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Genetic divergence of corn cultivars in relation to grain productivity, crude protein content and amino acid profile

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The aim of this study was to investigate the genetic variability among early-maturing and extremely early-maturing corn cultivars in relation to grain productivity, crude protein content and amino acid profile to obtain future crosses of cultivars with improved nutritional characteristics. Data from two experiments, which were both conducted in randomized block design and three replicates. Thirty-six early-maturing and 22 extremely early-maturing cultivars were evaluated of the experiments. Grain productivity, crude protein, lysine, methionine, cysteine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and arginine were evaluated. Analysis of variance was performed for each variable and means were compared by Scott-Knott test. Mahalanobis generalized distance matrix was calculated, and the cultivars were grouped using the unweighted pair group method with arithmetic mean (UPGMA). A dendrogram was then constructed, and the cophenetic correlation coefficient was calculated. The group means were compared using the t-test for independent samples. Genetic variability was detected in 13 variables for both maturity groups. For the early-maturing and extremely early-maturing cultivars, five and four groupings were obtained, respectively, based on grain productivity, crude protein content and amino acid profile, indicating that the crossbreeding of these cultivars may be used to improve the grain protein content and amino acid profile.

Key words: *Zea mays* L., genetic variability, nutritional variability, clustering analysis, near-infrared reflectance spectroscopy.

INTRODUCTION

Studies on the variability of the nutritional composition of corn are important because they contribute to increase nutrient use efficiency and minimize environmental damage. Although corn is a cereal used for energy

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purposes, studies on its protein contribution, particularly those related to its profile of essential amino acids are needed. Studies in this area are relevant because corn is the most expensive nutrient in the feed formulation (Jompuk et al., 2011), resulting in significant effects on production costs. In this direction, Pollak and Scott (2005) emphasized that breeding for grain quality provides end users with grain better suited to their needs and the improved amino acid balance lowers the cost of animal feed.

In recent decades, genetic improvement in corn has mainly focused on increasing grain productivity (PROD), which may have diminished the nutritional value of grain. For instance, Idikut et al. (2009) and Aliu et al. (2012) demonstrated that hybrids and corn populations show an inverse relationship between PROD and the crude proteins (CP) content of grain, and from 1930 to 1991, the level of this nutrient decreased 0.3% per decade (Duvick, 2005). Consequently, studies have been conducted to improve the protein quality and amino acid profile of corn grains. A review performed by Prasanna et al. (2001) shows that the QPM (Quality Protein Maize) cultivars with high quality protein provide benefits for human nutrition due to a significant increase on lysine and tryptophan levels compared to conventional corn cultivars. In order to improve the amino acid composition, Shewry (2007) demonstrates the possibility of using genetic engineering strategies to increase both the total protein content and composition of essential amino acids or even for exploitation of natural high lysine mutants.

Study done by Mittelmann et al. (2011), evaluating ten Brazilian corn populations under three environments, showed that these populations differ in relation to most of the nutritional traits evaluated, indicating the possibility of selecting genotypes with improved nutritional quality. In the study developed by Kil et al. (2014), with yellow corn from the United States and with yellow and white corn from South Africa, was observed that the concentrations of CP and AA (amino acids) varied among corn from different origins. Also, in a study of genetic divergence among modern inbred lines, teosinte accessions, and landraces, Flint-Garcia et al. (2009) verified that teosinte has very small seeds, but twice the protein content of landraces and inbred lines and a lower carbohydrate content, being considered a source of genetic variability for maize breeding programs.

Another important characteristic to be considered in a breeding program for corn is the texture of the grain, which is characteristic of fundamental importance to the industry, producers and processors of grain. Once the proteins, grain texture and hardness grains are inter-related, it is worth considering these features in a breeding program in order to address the storage pest resistance in maize (Mwololo et al., 2013). From the viewpoint of processing to feed grains, grains hard texture consume more energy in milling process and the difficulties in obtaining uniform grain size can affect the digestion of swine (Lawrence, 1972). In developing countries, is commonly used hard and dent grain, but the emergence of mutants with alterations in protein synthesis, thus causing changes in the texture of the grains, which is not always desirable (Sofi et al., 2009).

The study of genetic diversity is the basis of genetic improvement programs for corn plants (Subramanian and Subbaraman, 2010; Cruz and Carneiro, 2006). Clustering analysis using hierarchical methods is among the most commonly applied techniques for the description of genetic diversity patterns. By this technique, it is possible to classify individuals into distinct groups according to the variables of interest (Cruz and Carneiro, 2006) and to identify individuals that can be crossbred to maximize the gain in heterosis or hybrid vigor in the F_1 generation.

To estimate the distance between cultivars in replicated experiments, the Mahalanobis generalized distance (D^2), which takes into account the correlations between characters, is the most appropriate (Singh, 1981). These distances can be represented later in a dendrogram generated by different methods, such as the average linkage between groups clustering method [unweighted pair group method with arithmetic mean (UPGMA)], which is considered appropriate for the grouping of corn cultivars (Cargnelutti Filho and Guadagnin, 2011). This clustering method was used by Alves et al. (2014) to assess the variability of grain productivity and energy profile of maize cultivars. They indicated that it is possible to plan crossings between groups of genotypes in terms of productivity, ethereal extract and amylose, with the goal of maximizing heterosis. The UPGMA clustering method was also used by Osorno and Carena (2008) to determine the genetic diversity in early maturity maize, based on quality (percentage of protein, oil, and starch) and agronomic traits.

Despite the large number of genotypes, hybrids, cultivars and strains of corn grown, there is still missing data concerning their genetic variability in relation to PROD and nutritional characteristics. The aim of the present study was to investigate the genetic variability among early-maturing and extremely early-maturing cultivars of corn in relation to PROD and the CP content and the amino acid profiles of grain, thereby providing a basis for obtaining cultivars with improved nutritional characteristics in future crossbreeding studies.

MATERIALS AND METHODS

Field experiments

Two experiments were conducted with corn (*Zea mays* L.) cultivated during the 2009/2010 agricultural year in the experimental area of the Department of Plant Science, in the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul State, at 29°42'S, 53°49'W and an altitude of 95 m. The

experimental area is included in the physiographic region of the Central Depression of the state of Rio Grande do Sul, Brazil. Fundamentally, the region is characterized by a humid subtropical climate, type Cfa according to the Köppen classification, and the soil is classified as Paleudalf (Embrapa, 2006), with a sandy loam surface texture.

