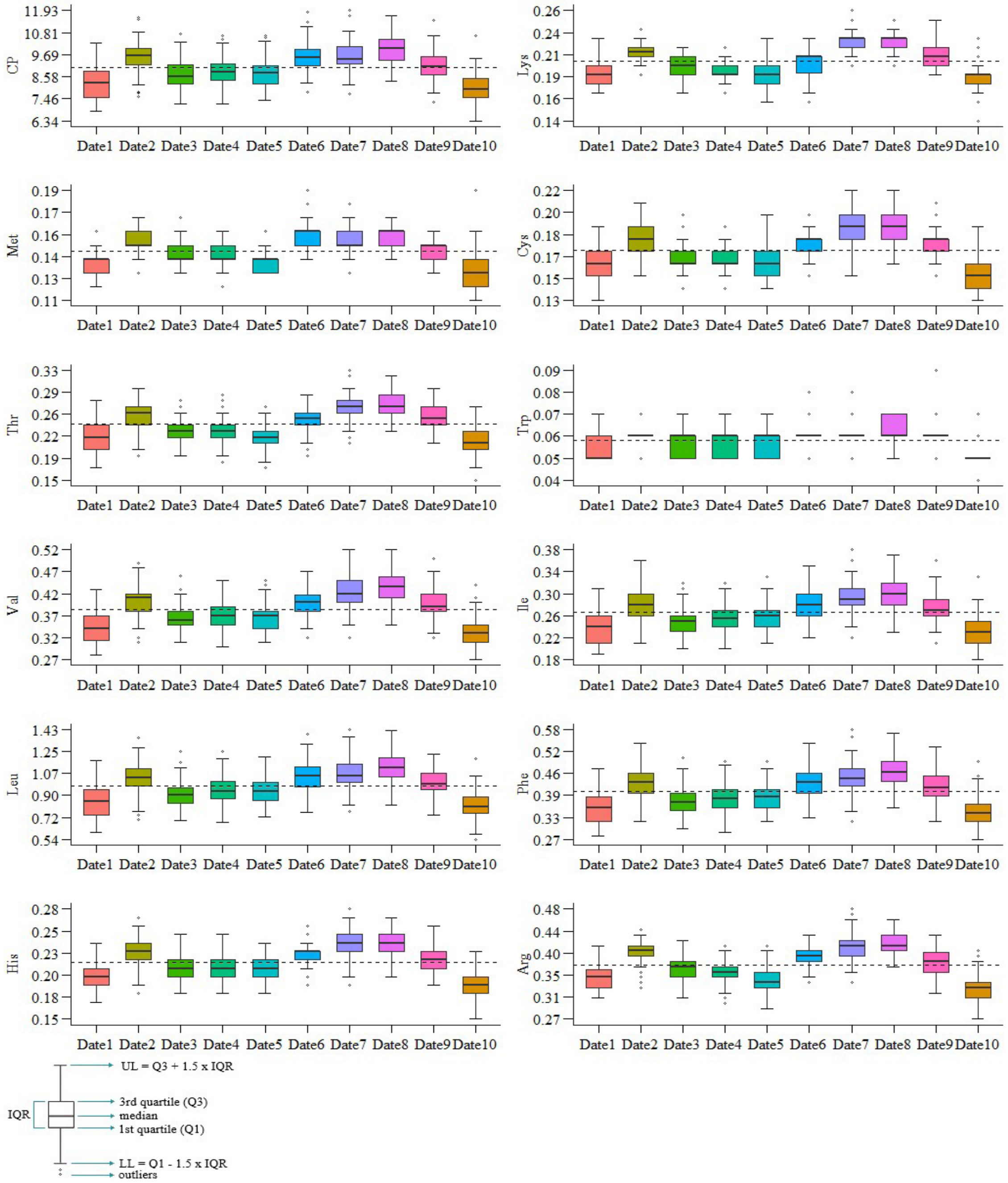

Figure 2. Boxplot representing the distribution of the data of the protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g), referring to ten sowing dates in the 2021/2022 and 2022/2023 seasons. Date1: 09/21/2021; Date2: 10/20/2021; Date3: 11/20/2021; Date4: 12/20/2021; Date5: 01/30/2022; Date6: 09/06/2022; Date7: 10/14/2022; Date8: 11/24/2022; Date9: 12/30/2022; Date10: 02/06/2023. UL: upper limit; LL: lower limit; and IQR: interquartile range. Dotted line represents the overall mean of each trait.

Table 2. Average contents of protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g), of single hybrids (SH), triple hybrids (TH), double hybrids (DH) and open pollinated varieties (VA) sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons.