The first experiment included 36 early-maturing corn cultivars, and the second included 22 extremely early-maturing corn cultivars. The cultivars used in each of the experiments (early-maturing and extremely early-maturing) belonged to the maize cultivar competition testing of the State of Rio Grande do Sul, Brazil. The relation of the cultivars included in each of the experiments was determined by the Agriculture Research State Foundation (Fundação Estadual de Pesquisa Agropecuária - FEPAGRO), which coordinates the maize cultivars evaluation testing in Rio Grande do Sul, based on information provided by the companies responsible for these cultivars, with respect to the cycle (early-maturing and extremely early-maturing). Further details of each cultivar, as commercial name, genetic basis, company and grain texture are presented in Table 1.

In both experiments, a randomized block design with three replications was used. The experimental plots consisted of two rows each 5 m long and spaced 0.8 m apart. Sowing was performed manually on October 26, 2009, with basic fertilization at a rate of 37.5 kg·ha^{-1} of nitrogen (N), 150 kg·ha^{-1} of phosphorus (P_2O_5) and 150 kg·ha^{-1} of potassium (K_2O). At the time of sowing, two seeds were placed every 0.20 m in each row, and after emergence, the plants were thinned to adjust the population to 62,500 plants ha^{-1}. The plants emerged between days 01 and 03 of November 2009 and were harvested on March 15, 2010. N fertilizer was applied as a top-dressing when plants were at the 3, 5 and 10 leaves, totaling 200 kg·ha^{-1} of N (Fancelli and Dourado Neto, 2004). All other cultivation practices were performed as locally recommended for the corn crop. During the experimental period, rainfall totaled 1,374 mm, and the average daily air temperature was 23.9°C [data collected at a conventional weather station belonging to the 8th District of Meteorology of the National Meteorological Institute (8° DISME/INMET), located 50 m from the experimental area] (Figure 1).

Measurement of variables and laboratory procedures

After the corn cob harvest, PROD was expressed in t·ha^{-1} for each repetition of each cultivar at 13% humidity. A sample of 500 g was collected, wrapped in a paper bag and maintained in a forced-air incubator (50°C) until 10% moisture was attained. After drying, the grain samples were ground in a micromill (model MA-630, Marconi, Piracicaba, São Paulo, Brazil) to obtain a particle size between 0.3 and 0.5 mm. Based on the percentage of raw material (% RM) in each milled sample, crude protein (CP) and the amino acids lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) were quantified using near infrared reflectance spectroscopy (NIR - Near Infrared reflectance), with specific methodology in equipment manual. The analyses were performed by the Laboratory CEAN - ADISSEO using the XDS EQUIPMENT FOSS® 2.5002, with wavelength range from 400 nm to 2500 nm, with calibration adjusted by analytical procedure CEAN 010 Adisseo Brazil SA.

Verification of assumptions, analysis of variance and comparison of means

For each variable measured in each experiment, analysis of variance was performed according to the mathematical model of a randomized complete block design given by: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, where Y_{ij} is the observed value of the response variable in the plot $_{ij}$, μ is the overall mean, τ_i is the effect of cultivar, β_j is the effect of block and ε_{ij} is the effect of experimental error (Storck et al., 2011). To ensure the reliability of the analysis of variance results, the assumptions of the mathematical model were verified. For this, the Kolmogorov-Smirnov test for verifying the normality of the errors (Campos, 1983) and Levene's test for checking the homogeneity of residual variances among the treatments (Steel et al., 1997) were performed. In addition, the selective accuracy (SA) was estimated by the equation $SA = (1\ (1 / Fc))^{0.5}$ and used to evaluate the experimental accuracy, in accordance to the limits postulated by Cargnelutti Filho and Storck (2009). SA can alternatively be obtained by square root of the heritability and accuracy is considered very high when the SA≥0.90, high when 0.70≤SA<0.90, moderate when 0.50≤SA<0.70 and low when the SA<0.50 (Cargnelutti Filho and Storck, 2009). After, the means of the cultivars were compared using the Scott-Knott test (1974) at the 5% probability level.

Genotypic correlation matrix and diagnosis of multicollinearity

In each experiment (early and extremely early maturing cultivars), a genotypic correlation matrix between the 13 variables was calculated, and the multicollinearity was diagnosed to identify and eliminate highly correlated variables. The degree of multicollinearity of the genotypic correlation matrix was evaluated based on the condition number (CN). CN an efficiente procedure for the diagnosis of multicollinearity should directly reflect the intensity of its effects and allow the identification of the independent variables involved whit this problem (Montgomery and Peck, 1982). The decision criterion used was based on the classification proposed by Montgomery and Peck (1982) and described by Cruz and Carneiro (2006).

Analysis and validation of clusters

For the cluster analysis, a Mahalanobis generalized distance matrix (D^2) between the cultivars of each experiment was determined first. Then, the D^2 matrix was used on a relative scale as the dissimilarity measure for cluster analysis of the cultivars by the average linkage between groups hierarchical method (UPGMA) (Cruz and Carneiro, 2006). Next, a dendrogram was constructed, and the cophenetic correlation coefficient (CCC) was calculated. Thereafter, using 50% dissimilarity (Cruz, 1990) as the cutoff criterion for the dendrogram, the cultivars were identified for each group. This percentage was set at 50%, because groups formed with more than 50% of genetic variability would present little practicality, due to the large genetic differentiation between genotypes considered in homogeneous groups (Barroso and Arts, 2003). The mean of each variable was then estimated within each group, and subsequently for grouping validation, the group means were compared two by two with Student's t test for independent samples. Next, these means were transformed into vectors, and the highest mean was considered equal to one. For each experiment, the group means were represented on a graph of means profiles (Barroso and Artes, 2003). In addition, multivariate analysis of variance (MANOVA) at a 5% probability level was used to test for differences between the profiles of group means using Wilk's criterion. The statistical analysis was performed using Genes (Cruz, 2013) and Bioestat 5.0 (Ayres et al., 2007) software and the Microsoft Office Excel application.

Table 1. Cultivar number, commercial name, genetic basis, company and grain texture for each of the 36 cultivars tested in the early-maturing experiment and for the 22 cultivars tested in the extremely early-maturing experiment, both conducted during the 2009/2010 harvest season.

Experiment early-maturing				
Cultivar number	**Commercial name**	**Genetic basis**	**Company**	**Grain texture**
1	20A55	Triple hydrid	Agromen	Semi flint
2	30A91	Simple hydrid	Agromen	Semi flint
3	ATL 200	Triple hydrid	Atlantica	Semi dent
4	BM 207	Double hydrid	Biomatrix	Semi flint
5	BM 822	Simple hydrid	Biomatrix	Semi dent
6	CD 321	Simple hydrid	Coodetec	Semi dent
7	CO 327	Simple modified hydrid	Coodetec	Flint
8	CO 388	Double hydrid	Coodetec	Semi dent
9	DKB 245	Simple hydrid	Dekalb	Flint
10	Dx 510	Triple hydrid	Delta	Semi flint
11	2B655	Triple hydrid	Dow	Semi flint
12	2B688	Triple hydrid	Dow	Semi flint
13	PMS 0219A54	Triple hydrid	Embrapa	Semi flint
14	FTH 404	Double hydrid	FT Sementes	Semi flint
15	FTH 900	Triple hydrid	FT Sementes	Semi flint
16	CEP M 128	Simple hydrid	Fundacep	Semi flint
17	CEP M 130	Simple hydrid	Fundacep	Semi flint
18	CEP M 143	Simple hydrid	Fundacep	Flint
19	GNZ 2005	Triple hydrid	Geneze Sementes	Semi flint
20	GNZ 2728	Double hydrid	Geneze Sementes	Semi flint
21	GNZX 0744	Double hydrid	Geneze Sementes	Semi flint
22	KSP 1356	Simple hydrid	KSP Sementes	Semi flint
23	KSP 3246	Triple hydrid	KSP Sementes	Semi flint
24	BX 945	Simple hydrid	Nidera	Semi dent
25	BG 7060	Triple hydrid	Pioneer	Semi flint
26	P 30B39	Simple modified hydrid	Pioneer	Semi flint
27	SHX 5121	Triple hydrid	Santa Helena	Flint
28	SHX 7222	Simple hydrid	Santa Helena	Flint
29	SHX 7323	Simple hydrid	Santa Helena	Flint
30	XB 6012	Simple hydrid	Semeali	Semi dent
31	XBX 70202	Simple hydrid	Semeali	Semi dent
32	AG 8025	Simple hydrid	Agroceres	Semi flint
33	AG 9040	Simple hydrid	Agroceres	Semi flint
34	P 30R50	Simple hydrid	Pioneer	Semi flint
35	AG 5011	Triple hydrid	Agroceres	Semi dent
36	AG 2020	Double hydrid	Agroceres	Semi flint
Experiment extremely early-maturing				
Cultivar number	**Commercial name**	**Genetic basis**	**Company**	**Grain texture**
1	BM 911	Simple hydrid	Biomatrix	Semi dent
2	Dx 915	Simple modified hydrid	Delta	Semi flint
3	2B433	Triple hydrid	Dow	Semi dent
4	PMS 3919	Simple hydrid	Embrapa	Semi flint
5	PMS 1635A08	Triple hydrid	Embrapa	Semi flint
6	FTH 960	Triple hydrid	FT Sementes	Flint
7	GNZ 0729	Simple hydrid	Geneze Sementes	Semi flint
8	GNZ 9505	Simple hydrid	Geneze Sementes	Semi flint

Table 1. Contd.

9	Bx 898	Simple hydrid	Nidera	Semi dent
10	HS 79707	Simple hydrid	Nidera	Semi dent
11	PRE 12S12	Simple hydrid	Prezzotto	Semi flint
12	PRE 22D11	Double hydrid	Prezzotto	Semi flint
13	PRE 22S11	Simple hydrid	Prezzotto	Semi flint
14	PRE 22T10	Triple hydrid	Prezzotto	Semi flint
15	RBX 79	Simple hydrid	Riber	Semi dent
16	SHS 7090	Simple hydrid	Santa Helena	Semi flint
17	SHX 7111	Simple hydrid	Santa Helena	Semi flint
18	AG 9045	Simple hydrid	Agroceres	Semi flint
19	BALU 7690	Simple hydrid	Sementes Balu	Flint
20	SG 6302	Triple hydrid	Sementes Guerra	Semi flint
21	AG 9020	Simple hydrid	Agroceres	Semi dent
22	BG 7060	Triple hydrid	Pioneer	Semi flint

Source: The relation of the cultivars included in each of the experiments was determined by the Agriculture Research State Foundation (Fundação Estadual de Pesquisa Agropecuária - FEPAGRO), which coordinates the maize cultivars evaluation testing in Rio Grande do Sul, for the 2009/2010 harvest season.

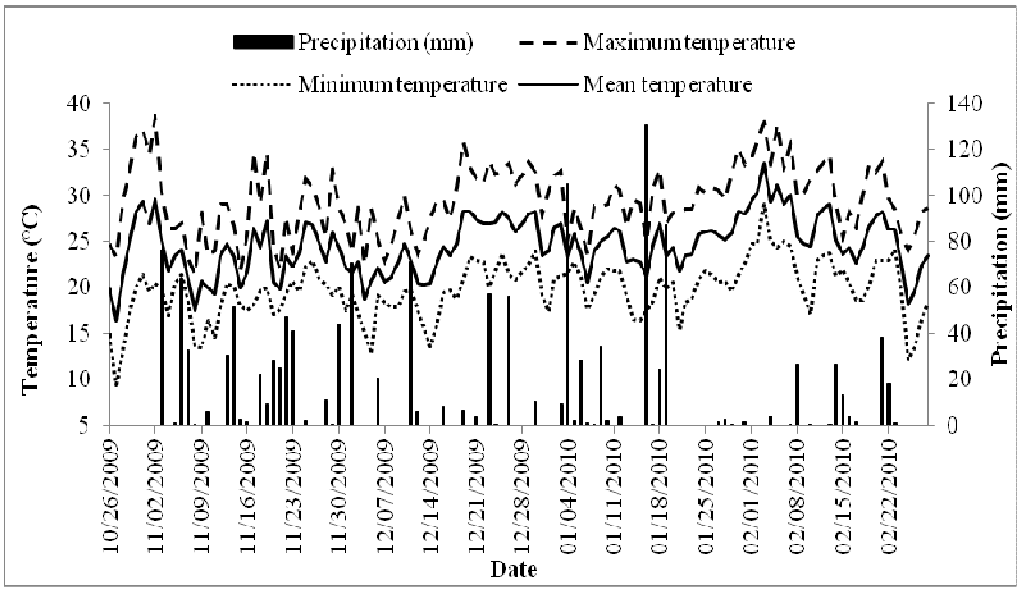

Figure 1. Minimum temperature, maximum temperature, mean daily air temperature (°C) and precipitation (mm).