Dates	GB	CP	Lys	Met	Cys	Thr	Trp	Val	Ile	Leu	Phe	His	Arg
	SH	8.023b	0.188b	0.137b	0.154b	0.211b	0.054b	0.335b	0.229b	0.807b	0.343b	0.197b	0.343b
	TH	8.166b	0.191b	0.136b	0.157b	0.216b		0.341b	0.233b	0.826b	0.350b	0.199b	0.347b
	DH				0.170a			0.377a	0.263a			0.217a	
	VA	9.414a	0.207a	0.152a	0.175a	0.252a	0.060a	0.395a	0.276a	1.021a	0.420a	0.226a	0.386a
	SH	9.487a	0.214a			0.250a		0.398a	0.277a		0.423a		0.394a
	TH	9.512a	0.214a	0.151b	0.177b	0.253a	0.060b	0.401a	0.279a	1.019b	0.422a	0.229b	0.397a
	DH	9.797a	0.212a	0.158a		0.260a	0.062a	0.415a	0.290a		0.441a		0.403a
	VA	9.954a	0.219a	0.159a	0.185a	0.265a	0.062a	0.420a	0.295a	1.093a	0.448a	0.239a	0.411a
	SH	8.601b	0.199b	0.141b	0.163b	0.227b	0.055b	0.362b	0.248b	0.892b	0.375b	0.209b	0.364b
	TH		0.198b	0.142b	0.165b		0.056b	0.369b				0.213b	0.365b
	DH	8.523b	0.199b		0.163b	0.225b	0.055b	0.360b	0.244b	0.876b	0.370b	0.210b	0.366b
	VA	9.352a	0.210a	0.150a	0.176a	0.249a	0.061a	0.395a	0.273a	1.001a	0.414a	0.228a	0.391a
	SH	8.749b		0.142b	0.162b	0.227b	0.056b	0.365b	0.254b	0.920b	0.382b	0.210b	0.359b
	TH	8.763b	0.190b	0.140b	0.162b	0.231b	0.055b	0.367b	0.254b	0.931b	0.383b	0.210b	0.354b
	DH	8.686b		0.141b	0.163b	0.226b	0.055b	0.365b	0.249b	0.916b	0.378b	0.212b	0.356b
	VA	9.853a	0.203a	0.155a	0.177a	0.261a	0.060a	0.412a	0.292a	1.100a	0.444a	0.234a	0.388a
	SH		0.193a	0.139b			0.056a					0.205b	
	TH		0.192a				0.054a					0.205b	
	DH	8.350b	0.180b	0.138b	0.155b	0.207b	0.054a	0.349b	0.240b	0.881b	0.365b	0.201b	0.333b
	VA	9.192a	0.195a	0.145a	0.173a	0.233a	0.057a	0.385a	0.273a	1.006a	0.411a	0.220a	0.361a
	SH	9.385b	0.201a	0.157b	0.173b	0.243b	0.061b	0.388c		1.015b	0.417b	0.221c	0.381b
	TH	9.541b	0.199a	0.158b	0.174b	0.247b	0.061b	0.394c	0.279c	1.038b	0.423b	0.225c	0.385b
	DH	9.876b	0.205a		0.177b	0.260a		0.410b	0.290b	1.094b	0.442b	0.232b	
	VA		0.209a	0.173a	0.192a	0.270a	0.066a	0.438a	0.312a	1.196a	0.480a	0.249a	0.412a
	SH	9.415b	0.224b	0.150b	0.184b	0.264b	0.060b	0.411b	0.284b	1.037b	0.431b	0.231b	0.401b
	TH	9.599b	0.226b	0.153b	0.188b	0.271b	0.061b	0.423b	0.290b	1.065b	0.444b	0.237b	0.407b
	DH		0.229b										0.416b
	VA		0.244a	0.161a	0.204a	0.294a	0.069a	0.461a	0.323a	1.191a	0.494a	0.255a	0.442a
	SH	9.768b	0.224b	0.154b	0.188b	0.269b	0.062b	0.425b	0.294b	1.089b	0.449b	0.237b	0.407b
	TH		0.227b		0.196a	0.283a	0.065a	0.448a	0.316a	1.171a	0.480a	0.246a	
	DH		0.229b			0.282a							0.416b
	VA		0.237a	0.164a	0.204a	0.291a	0.068a	0.465a	0.325a	1.209a	0.497a	0.252a	0.436a
	SH	9.104b	0.210a	0.145b	0.176b	0.253b	0.060b	0.394b	0.273b	0.999b	0.415b	0.219b	0.377b
	TH	9.015b	0.208a	0.143b	0.173b	0.253b	0.057b	0.393b	0.270b	0.989b	0.413b	0.219b	0.373b
	DH		0.209a	0.144b	0.175b	0.251b		0.391b		0.986b	0.411b	0.220b	0.374b
	VA	9.821a	0.217a	0.151a	0.189a	0.273a	0.065a	0.429a	0.297a	1.109a	0.456a	0.238a	0.399a
	SH	7.976b	0.189a	0.130b	0.151a	0.209a	0.052a					0.186b	0.327b
	TH	7.767b	0.184a	0.128b	0.149a	0.207a	0.051a					0.183b	0.321b
	DH	7.690b	0.184a		0.149a	0.205a	0.050a	0.329b	0.224b	0.789b	0.339b	0.186b	
	VA	8.570a	0.190a	0.144a	0.160a	0.222a	0.054a	0.359a	0.249a	0.900a	0.380a	0.203a	0.348a
Overall	SH	8.931b	0.204b	0.145c	0.169b	0.237b	0.058b	0.378b	0.262b	0.952b	0.397b	0.214c	0.370b
	TH	9.018b	0.203b		0.171b	0.242b	0.058b	0.383b	0.266b	0.969b	0.403b		0.372b
	DH	9.081b	0.204b	0.148b	0.172b	0.244b	0.058b	0.388b	0.268b	0.982b	0.408b	0.220b	0.376b
	VA	9.787a	0.213a	0.155a	0.183a	0.261a	0.062a	0.416a	0.291a	1.083a	0.444a	0.234a	0.398a

GB: genetic base. Means not followed by the same lowercase letter in the column differ by the Student's t-test at 5% significance level.

Table 3. Coefficient of variation (%) of protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g), of single hybrids (SH), triple hybrids (TH), double hybrids (DH) and open pollinated varieties (VA) sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons.

Dates	GB	n	CP	Lys	Met	Cys	Thr	Trp	Val	Ile	Leu	Phe	His	Arg
09/21/2021	SH	46	9.359	6.241	6.132	8.271	11.270	9.152	9.615	11.605	14.980	11.910	8.185	6.351
	TH	14	7.356	4.959	4.642	5.816	9.220	9.218	7.232	9.739	12.059	9.443	5.879	4.580
	DH	3	4.532	2.839	4.028	5.882	7.217	10.189	5.527	5.801	8.603	6.784	5.329	3.149
	VA	8	7.402	6.693	4.637	5.290	8.401	8.909	7.287	9.467	10.934	8.999	6.223	5.349
10/20/2021	SH	47	9.747	5.211	6.950	8.284	10.059	8.889	10.246	11.828	14.268	12.114	8.987	6.931
	TH	15	4.742	3.870	3.038	3.494	5.714	0.000	4.853	6.196	7.288	6.153	4.331	3.221
	DH	8	4.857	3.328	4.490	3.536	5.036	7.407	5.152	6.646	7.350	6.345	3.940	3.450
	VA	8	6.227	5.147	4.037	6.461	4.510	7.407	6.235	7.018	8.428	7.531	5.221	4.765
11/20/2021	SH	47	8.183	5.470	5.833	6.880	8.159	9.866	7.915	9.383	12.392	10.153	6.786	6.105
	TH	15	7.991	5.122	4.761	5.991	8.823	9.055	7.553	9.717	12.983	10.299	5.786	5.054
	DH	8	5.276	4.986	4.962	4.351	5.312	9.719	4.924	6.556	7.690	6.928	3.600	4.112
	VA	8	5.282	4.409	5.040	4.221	5.011	5.772	5.579	6.724	7.818	6.051	3.896	3.726
12/20/2021	SH	47	6.517	6.756	4.792	5.597	6.704	9.616	6.483	7.901	9.841	8.091	5.774	5.533
	TH	15	8.688	5.263	6.037	7.451	10.294	9.332	8.967	10.395	13.231	11.400	7.199	6.564
	DH	8	7.326	3.673	4.537	6.370	7.063	9.719	6.549	10.179	11.505	9.577	4.871	3.952
	VA	8	6.177	5.753	4.877	5.832	7.216	0.000	6.702	7.479	9.078	7.801	6.443	5.645
01/30/2022	SH	47	9.156	7.992	5.460	8.002	9.975	11.354	9.003	11.165	12.580	11.177	7.469	7.663
	TH	15	9.546	7.675	6.342	8.527	11.491	9.391	9.409	11.330	13.658	11.596	8.205	8.900
	DH	8	7.495	5.143	5.143	7.711	8.044	9.629	7.730	8.909	11.043	9.027	5.595	6.174
	VA	8	6.770	3.877	3.686	5.139	8.525	8.051	6.942	8.028	10.217	8.058	4.859	4.546
09/06/2022	SH	47	7.755	7.805	7.681	6.991	8.953	6.706	7.486	8.773	11.514	9.137	5.856	4.892
	TH	15	5.438	8.578	6.419	3.635	5.665	5.737	4.047	4.865	7.946	6.292	3.711	3.518
	DH	8	3.001	8.648	7.888	4.994	5.036	5.772	3.449	3.192	4.058	3.772	3.812	4.688
	VA	8	6.309	10.061	8.626	3.673	5.599	11.231	4.363	6.343	8.129	6.682	3.355	3.833
10/14/2022	SH	47	7.094	4.971	5.361	6.264	7.267	8.114	7.947	8.825	10.051	9.295	6.928	5.904
	TH	15	5.955	4.028	4.610	5.395	5.492	5.737	5.896	7.019	7.940	7.604	5.152	5.287
	DH	8	6.887	4.332	4.490	7.214	6.399	11.314	7.165	8.215	10.026	8.471	6.178	4.963
	VA	8	8.196	4.351	6.983	5.829	7.707	12.139	8.148	10.048	11.960	10.380	6.952	6.010
11/24/2022	SH	47	7.229	4.978	4.974	6.801	6.836	8.177	7.524	8.960	10.200	9.113	6.187	5.577
	TH	15	6.276	5.379	4.279	5.029	5.764	7.904	6.707	7.733	8.883	8.030	5.049	4.163
	DH	8	5.251	2.802	2.939	3.877	5.267	8.118	5.634	6.446	8.658	6.443	5.343	3.382
	VA	8	6.871	2.977	4.544	5.206	6.973	6.858	7.270	8.546	10.020	8.646	5.893	4.891
12/30/2022	SH	47	7.312	5.859	5.554	6.264	6.980	7.390	7.345	8.364	10.232	8.779	6.746	6.588
	TH	15	8.643	5.512	4.161	6.419	7.258	10.354	8.080	9.997	11.490	9.919	6.437	5.514
	DH	8	6.430	5.394	3.600	5.290	6.873	0.000	6.326	7.919	9.603	7.403	4.859	5.709
	VA	8	9.533	8.424	4.237	7.185	7.533	16.447	9.448	11.884	9.428	10.409	7.028	6.060
02/06/2023	SH	47	10.101	7.782	8.039	8.676	10.943	10.911	10.339	12.578	15.072	13.237	9.086	8.257
	TH	15	9.399	4.500	6.052	9.627	13.819	6.855	11.285	12.270	17.639	13.258	9.372	5.746
	DH	8	5.673	4.986	5.815	6.662	10.099	0.000	6.790	6.292	9.970	7.800	7.559	4.581
	VA	8	6.123	11.599	13.882	7.470	7.877	9.629	7.805	8.712	10.919	10.240	6.330	5.492
Geral	SH	469	10.460	8.881	8.265	9.957	12.117	10.539	11.343	12.613	15.263	13.265	10.052	9.324
	TH	149	10.626	8.994	8.130	9.943	12.603	10.227	11.524	12.903	15.521	13.477	10.113	9.525
	DH	75	10.165	9.253	8.544	10.210	12.583	10.840	11.138	12.531	14.387	12.792	9.681	9.260
	VA	80	9.311	9.893	8.316	9.057	10.692	11.706	10.250	11.399	12.806	11.629	8.505	8.636