RESULTS

Assumptions verification and analysis of variance

In the experiment with the early maturing cultivars, the errors in nine of the 13 variables (69.23%) conformed to a normal distribution based on the Kolmogorov-Smirnov test. In the experiment with the extremely early-maturing cultivars, the errors in 12 variables (92.31%) conformed to the normal distribution (Table 2). In both experiments, the residual variances of the variables were homogeneous by Levene's test. Therefore, in general, the assumptions of normality of errors and homogeneity of variances were met, which offered credibility to the results of the analysis of variance.

The high values for selective accuracy (SA) indicated that the experimental precision was high at 7.69% (one variable) and 15.38% (two variables) for the 13 variables

Table 2. Summary of the analysis of variance [number of degrees of freedom (DF) and the mean square for the sources of variation for blocks, cultivars and error], mean, experimental coefficient of variation (CV%), selective accuracy (SA), experimental precision, normality of errors by the Kolmogorov-Smirnov test and homogeneity of residual variances by Levene's test in one experiment with 36 early-maturing corn cultivars and another experiment with 22 extremely early-maturing corn cultivars, both conducted during the 2009/2010 harvest season.

Experiment early-maturing									
Variable[a]	Blocks (DF = 2)	Cultivars (DF = 35)	Error (DF = 70)	Mean	CV(%)	SA	Precision[b]	Normality	Homogeneity
PROD	1.420.030	3.614059*	0.443559	5	13.32	0.94	VH	Normal	Homogeneous
CP	0.115833	0.264911*	0.101864	7.54	4.24	0.78	H	Normal	Homogeneous
Lys	0.000036	0.000136*	0.000026	0.21	2.4	0.9	VH	Non-Normal	Homogeneous
Met	0.00004	0.000067*	0.000011	0.14	2.37	0.91	VH	Non-Normal	Homogeneous
Cys	0.000011	0.000104*	0.00002	0.16	2.81	0.91	VH	Non-Normal	Homogeneous
Thr	0.000118	0.000551*	0.000107	0.21	4.89	0.9	VH	Normal	Homogeneous
Trp	0.000004	0.000071*	0.00001	0.05	6.49	0.93	VH	Non-Normal	Homogeneous
Val	0.000169	0.000965*	0.000087	0.3	3.12	0.95	VH	Normal	Homogeneous
Ile	0.000181	0.000543*	0.000063	0.18	4.4	0.94	VH	Normal	Homogeneous
Leu	0.000381	0.007932*	0.000901	0.79	3.78	0.94	VH	Normal	Homogeneous
Phe	0.000311	0.001372*	0.00012	0.27	4.02	0.96	VH	Normal	Homogeneous
His	0.000045	0.000351*	0.000033	0.19	3.02	0.95	VH	Normal	Homogeneous
Arg	0.000073	0.000611*	0.000063	0.33	2.4	0.95	VH	Normal	Homogeneous
Experiment extremely early-maturing									
Variable[a]	Blocks (DF = 2)	Cultivars (DF = 35)	Error (DF = 70)	Mean	CV(%)	SA	Precision[b]	Normality	Homogeneity
PROD	0.703974	3.557506*	0.39626	5.92	10.64	0.94	VH	Normal	Homogeneous
CP	0.18138	0.217603*	0.051776	7.31	3.11	0.87	H	Normal	Homogeneous
Lys	0.000006	0.000217*	0.000043	0.22	3.02	0.9	VH	Normal	Homogeneous
Met	0.000041	0.000089*	0.000024	0.15	3.34	0.86	H	Normal	Homogeneous
Cys	0.000002	0.000158*	0.000021	0.16	2.92	0.93	VH	Normal	Homogeneous
Thr	0.000024	0.000866*	0.000162	0.22	5.77	0.9	VH	Normal	Homogeneous
Trp	0.000011	0.000097*	0.000019	0.05	8.4	0.9	VH	Non-Normal	Homogeneous
Val	0.000156	0.001091*	0.000143	0.31	3.81	0.93	VH	Normal	Homogeneous
Ile	0.000056	0.000657*	0.000069	0.19	4.37	0.95	VH	Normal	Homogeneous
Leu	0.001156	0.006512*	0.000902	0.84	3.56	0.93	VH	Normal	Homogeneous
Phe	0.000505	0.001380*	0.000152	0.29	4.33	0.94	VH	Normal	Homogeneous
His	0.000018	0.000505*	0.000033	0.19	3.11	0.97	VH	Normal	Homogeneous
Arg	0.000029	0.000945*	0.000079	0.33	2.67	0.96	VH	Normal	Homogeneous

[a]PROD, grain productivity in $t \cdot ha^{-1}$; CP, crude protein as a percentage of raw materials (% RM); Lys, lysine as % RM; Met, methionine as % RM; Cys, cysteine as % RM; Thr, threonine as % RM; Trp, tryptophan as % RM; Val, valine as % RM; Ile, isoleucine as % RM; Leu, leucine as % RM; Phe, phenylalanine as % RM; His, histidine as % RM; and Arg, arginine as % RM. *Significant difference by the F test at the 5% probability of error. [b]Class limits established by Resende and Duarte (2007), with VH being very high (SA ≥ 0.90), H high (0.70 ≤ SA < 0.90), M moderate (0.50 ≤ SA < 0.70) and L low (SA < 0.50).

measured in the experiments with the early and extremely early-maturing cultivars, respectively (Table 2). Likewise, the precision was quite high at 92.31% and 84.62% for the variables measured in the experiments with the early and extremely early-maturing cultivars, respectively (Resende and Duarte, 2007; Cargnelutti Filho and Storck, 2009). Furthermore, the coefficient of variation (CV) was low for all the variables measured in the two experiments (CV ≤ 13.32%).