GB: genetic base; n: number of samples.

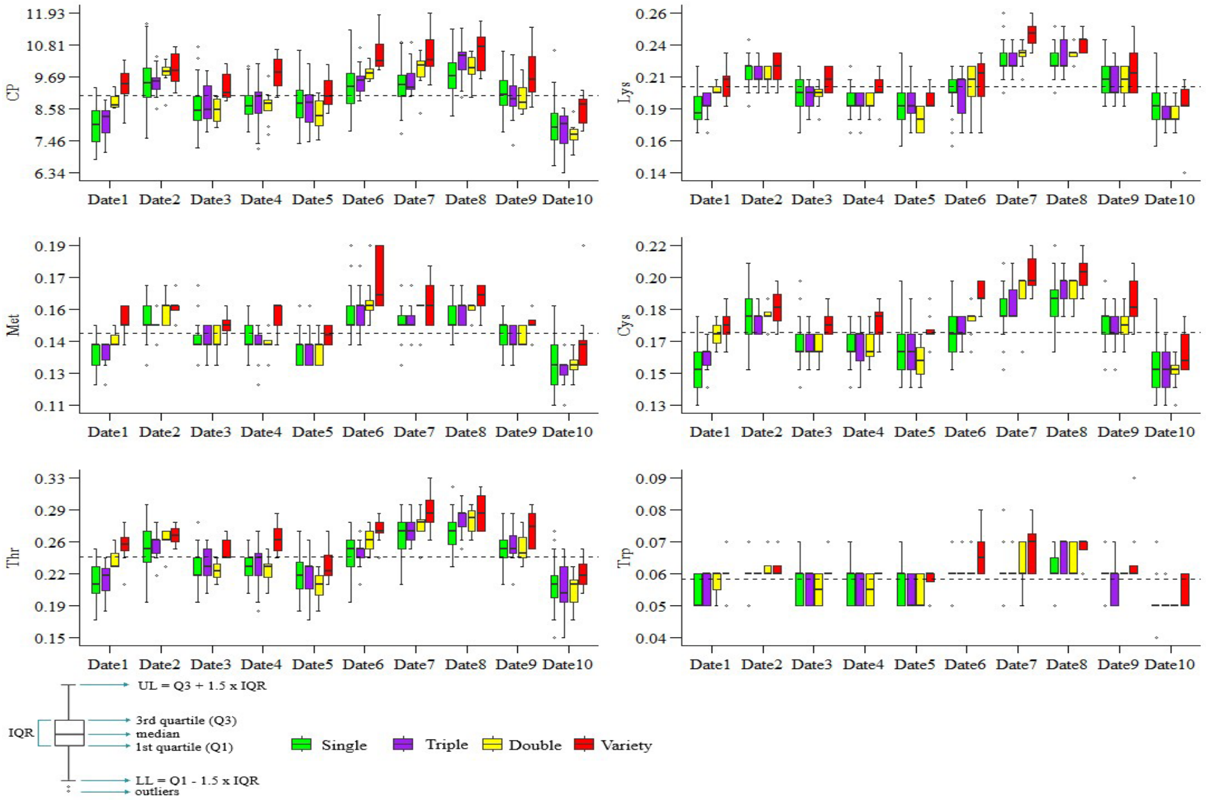

Figure 3. Boxplot representing the distribution of the data of the protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g) and tryptophan (Trp, g/100 g), of single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons. Date1: 09/21/2021; Date2: 10/20/2021; Date3: 11/20/2021; Date4: 12/20/2021; Date5: 01/30/2022; Date6: 09/06/2022; Date7: 10/14/2022; Date8: 11/24/2022; Date9: 12/30/2022; Date10: 02/06/2023. UL: upper limit; LL: lower limit; and IQR: interquartile range. Dotted line represents the overall mean of each trait.