The effect of cultivar was significant for all the variables measured in the two experiments (Table 2), which indicated that there was genetic variability among the early and extremely early-maturing cultivars for PROD and for the grain CP and amino acid levels. Considering that the assumptions of the model were met, the experimental precisions were high, and there was variability among the cultivars for all the variables, it can be inferred that this database is suitable for genetic diversity studies among cultivars by clustering analysis.

For the experiments with the early and extremely early-maturing cultivars, the PROD averaged 5.00 and 5.92 $t \cdot ha^{-1}$, respectively, and the CV (experimental coefficient of variation) was 13.32% and 10.64% (Table 2). Despite the higher CV for the PROD in relation to the other

variables, the SA was 0.94 for the two experiments, which indicated quite high experimental precision in the measurement of PROD (Resende and Duarte, 2007; Cargnelutti Filho and Storck, 2009). The PROD ranged between 2.7581 and 7.1936 $t \cdot ha^{-1}$ for the early-maturing cultivars (Table 3) and between 3.8391 and 7.8526 $t \cdot ha^{-1}$ for the extremely early-maturing cultivars (Table 4). These results indicated wide variability in the PROD values of the cultivars evaluated in both experiments.

The mean CP content for the early and extremely early-maturing cultivars was 7.54 and 7.31%, respectively (Table 2). The values ranged between 7.0467% and 8.5667% for the early-maturing cultivars (Table 3) and between 6.7600% and 7.9933% for the extremely early-maturing cultivars (Table 4). In both experiments, the experimental precision was high (SA = 0.78 and 0.87) according to the criteria of Resende and Duarte (2007) and Cargnelutti Filho and Storck (2009) (Table 2).

In the experiment with the early-maturing cultivars, the mean % RM value for each amino acid was 0.21 for Lys, 0.14 for Met, 0.16 for Cys, 0.21 for Thr, 0.05 for Trp, 0.30 for Val, 0.18 for Ile, 0.79 for Leu, 0.27 for Phe, 0.19 for His and 0.33 for Arg. In the experiment with the extremely early-maturing cultivars, the mean % RM value for each amino acid was 0.22 for Lys, 0.15 for Met, 0.16 for Cys, 0.22 for Thr, 0.05 for Trp, 0.31 for Val, 0.19 for Ile, 0.84 for Leu, 0.29 for Phe, 0.19 for His and 0.33 for Arg (Table 2). Thus, the mean percentage for each amino acid was found to be similar between the early and extremely early-maturing cultivars. However, these values fluctuated widely (variability) among the early-maturing cultivars (Table 3) and among the extremely early-maturing cultivars (Table 4).

The CV values associated with the amino acid measurements ranged from 2.37 to 6.49% in the early-maturing cultivars, resulting from the Met and Trp variables, respectively, and from 2.67 to 8.40% in the extremely early-maturing cultivars, resulting from the Arg and Trp variables, respectively (Table 2). The SA in the experiment with the early-maturing cultivars ranged between 0.90 and 0.96, indicating that the experimental precision was quite high for all the variables. In the experiment with the extremely early-maturing cultivars, the SA varied between 0.86 for the Met variable and 0.97 for the His variable, indicating that for most of the amino acids (90.9%), the experimental precision was quite high according to the criteria established by Resende and Duarte (2007) and Cargnelutti Filho and Storck (2009).

From the Scott-Knott means comparison test, two or more groupings of the cultivars were obtained for all the variables in both experiments (Tables 3 and 4). These results confirmed the above inference that there was genetic variability among the cultivars of the same plant maturity class for PROD and for grain CP and amino acid levels. Thus, it is possible to investigate the genetic variability among cultivars through cluster analysis.

Diagnosis of multicollinearity and elimination of variables

The diagnosis of multicollinearity based on the matrices of genetic correlations between the 13 variables resulted in a CN of 40,261 and 2,220, respectively, for the early and extremely early-maturing cultivars. Therefore, in both experiments, multicollinearity was severe according to the criteria of Montgomery and Peck (1982), and clustering analysis is not recommended under this condition because the multicollinear variables contribute greater weight in the clustering process (Cruz and Carneiro, 2006). With the elimination of the highly correlated variables (Cys, Thr, Trp, Ile, Phe, His and Arg for the early-maturing cultivars and Thr, Trp, Val, Ile, Phe, His and Arg for the extremely early-maturing cultivars), the CN was reduced to 86 and 93, respectively, which indicated weak collinearity and therefore enabled the clustering analysis to proceed satisfactorily.

Analysis and validation of clusters

In both experiments, the dendrogram was generated by the average linkage between groups method of grouping (UPGMA) using D^2 as a measure of dissimilarity. For the 36 early-maturing cultivars (Figure 3A), five groupings were obtained based on the PROD, CP, Lys, Met, Val and Leu variables, using 50% as the criterion of dissimilarity. The number of cultivars in Groups 1 through 5 was 21, 10, 1, 3 and 1, respectively. The 22 extremely early-maturing cultivars were divided into four groups (Figure 2B) based on the PROD, CP, Lys, Met, Cys and Leu variables and using 50% dissimilarity as the criterion for defining the groups. The number of cultivars in Groups 1 through 4 was 13, 2, 6 and 1, respectively. The CCCs of 0.67 for the early-maturing cultivar groups (Figure 2A) and of 0.58 for the extremely early-maturing cultivar groups (Figures 2B), were statistically significant, which indicated good relationships between the D^2 matrix and the graphical distance matrix and, consequently, good group consistency.

The means of the six variables used in the clustering of the early-maturing cultivars and of the six variables used in the clustering of the extremely early-maturing cultivars differed between the groups, which revealed contrasting groups (Table 5). For the early-maturing cultivars, Groups 2 and 3 had the highest mean PROD, followed by Groups 1, 4 and 5. Group 5 had the highest mean CP. The highest mean Lys values were observed in Groups 3, 4 and 5, followed by Groups 1 and 2. Met showed the highest means in groups 3 and 4, followed by Group 1 and then Groups 2 and 5. Group 4 showed the highest mean Val value and Group 2, the lowest. For Leu, Groups 4 and 5 showed the highest mean values, followed by Group 1 and then Groups 2 and 3.

Table 3. Means for grain productivity (PROD) in t·ha^{-1}, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) in 36 early-maturing corn cultivars evaluated during the 2009/2010 harvest.