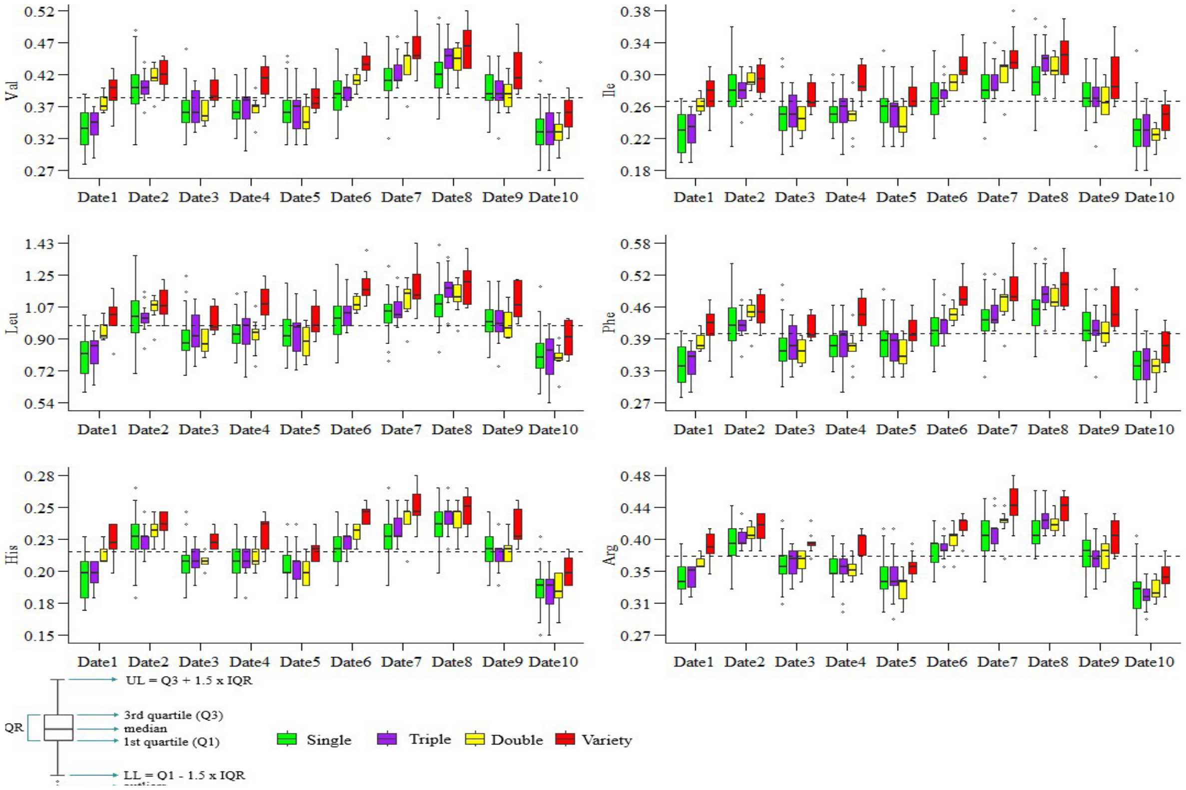

Figure 4. Boxplot representing the distribution of the data of the protein nutritional traits: valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g), of single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons. Date1: 09/21/2021; Date2: 10/20/2021; Date3: 11/20/2021; Date4: 12/20/2021; Date5: 01/30/2022; Date6: 09/06/2022; Date7: 10/14/2022; Date8: 11/24/2022; Date9: 12/30/2022; Date10: 02/06/2023. UL: upper limit; LL: lower limit; and IQR: interquartile range. Dotted line represents the overall mean of each trait.

There was a significant difference between the genetic bases for the contents of the protein nutritional traits. The open pollinated varieties showed the highest means of CP and digestible amino acid contents on the ten sowing dates. In the overall mean of the ten sowing dates, the open pollinated varieties had a statistically higher mean than the other maize genetic bases for all protein nutritional traits. In addition, the mean of the open pollinated varieties was statistically higher than the overall mean of the hybrids (single hybrids + triple hybrids + double hybrids) for all traits.

The superiority of the open pollinated varieties over the single, triple and double hybrids ranged from 4.65 % to 13.92 %, 4.97 % to 12.21 %, and 4.40 % to 10.92 %, respectively, considering all traits. The biggest difference was recorded for the Leu trait, between varieties and single hybrids, for the sowing carried out on November 21, 2021, with 26.59 %. The greatest differences between the varieties and the other genetic bases were recorded, in descending order, for the traits Leu, Phe, Ile, Val, CP, Thr, His, Cys, Trp, Met, Arg and Lys. Variation in the contents of the traits was observed in all maize genetic bases, with the magnitude of these variations influenced both by the genetic bases and by the sowing dates.

The first two principal components explained 78.60% of the total variation in the data (Figure 5). The open pollinated varieties had the highest means for the protein nutritional traits. In the two agricultural seasons analyzed, the sowing dates were distributed in five periods: the first in September (09/21/2021 and 09/06/2022), the second in October (10/20/2021 and 10/14/2022), the third in November (11/20/2021 and 11/24/2022), the fourth in December (12/20/2021 and 12/30/2022) and the fifth between January and February (01/30/2022 and 02/06/2023). The differences in sowing dates between the two seasons, in terms of day and month, were minimal, with variations of 15, 6, 4, 10 and 7 days for the first, second, third, fourth and fifth sowing dates, respectively.

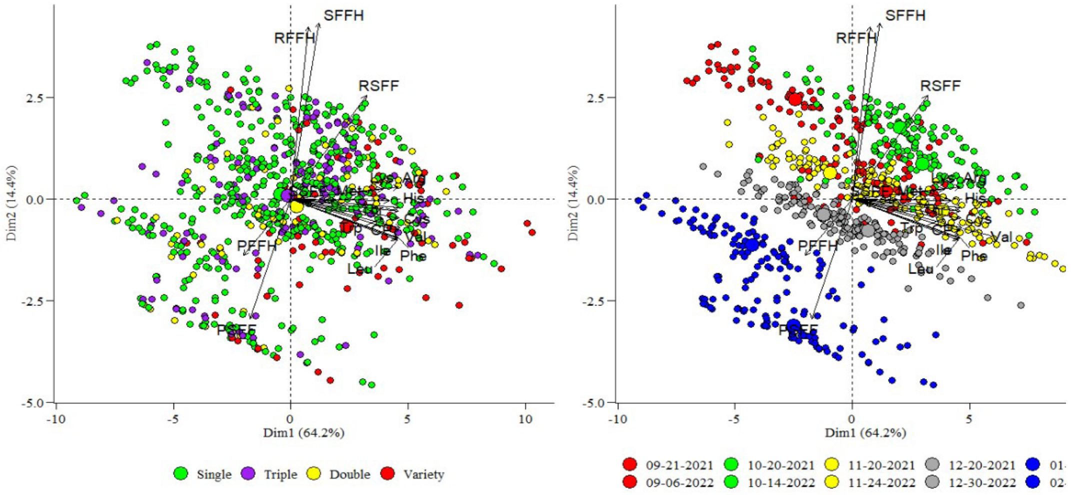

Figure 5. Data variability (n = 773 samples) represented by two principal components stratified by four genetic bases and ten sowing dates. Meteorological variables: cumulative global solar radiation from sowing to female flowering (RSFF, MJ m-2), cumulative global solar radiation from female flowering to harvest (RFFH, MJ m-2), thermal sum from sowing to female flowering (SSFF, °C day), thermal sum from female flowering to harvest (SFFH, °C day), cumulative precipitation from sowing to female flowering (PSFF, mm) and cumulative precipitation from female flowering to harvest (PFFH, mm). Protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g). Larger circles correspond to the average performance of each group.

Sowing dates had a greater influence on the expression of CP and digestible amino acids, compared to genetic bases. The highest rainfall and lowest values of RSFF, RFFC, SSFF and SFFH were recorded in the sowings of December, January and February. The lowest rainfall and highest values of RSFF and SSFF were recorded in the sowings of October and November, associated with the highest levels of protein nutritional traits.

CP and digestible amino acids showed positive correlations with each other, regardless of the genetic base evaluated (Figure 6A, 6B, 6C and 6D). The variables RSFF, RFFH, SSFF and SFFH showed a positive correlation with CP and digestible amino acids, while the variables PSFF and PFFH showed a negative correlation with these traits in all maize genetic bases. RSFF was the meteorological variable that showed the highest correlation with protein nutritional traits

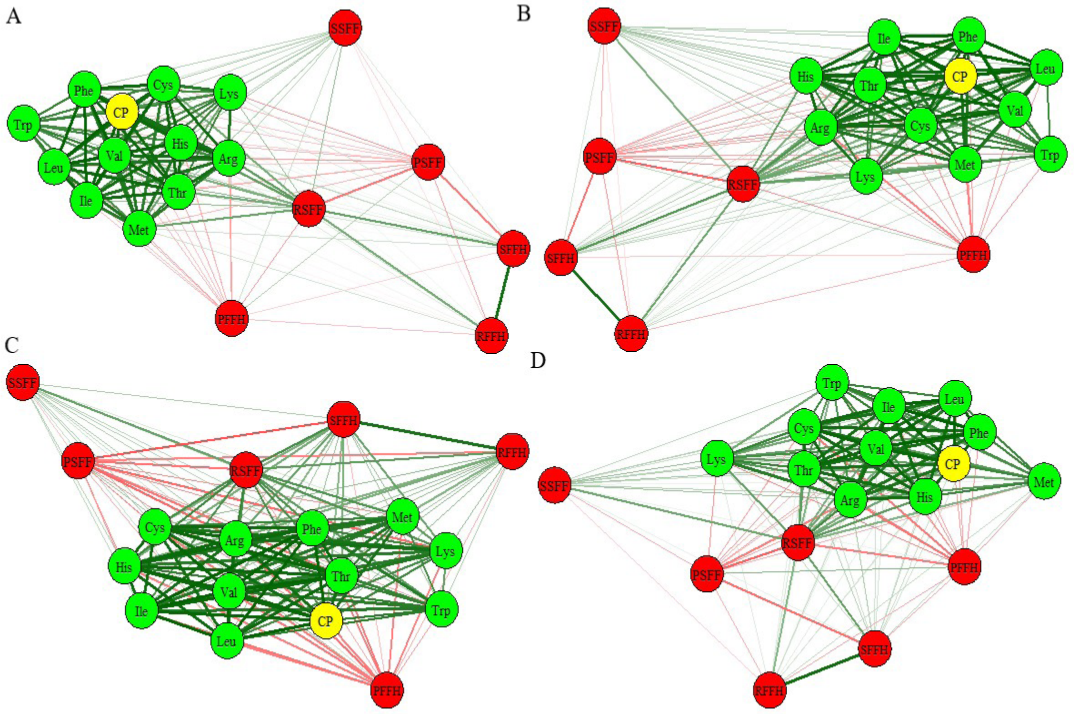

Figure 6. Network of Pearson's linear correlation between meteorological variables and protein nutritional traits in maize genetic bases (A: single hybrids, B: triple hybrids, C: double hybrids, D: open pollinated varieties). Meteorological variables: cumulative global solar radiation from sowing to female flowering (RSFF, MJ m-2), cumulative global solar radiation from female flowering to harvest (RFFH, MJ m-2), thermal sum from sowing to female flowering (SSFF, °C day), thermal sum from female flowering to harvest (SFFH, °C day), cumulative precipitation from sowing to female flowering (PSFF, mm) and cumulative precipitation from female flowering to harvest (PFFH, mm). Protein nutritional traits: crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g). Red lines represent negative linear correlations between the traits, and blue lines indicate positive linear correlations. The thickness of the lines reflects the magnitude of the correlation coefficients: thin lines correspond to lower intensity correlations, thicker lines indicate stronger correlations.

3.2 Models for predicting amino acids

The coefficient of determination ranged from 0.39 to 0.97, being equal to or greater than 0.78 in eight of the eleven digestible amino acids for all maize genetic bases (Figures 7 and 8). The amino acids Val, Ile, Leu, Phe and His showed the highest degrees of association with CP, evidenced by the high coefficients of determination ($0.89 \leq R^2 \leq 0.97$). However, Lys exhibited a low association with CP in all genetic bases, with coefficients of determination equal to or lower than 0.57. Double hybrids showed higher values of the angular coefficient for the amino acids Lys, Met, Cys, Thr, His and Arg. Open pollinated varieties showed higher values of the angular coefficient for the amino acids Trp, Val and Ile and Phe, indicating a higher rate of increase of these amino acids as the CP increases. It is important to note that all traits were evaluated using the same unit of measurement, ensuring comparability of the results.