Nº	Cultivar	PROD	CP	Lys	Met	Cys	Thr	Trp
1	20A55	5.2847^{ba}	7.2767^{b}	0.2100^{c}	0.1400^{b}	0.1600^{b}	0.2133^{b}	0.0500^{c}
2	30A91	4.8165^{b}	8.0000^{a}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2167^{b}	0.0500^{c}
3	ATL 200	4.3258^{c}	7.6167^{b}	0.2133^{b}	0.1467^{a}	0.1633^{b}	0.2133^{b}	0.0533^{b}
4	BM 207	5.0614^{b}	7.3833^{b}	0.2100^{c}	0.1467^{a}	0.1567^{c}	0.2133^{b}	0.0500^{c}
5	BM 822	6.5684^{a}	7.3333^{b}	0.2067^{c}	0.1433^{b}	0.1533^{c}	0.1967^{c}	0.0400^{d}
6	CD 321	3.1912^{d}	7.4467^{b}	0.2000^{d}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
7	CO 327	3.8575^{c}	7.5333^{b}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2200^{b}	0.0500^{c}
8	CO 388	3.4642^{d}	7.4333^{b}	0.2133^{b}	0.1433^{b}	0.1567^{c}	0.2267^{a}	0.0500^{c}
9	DKB 245	6.0506^{a}	7.0467^{b}	0.2033^{d}	0.1400^{b}	0.1500^{c}	0.1933^{c}	0.0400^{d}
10	Dx 510	3.4902^{d}	7.4733^{b}	0.2300^{a}	0.1533^{a}	0.1733^{a}	0.2400^{a}	0.0600^{a}
11	2B655	5.1640^{b}	7.1667^{b}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2100^{b}	0.0500^{c}
12	2B688	5.8645^{b}	7.3767^{b}	0.2100^{c}	0.1400^{b}	0.1533^{c}	0.2100^{b}	0.0500^{c}
13	PMS0219A54	4.5828^{c}	7.5567^{b}	0.2133^{b}	0.1400^{b}	0.1533^{c}	0.2133^{b}	0.0467^{c}
14	FTH 404	4.5645^{c}	7.3033^{b}	0.2100^{c}	0.1400^{b}	0.1567^{c}	0.2067^{c}	0.0500^{c}
15	FTH 900	4.5317^{c}	7.5367^{b}	0.2167^{b}	0.1433^{b}	0.1600^{b}	0.2200^{b}	0.0500^{c}
16	CEPM128	5.2400^{b}	7.5100^{b}	0.2133^{b}	0.1500^{a}	0.1600^{b}	0.2333^{a}	0.0533^{b}
17	CEPM130	5.1388^{b}	7.5733^{b}	0.2100^{c}	0.1467^{a}	0.1600^{b}	0.2167^{b}	0.0500^{c}
18	CEPM143	3.8565^{c}	7.4100^{b}	0.2100^{c}	0.1367^{b}	0.1567^{c}	0.2133^{b}	0.0500^{c}
19	GNZ 2005	5.2137^{b}	7.4367^{b}	0.2200^{a}	0.1500^{a}	0.1633^{b}	0.2267^{a}	0.0500^{c}
20	GNZ 2728	3.3693^{d}	7.6467^{b}	0.2233^{a}	0.1500^{a}	0.1667^{a}	0.2233^{a}	0.0533^{b}
21	GNZX0744	2.7581^{d}	7.5133^{b}	0.2200^{a}	0.1533^{a}	0.1700^{a}	0.2367^{a}	0.0567^{a}
22	KSP 1356	5.3924^{b}	7.1033^{b}	0.2000^{d}	0.1367^{b}	0.1500^{c}	0.1900^{c}	0.0400^{d}
23	KSP 3246	6.7217^{a}	7.5400^{b}	0.2067^{c}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
24	BX 945	5.7612^{b}	7.3767^{b}	0.2033^{d}	0.1400^{b}	0.1600^{b}	0.1933^{c}	0.0433^{d}
25	BG 7060	5.1401^{b}	8.0933^{a}	0.2167^{b}	0.1400^{b}	0.1633^{b}	0.2067^{c}	0.0500^{c}
26	P30B39	3.4087^{d}	7.5800^{b}	0.2167^{b}	0.1433^{b}	0.1633^{b}	0.2233^{a}	0.0500^{c}
27	SHX 5121	5.1536^{b}	7.8400^{a}	0.2067^{c}	0.1400^{b}	0.1600^{b}	0.2000^{c}	0.0500^{c}
28	SHX 7222	5.6476^{b}	7.6000^{b}	0.2033^{d}	0.1367^{b}	0.1533^{c}	0.1833^{c}	0.0400^{d}
29	SHX 7323	4.1077^{c}	8.5667^{a}	0.2200^{a}	0.1400^{b}	0.1700^{a}	0.2267^{a}	0.0533^{b}
30	XB 6012	5.9336^{b}	8.0867^{a}	0.2067^{c}	0.1400^{b}	0.1633^{b}	0.2167^{b}	0.0500^{c}
31	XBX 70202	5.7330^{b}	7.6933^{b}	0.2200^{a}	0.1500^{a}	0.1600^{b}	0.2167^{b}	0.0500^{c}
32	AG 8025	7.1936^{a}	7.5867^{b}	0.2000^{d}	0.1400^{b}	0.1567^{c}	0.1933^{c}	0.0400^{d}
33	AG 9040	6.4125^{a}	7.6200^{b}	0.2100^{c}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
34	P30R50	6.5827^{a}	7.5300^{b}	0.2133^{b}	0.1433^{b}	0.1633^{b}	0.2067^{c}	0.0500^{c}
35	AG 5011	5.1031^{b}	7.3233^{b}	0.2133^{b}	0.1500^{a}	0.1600^{b}	0.2233^{a}	0.0533^{b}
36	AG 2020	5.3447^{b}	7.1467^{b}	0.2100^{c}	0.1400^{b}	0.1633^{b}	0.2033^{c}	0.0500^{c}
Nº	Groups	4	2	4	2	3	3	4

Nº	Cultivar	Val	Ile	Leu	Phe	His	Arg
1	20A55	0.3100$^{b\,a}$	0.1867^{b}	0.8500^{b}	0.2833^{c}	0.1933^{b}	0.3267^{c}
2	30A91	0.3067^{b}	0.1833^{b}	0.8367^{b}	0.2867^{c}	0.1867^{c}	0.3267^{c}
3	ATL200	0.3200^{a}	0.1900^{b}	0.8367^{b}	0.2900^{c}	0.2000^{b}	0.3400^{b}
4	BM 207	0.2933^{c}	0.1733^{c}	0.7733^{c}	0.2733^{c}	0.1867^{c}	0.3267^{c}
5	BM 822	0.2800^{d}	0.1700^{c}	0.7667^{c}	0.2633^{d}	0.1700^{d}	0.3133^{d}
6	CD 321	0.2800^{d}	0.1733^{c}	0.7700^{c}	0.2533^{d}	0.1800^{d}	0.3133^{d}
7	CO327	0.3033^{b}	0.1867^{b}	0.8000^{c}	0.2767^{c}	0.1933^{b}	0.3300^{c}
8	CO388	0.3000^{c}	0.1867^{b}	0.8033^{c}	0.2767^{c}	0.1867^{c}	0.3367^{b}

Table 3. Contd.