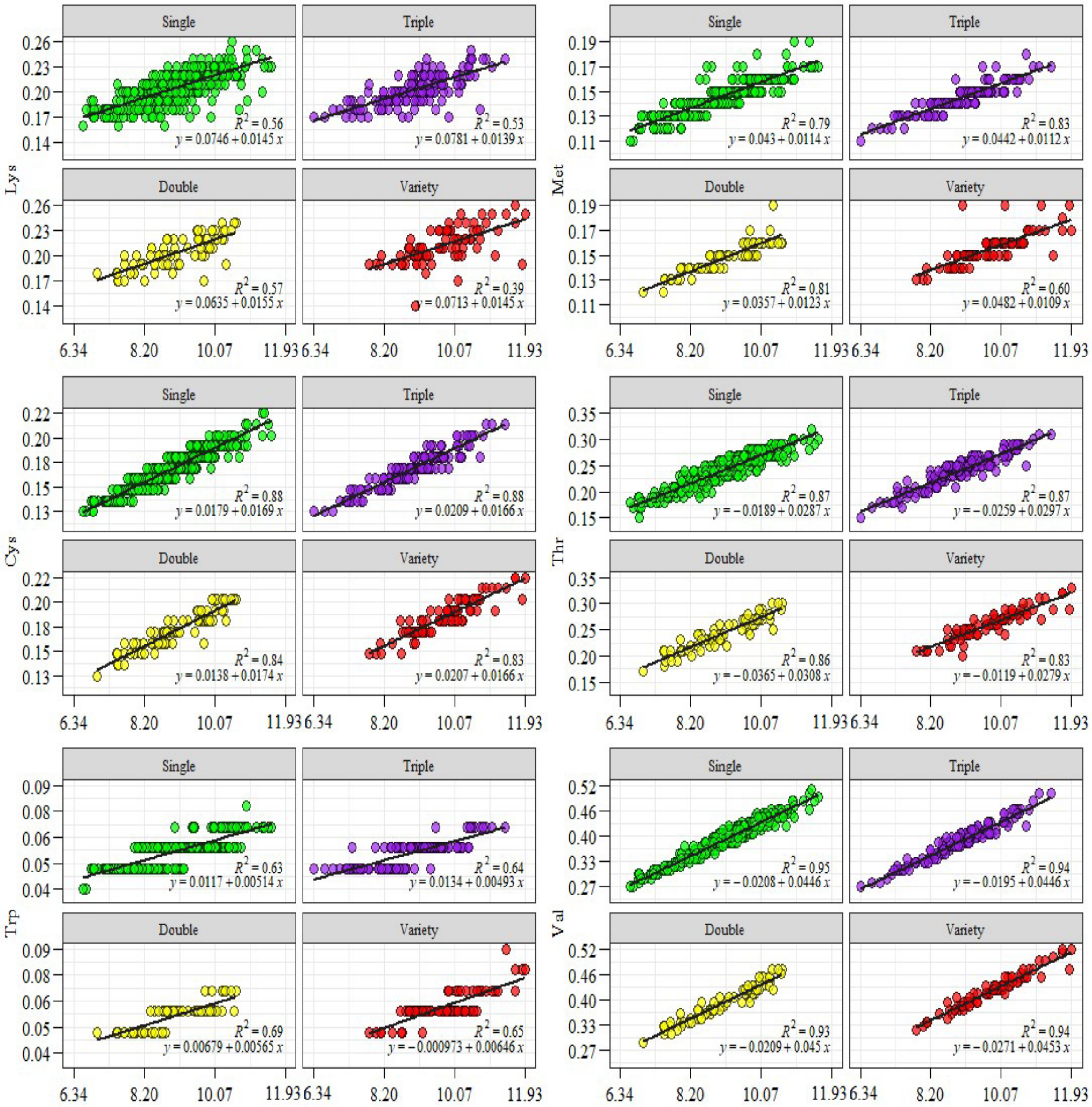

Figure 7. Simple linear regression between crude protein (CP, g/100 g) (x) and lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), for single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons. Estimates of the parameters of all simple linear regression models were significant by Student's t-test at 5% significance level.

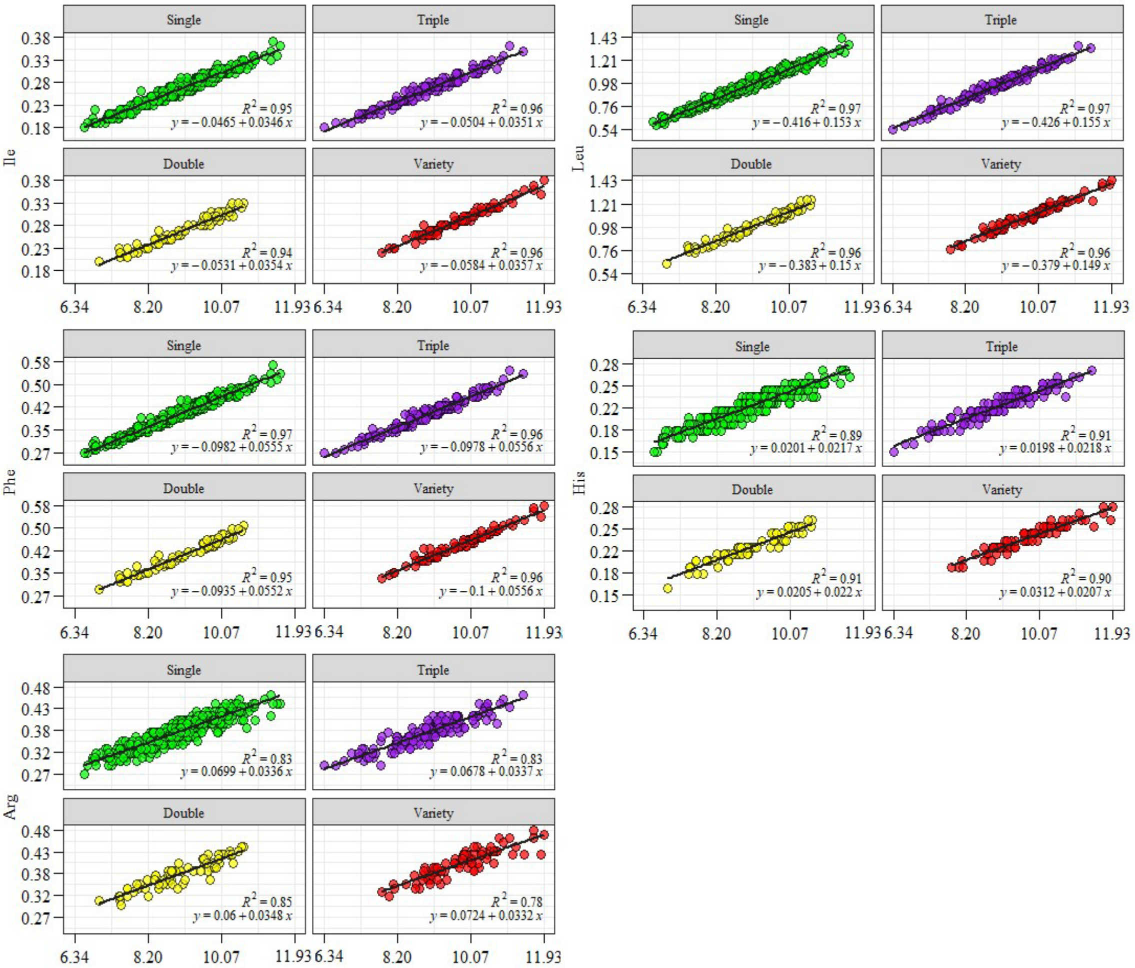

Figure 8. Simple linear regression between crude protein (CP, g/100 g) (x) and isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g), for single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates in the 2021/2022 and 2022/2023 seasons. Estimates of the parameters of all simple linear regression models were significant by the Student's t-test at 5% significance level.

The results revealed that the influence of meteorological variables (RSFF, RFFH, SSFF, SFFH, PSFF and PFFH) on protein nutritional traits varies among the maize genetic bases (single, triple, double hybrids and open pollinated varieties) (Table 5). In general, single hybrids had the lowest values of adjusted coefficients of determination (0.25 ≤ R2 ≤ 0.47) and cross-validation coefficients of determination (0.24 ≤ R^{2}cv ≤ 0.46), indicating lower predictive capacity and greater variation not explained by the fitted models. Double hybrids exhibited higher values of R2 (0.46 ≤ R2 ≤ 0.72) and R^{2}cv (0.40 ≤ R^{2}cv ≤ 0.68), suggesting greater stability and accuracy in the modeling of nutritional traits in response to meteorological variables. Open pollinated varieties stood out with the highest angular coefficients for variables such as RSFF and PFFH, reflecting greater sensitivity to these meteorological variables and genetic potential for protein and amino acid accumulation in the grains. RSFF was the only meteorological variable selected in all models for predicting CP and digestible amino acids. In all maize genetic bases, the highest R^2 and R^{2}cv were observed in the prediction of Arg, while the lowest values were observed in the prediction of Trp.

Table 4. Stepwise forward multiple linear regression to predict crude protein (CP, g/100 g), lysine (Lys, g/100 g), methionine (Met, g/100 g), cystine (Cys, g/100 g), threonine (Thr, g/100 g), tryptophan (Trp, g/100 g), valine (Val, g/100 g), isoleucine (Ile, g/100 g), leucine (Leu, g/100 g), phenylalanine (Phe, g/100 g), histidine (His, g/100 g) and arginine (Arg, g/100 g) by means of the meteorological variables: cumulative global solar radiation from sowing to female flowering (RSFF, MJ m-2), cumulative global solar radiation from female flowering to harvest (RFFH, MJ m-2), thermal sum from sowing to female flowering (SSFF, °C day), thermal sum from female flowering to harvest (SFFH, °C day), cumulative precipitation from sowing to female flowering (PSFF, mm) and cumulative precipitation from female flowering to harvest (PFFH, mm) for single hybrids, triple hybrids, double hybrids and open pollinated varieties sown on ten sowing dates (2021/2022 and 2022/2023).