9	DKB245	0.2667[d]	0.1533[d]	0.7167[d]	0.2367[e]	0.1733[d]	0.3100[d]
10	Dx5 10	0.3400[a]	0.2100[a]	0.8933[a]	0.3200[a]	0.2133[a]	0.3567[a]
11	2B655	0.2967[c]	0.1800[c]	0.7833[c]	0.2700[d]	0.1867[c]	0.3200[c]
12	2B688	0.2900[c]	0.1733[c]	0.7900[c]	0.2667[d]	0.1833[c]	0.3133[d]
13	PMS0219A54	0.2833[d]	0.1700[c]	0.7200[d]	0.2533[d]	0.1800[d]	0.3267[c]
14	FTH 404	0.2867[c]	0.1700[c]	0.7800[c]	0.2633[d]	0.1867[c]	0.3267[c]
15	FTH 900	0.2967[c]	0.1833[b]	0.7633[c]	0.2767[c]	0.1933[b]	0.3367[b]
16	CEPM128	0.3233[a]	0.2033[a]	0.8500[b]	0.3033[b]	0.2000[b]	0.3467[a]
17	CEPM130	0.3067[b]	0.1867[b]	0.8067[c]	0.2833[c]	0.1967[b]	0.3367[b]
18	CEPM143	0.2933[c]	0.1767[c]	0.7933[c]	0.2633[d]	0.1867[c]	0.3267[c]
19	GNZ 2005	0.3200[a]	0.1900[b]	0.8567[b]	0.3000[b]	0.2000[b]	0.3500[a]
20	GNZ 2728	0.3267[a]	0.1933[b]	0.8700[a]	0.3067[b]	0.2033[b]	0.3533[a]
21	GNZX 0744	0.3300[a]	0.2100[a]	0.8933[a]	0.3167[a]	0.2100[a]	0.3567[a]
22	KSP 1356	0.2700[d]	0.1600[d]	0.7000[d]	0.2367[e]	0.1733[d]	0.3100[d]
23	KSP 3246	0.2833[d]	0.1700[c]	0.7833[c]	0.2533[d]	0.1800[d]	0.3233[c]
24	BX 945	0.2833[d]	0.1733[c]	0.7600[c]	0.2567[d]	0.1800[d]	0.3100[d]
25	BG 7060	0.3067[b]	0.1800[c]	0.8167[b]	0.2767[c]	0.2033[b]	0.3467[a]
26	P30B39	0.3100[b]	0.1867[b]	0.7800[c]	0.2767[c]	0.2000[b]	0.3433[b]
27	SHX 5121	0.2933[c]	0.1700[c]	0.8100[c]	0.2667[d]	0.1900[c]	0.3267[c]
28	SHX 7222	0.2733[d]	0.1600[d]	0.7567[c]	0.2400[e]	0.1800[d]	0.3100[d]
29	SHX 7323	0.3167[b]	0.1867[b]	0.8967[a]	0.2967[b]	0.2100[a]	0.3567[a]
30	XB 6012	0.3133[b]	0.1900[b]	0.8000[c]	0.2767[c]	0.2000[b]	0.3400[b]
31	XBX 70202	0.2933[c]	0.1800[c]	0.7433[d]	0.2633[d]	0.1867[c]	0.3367[b]
32	AG 8025	0.2867[c]	0.1667[c]	0.7300[d]	0.2500[e]	0.1867[c]	0.3200[c]
33	AG 9040	0.2800[d]	0.1600[d]	0.7133[d]	0.2400[e]	0.1767[d]	0.3167[d]
34	P30R50	0.2900[c]	0.1733[c]	0.7633[c]	0.2633[d]	0.1867[c]	0.3300[c]
35	AG 5011	0.3200[a]	0.2000[a]	0.8233[b]	0.2933[b]	0.1833[c]	0.3333[b]
36	AG 2020	0.2967[c]	0.1800[c]	0.7733[c]	0.2667[d]	0.1900[c]	0.3267[c]
Nº	Groups	4	4	4	5	4	4

[a]Means followed by the same letter do not differ by the Scott-Knott test at the 5% level of probability.

Table 4. Means for grain productivity (PROD) in $t \cdot ha^{-1}$, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) in 22 extremely early-maturing corn cultivars evaluated during the 2009/2010 harvest.

Nº	Cultivar	PROD	CP	Lys	Met	Cys	Thr	Trp
1	BM 911	7.2870[a a]	7.7833[a]	0.2167[b]	0.1433[b]	0.1600[b]	0.2200[a]	0.0500[b]
2	Dx 915	5.3894[c]	7.4633[b]	0.2267[a]	0.1500[a]	0.1667[a]	0.2400[a]	0.0567[a]
3	2B433	6.6780[b]	7.4233[b]	0.2233[a]	0.1433[b]	0.1567[c]	0.2300[a]	0.0533[a]
4	PMS 3919	6.9009[a]	7.2167[c]	0.2167[b]	0.1500[a]	0.1500[c]	0.2067[b]	0.0467[b]
5	PMS 1635A08	5.7010[c]	7.2733[c]	0.2033[c]	0.1433[b]	0.1500[c]	0.2067[b]	0.0467[b]
6	FTH 960	6.1784[b]	7.1033[c]	0.2100[c]	0.1433[b]	0.1567[c]	0.2167[b]	0.0533[a]
7	GNZ 0729	4.4088[d]	7.9933[a]	0.2300[a]	0.1567[a]	0.1733[a]	0.2467[a]	0.0600[a]
8	GNZ 9505	5.1994[c]	7.4800[b]	0.2333[a]	0.1500[a]	0.1633[b]	0.2433[a]	0.0600[a]
9	Bx 898	7.0347[a]	7.2600[c]	0.2000[c]	0.1333[b]	0.1500[c]	0.1833[b]	0.0400[c]
10	HS 79707	6.3075[b]	7.2633[c]	0.2100[c]	0.1367[b]	0.1533[c]	0.2033[b]	0.0500[b]
11	PRE 12S12	3.8391[d]	7.0567[c]	0.2200[a]	0.1467[a]	0.1633[b]	0.2300[a]	0.0567[a]
12	PRE 22D11	4.3221[d]	7.4433[b]	0.2233[a]	0.1500[a]	0.1667[a]	0.2400[a]	0.0567[a]

Table 4. Contd.