Model	CP	Lys	Met	Cys	Thr	Trp	Val	Ile	Leu	Phe	His	Arg
						Single Hybrids						
Interc	7.2E+00	1.9E-01	1.2E-01	1.3E-01	1.9E-01	5.7E-02	2.8E-01	2.1E-01	7.0E-01	3.1E-01	1.3E-01	2.9E-01
RSFF	3.3E-03	2.9E-05	5.3E-05	5.0E-05	8.5E-05	2.1E-05	1.4E-04	1.1E-04	5.1E-04	1.8E-04	6.8E-05	1.2E-04
RFFH	-3.1E-03	-4.7E-05	-4.1E-05	-5.4E-05	-1.1E-04	-2.9E-05	-1.5E-04	-1.2E-04	-5.4E-04	-1.9E-04	-2.3E-05	-1.1E-04
SSFF	-2.3E-03	-	-5.1E-05	-2.1E-05	-4.4E-05	-2.6E-05	-7.1E-05	-7.9E-05	-3.7E-04	-1.2E-04	-	-6.8E-05
SFFH	2.5E-03	5.0E-05	3.6E-05	5.1E-05	1.1E-04	3.1E-05	1.4E-04	1.0E-04	4.6E-04	1.6E-04	-	1.2E-04
PSFF	9.5E-04	-3.5E-05	1.7E-05	1.6E-05	-	9.7E-06	4.6E-05	3.4E-05	2.0E-04	5.4E-05	-	-
PFFH	-	-3.3E-05	6.7E-06	-1.6E-05	-2.5E-05	-	-2.0E-05	-	-	-7.3E-06	-9.3E-06	-2.2E-05
R^2	0.33	0.36	0.44	0.39	0.42	0.25	0.39	0.32	0.33	0.33	0.45	0.47
R^2cv	0.31	0.34	0.42	0.37	0.40	0.24	0.37	0.30	0.31	0.31	0.44	0.46
						Triple Hybrids						
Interc	8.2E+00	1.7E-01	1.4E-01	1.5E-01	2.3E-01	5.9E-02	3.6E-01	2.0E-01	9.2E-01	3.0E-01	1.7E-01	2.6E-01
RSFF	4.1E-03	3.0E-05	5.8E-05	5.1E-05	9.6E-05	1.7E-05	1.5E-04	8.6E-05	6.7E-04	1.3E-04	9.0E-05	1.0E-04
RFFH	-4.8E-03	-4.2E-05	-4.8E-05	-5.9E-05	-1.5E-04	-2.2E-05	-2.0E-04	-5.5E-05	-8.9E-04	-8.8E-05	-9.5E-05	-3.7E-05
SSFF	-4.2E-03	7.0E-06	-6.8E-05	-3.2E-05	-8.5E-05	-2.1E-05	-1.2E-04	-	-7.6E-04	-	-7.2E-05	-
SFFH	4.3E-03	4.6E-05	3.1E-05	4.9E-05	1.6E-04	2.4E-05	1.7E-04	-	8.2E-04	-	9.0E-05	-
PSFF	1.7E-03	-	-	-	-	-1.1E-06	-	-	2.7E-04	-	4.1E-05	-
PFFH	-4.1E-04	-5.1E-05	-	-3.1E-05	-4.3E-05	-9.2E-06	-5.4E-05	-6.3E-05	-	-1.0E-04	-1.9E-05	-7.1E-05
R^2	0.44	0.43	0.55	0.46	0.48	0.38	0.47	0.40	0.40	0.40	0.52	0.57
R^2cv	0.38	0.38	0.52	0.42	0.43	0.32	0.43	0.37	0.35	0.37	0.48	0.55
						Double Hybrids						
Interc	8.0E+00	1.7E-01	1.3E-01	1.2E-01	1.8E-01	4.2E-02	3.3E-01	1.7E-01	8.7E-01	2.6E-01	1.5E-01	2.9E-01
RSFF	4.8E-03	2.7E-05	5.7E-05	4.1E-05	6.3E-05	1.4E-05	1.7E-04	8.2E-05	7.3E-04	1.3E-04	5.0E-05	7.1E-05
RFFH	-5.0E-03	-4.1E-05	-2.0E-05	-8.4E-06	-1.8E-05	-4.2E-06	-1.5E-04	-	-8.2E-04	-	-	-
SSFF	-6.2E-03	-4.8E-06	-6.0E-05	-	-	-	-1.6E-04	-	-9.3E-04	-	-	-
SFFH	5.3E-03	7.4E-05	-	-	-	-	1.3E-04	-3.6E-05	8.4E-04	-6.3E-05	-	-
PSFF	2.8E-03	-1.5E-05	-	-	-6.0E-05	-	-	-	4.7E-04	-	-	-7.6E-05
PFFH	-5.2E-06	-4.8E-05	-	-4.0E-05	-7.7E-05	-1.2E-05	-4.4E-05	-8.2E-05	-4.4E-05	-1.3E-04	-3.9E-05	-7.2E-05
R^2	0.68	0.62	0.65	0.59	0.64	0.46	0.65	0.61	0.62	0.60	0.64	0.72
R^2cv	0.62	0.52	0.61	0.56	0.59	0.40	0.59	0.57	0.55	0.56	0.61	0.68
						Open pollinated varieties						
Interc	1.0E+01	1.7E-01	1.6E-01	1.8E-01	2.5E-01	5.1E-02	4.2E-01	2.2E-01	1.2E+00	3.4E-01	2.2E-01	3.4E-01
RSFF	5.1E-03	4.0E-05	5.6E-05	7.3E-05	1.2E-04	1.2E-05	2.1E-04	6.9E-05	7.8E-04	1.0E-04	1.0E-04	1.4E-04
RFFH	-7.7E-03	-3.9E-05	-7.7E-05	-1.1E-04	-2.1E-04	-5.8E-06	-3.7E-04	-	-1.3E-03	-	-1.5E-04	-1.7E-04
SSFF	-8.4E-03	-	-9.8E-05	-1.0E-04	-1.7E-04	-	-3.3E-04	-	-1.4E-03	-	-1.5E-04	-1.5E-04
SFFH	8.9E-03	3.6E-05	9.1E-05	1.2E-04	2.6E-04	-	4.4E-04	-5.0E-05	1.5E-03	-7.4E-05	1.8E-04	1.9E-04
PSFF	4.5E-03	-	4.8E-05	6.7E-05	9.3E-05	-	2.2E-04	-	8.1E-04	-	1.1E-04	7.7E-05
PFFH	1.1E-03	-4.9E-05	-	-2.0E-05	-6.3E-07	-1.8E-05	-	-5.5E-05	1.8E-04	-1.0E-04	-	-3.3E-05
R^2	0.48	0.47	0.38	0.55	0.51	0.35	0.51	0.34	0.44	0.35	0.56	0.64
R^2cv	0.38	0.40	0.29	0.47	0.41	0.28	0.43	0.28	0.33	0.29	0.49	0.58

Estimates of the parameters of all multiple linear regression models were significant by the Student's t-test at 5% significance level. Interc: intercept; R^2: adjusted coefficient of determination; R^2cv: coefficient of determination of leave-one-out cross-validation.

4. Discussion

4.1 Variability of protein nutritional traits

Many factors contribute to the existence of variability in the CP and digestible amino acid contents for swine in maize grains. Therefore, it was predicted that the use of a wide range of sources of maize grain samples could capture much of the existing variability. This allows the development of an acceptable prediction, since the study was based on 773 samples of maize grains, from four genetic bases cultivated on ten sowing dates. Knowledge of this variation is important to determine which environmental conditions promote an increase in the contents of protein nutritional traits in maize.

The results of this study revealed a clear influence of sowing dates and genetic bases on the CP and digestible amino acid contents in maize, with significant variations between the 2021/2022 and 2022/2023 seasons and between single, triple, double hybrids and open pollinated varieties. Wu *et al*. Vasconcelos *et al*.(19) also found variations in the contents of total amino acids in maize grains as a function of the sowing dates. This reflects distinct regulatory patterns in response to environmental conditions (25, 26). These patterns highlight the influence of environmental conditions on differential expression between genotypes.

Studies have indicated the variation of the digestible amino acid content in maize grains, as a function of their oil content (10), the type of endosperm (2), country of origin (11, 8), genotype (12) and extrusion process (13). However, no studies were identified evaluating the CP and digestible amino acid contents for swine in grains of maize genetic bases (single, triple, double hybrids and open pollinated varieties) grown under different meteorological conditions.

Maize open pollinated varieties stood out with the highest means of digestible amino acid contents for the ten sowing dates, indicating a superior genetic potential for the synthesis of proteins and amino acids. Adequate intake of digestible amino acids can vary according to feed ingredients, and understanding the composition of maize grains of different genetic bases under different climatic conditions allows nutritionists to optimize the use of feed additives, better meeting the needs of pigs.

The negative relationship between crude protein and yield of maize grains (27, 28, 15, 2) suggests that the exclusive focus on increasing maize grain yield results in an indirect selection of plants with lower protein and amino acid content in the grains. The lower contents of crude protein and digestible amino acids observed, especially in single hybrids, reflect this dynamic, as breeding programs prioritize high yield. In contrast, the open pollinated varieties, which undergo fewer breeding processes, have higher contents of crude protein and digestible amino acids in the grains, becoming important sources of favorable alleles for nutritional quality.

RSFF, as the main correlated variable, highlights its importance as an indicator for the selection of environments. This indicates that sites with high radiation in the vegetative stage of maize favor a greater synthesis of CP and digestible amino acids. This explains the higher means of protein nutritional traits for the sowings carried out in October and November (> RSFF and RFFH and < PSFF and PFFH). The results are consistent with those of Safian *et al.* (16) and Guo *et al.* (15), who reported higher protein content in maize grains under water deficit, associated with a reduction in starch synthesis and an increase in protein synthesis. Positive relationships were also observed between crude protein and cumulative temperature before anthesis (18) and between amino acid contents and global solar radiation (19). This reinforces the importance of synchrony between maize development and environmental conditions to optimize nutritional traits.

4.2 Models for predicting amino acids

The predictions showed high accuracy for all digestible amino acids, except for Lys and Trp ($R^2 \leq 0.69$). Thus, the use of CP as a predictor of digestible amino acid contents can be efficient in the formulation of animal feeds, especially in regression models with $R^2 \geq 0.80$. Linear prediction equations based on protein content have been developed for maize, such as the model for predicting the digestible content of histidine: $y = - 0.0877 + 0.0293 \times CP$, R2 = 0.94 (8). Zuber and Rodehutscord (7) observed that the digestibility coefficient of amino acids showed a positive correlation with the CP content in the maize samples. Ebadi *et al.*(9) identified the best relationships between digestible amino acids and the chemical composition in sorghum grains for most amino acids. The low linear relationship between CP and Lys, observed by the low coefficient of determination, suggests that Lys can be determined by other factors. The low linear relationship between CP and total lysine has also been reported in wheat (6) and maize (28, 2), and may be associated with the absence of lysine in the constitution of zein protein, which makes up more than 60% of the protein in the maize grain (29).

The different responses of the genetic bases to the increase in CP, evidenced by the higher angular coefficients, indicate that the profile of response to CP is not uniform among the genetic bases. From these models, it is possible to reduce the costs and time required for the evaluation of genotypes in breeding programs. In addition, this approach improves the accuracy in the formulation of diets for swine, which are usually based on average reference contents for maize grains (30), without considering the variability between the different genetic bases and the cultivation conditions.

Among the meteorological variables, RSFF had a strong positive effect on protein nutritional traits, being selected in all prediction models. In addition, all meteorological variables (RSFF, RFFH, SSFF, SFFH, PSFF and PFFH) were selected in some models, suggesting the complexity of the interaction between the synthesis of protein nutritional traits and meteorological variables. Predictive models corroborate this relationship, showing positive relationships between amino acid content and global solar radiation (19), as well as between crude protein and cumulative temperature before anthesis ($R^2 = 0.31$) and average daily temperature after anthesis ($R^2 = 0.83$) (18). These results reinforce the importance of aligning the development stage of maize with environmental conditions, in order to optimize the nutritional quality of grains.

The differentiated response of the genetic bases to meteorological variables suggests that genotypes more adapted to certain environmental conditions can be selected to maximize the nutritional quality of the grains. The higher accuracy of multiple regression models for double hybrids, evidenced by the high values of R^2 and R^2cv, reinforces the importance of exploring genetic variability and genotype × environment interactions in breeding programs. The higher values of R^2 and R^2cv for the prediction of Arg in all genetic bases indicate that this amino acid has a more stable and predictable relationship with the meteorological variables considered in the study. The lower values of R^2 and R^2cv for the prediction of Trp suggest greater complexity in the determination of this trait, possibly due to a more intense interaction between genetic and environmental factors.

The results show that the strategic choice of the sowing date, combined with the appropriate selection of the genetic base, can optimize the nutritional quality of maize in different crop scenarios. This information is important for developing management strategies and breeding programs that prioritize obtaining maize genotypes with high nutritional quality, especially in terms of crude protein and digestible amino acids for swine. There is a need for further research that explores genetic and environmental variability, in addition to improving the prediction models of digestible amino acids, especially Lys, which showed lower accuracy in the predictions. To date, no studies have been found in the literature that evaluated differences between maize genetic bases regarding the protein nutritional composition of the grains, which gives an innovative and pioneering character to the present study.

5. Conclusion

There is variation in the digestible contents of lysine, methionine, cystine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and arginine in the grains between maize genetic bases (single hybrids, triple hybrids, double hybrids and open pollinated varieties) and sowing dates. Open-pollinated varieties of maize have the highest digestible contents of lysine, methionine, cystine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and arginine in the grains, compared to single hybrids, triple hybrids and double hybrids, regardless of the sowing date.

Sowings carried out in October and November led to higher digestible contents of lysine, methionine, cystine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and arginine in maize grains, compared to sowings in September, December, January and February, regardless of the genetic base. The digestible contents of methionine, cystine, threonine, valine, isoleucine, leucine, phenylalanine, histidine and arginine in maize grains can be predicted from crude protein with high accuracy, in all genetic bases (single hybrids, triple hybrids, double hybrids and open pollinated varieties). The global solar radiation accumulated in the period from sowing to female flowering is the meteorological variable that presents the greatest predictive capacity for the levels of digestible amino acids for pigs in grains from different genetic bases of maize.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The full dataset supporting the results of this study is available upon request from the corresponding author.

Author contributions

Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project management, Writing (original draft) and Writing (review and editing): M. V. Loro and A. Cargnelutti Filho.

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