13	PRE 22S11	5.9081[b]	7.3967[b]	0.2167[b]	0.1433[b]	0.1600[b]	0.2267[a]	0.0500[b]
14	PRE 22T10	5.1617[c]	7.4400[b]	0.2233[a]	0.1467[a]	0.1633[b]	0.2267[a]	0.0567[a]
15	RBX 79	7.3071[a]	6.7600[d]	0.2067[c]	0.1433[b]	0.1467[c]	0.2000[b]	0.0400[c]
16	SHS 7090	5.1798[c]	7.3833[b]	0.2167[b]	0.1500[a]	0.1600[b]	0.2300[a]	0.0567[a]
17	SHX 7111	6.1691[b]	7.1933[c]	0.2133[b]	0.1400[b]	0.1533[c]	0.2000[b]	0.0500[b]
18	AG 9045	7.8526[a]	7.3567[b]	0.2100[c]	0.1433[b]	0.1567[c]	0.2100[b]	0.0500[b]
19	BALU 7690	5.7573[c]	7.1533[c]	0.2133[b]	0.1433[b]	0.1533[c]	0.2100[b]	0.0467[b]
20	SG 6302	4.4419[d]	7.3900[b]	0.2267[a]	0.1533[a]	0.1700[a]	0.2433[a]	0.0567[a]
21	AG 9020	6.3993[b]	6.8167[d]	0.2167[b]	0.1500[a]	0.1500[c]	0.2133[b]	0.0500[b]
22	BG 7060	6.7783[a]	7.2367[c]	0.2200[a]	0.1500[a]	0.1633[b]	0.2267[a]	0.0567[a]
Nº	Groups	4	4	3	2	3	2	3

Nº	Cultivar	Val	Ile	Leu	Phe	His	Arg
1	BM 911	0.3067[ba]	0.1867[c]	0.7967[d]	0.2767[c]	0.1900[b]	0.3367[b]
2	Dx 915	0.3300[a]	0.2000[b]	0.8700[b]	0.3000[b]	0.1967[a]	0.3533[a]
3	2B433	0.3100[b]	0.1867[c]	0.8167[c]	0.2800[c]	0.1833[c]	0.3300[c]
4	PMS 3919	0.3067[b]	0.1800[c]	0.8367[c]	0.2733[c]	0.1833[c]	0.3267[c]
5	PMS 1635A08	0.3033[b]	0.1867[c]	0.8300[c]	0.2733[c]	0.1733[d]	0.3133[d]
6	FTH 960	0.3033[b]	0.1833[c]	0.8200[c]	0.2733[c]	0.1867[b]	0.3300[c]
7	GNZ 0729	0.3467[a]	0.2200[a]	0.9267[a]	0.3233[a]	0.2067[a]	0.3600[a]
8	GNZ 9505	0.3400[a]	0.2067[b]	0.9100[a]	0.3133[a]	0.1933[b]	0.3533[a]
9	Bx 898	0.2767[c]	0.1567[d]	0.7800[d]	0.2467[d]	0.1667[d]	0.3000[d]
10	HS 79707	0.3000[b]	0.1833[c]	0.8367[c]	0.2733[c]	0.1700[d]	0.3067[d]
11	PRE 12S12	0.3300[a]	0.2000[b]	0.8667[b]	0.2967[b]	0.1967[a]	0.3433[b]
12	PRE 22D11	0.3367[a]	0.2067[b]	0.9100[a]	0.3133[a]	0.2000[a]	0.3500[a]
13	PRE 22S11	0.3067[b]	0.1900[c]	0.8433[c]	0.2833[c]	0.1867[b]	0.3300[c]
14	PRE 22T10	0.3267[a]	0.1967[b]	0.8767[b]	0.2967[b]	0.1967[a]	0.3400[b]
15	RBX 79	0.2867[c]	0.1700[d]	0.7667[d]	0.2500[d]	0.1667[d]	0.3067[d]
16	SHS 7090	0.3167[b]	0.1933[b]	0.8500[c]	0.2967[b]	0.1900[b]	0.3433[b]
17	SHX 7111	0.2967[b]	0.1767[c]	0.8233[c]	0.2700[c]	0.1733[d]	0.3167[d]
18	AG 9045	0.3033[b]	0.1833[c]	0.8233[c]	0.2767[c]	0.1833[c]	0.3233[c]
19	BALU 7690	0.2967[b]	0.1767[c]	0.7667[d]	0.2567[d]	0.1700[d]	0.3133[d]
20	SG 6302	0.3467[a]	0.2167[a]	0.9233[a]	0.3200[a]	0.2067[a]	0.3567[a]
21	AG 9020	0.3100[b]	0.1900[c]	0.8267[c]	0.2767[c]	0.1700[d]	0.3233[c]
22	BG 7060	0.3260[a]	0.1967[b]	0.8633[b]	0.3000[b]	0.2000[a]	0.3467[b]
Nº	Groups	2	4	4	4	4	4

[a]Means followed by the same letter do not differ by the Scott-Knott test at the 5% level of probability.

For the extremely early-maturing cultivars, group 2 had the highest mean PROD, followed by Group 1 and then Groups 3 and 4 (Table 5). The mean CP value was highest in Group 4, followed by Groups 1 and 3, and was lowest in Group 2. The mean Lys value was highest in Groups 4 and 5, followed by Group 1, and was lowest in Group 2. For the Met, Cys and Leu variables, the highest means were in Group 4, followed by Group 3, then Group 1 and finally Group 2, with the lowest mean.

In the early-maturing (PROD, CP, Lys, Met, Val and Leu variables) and extremely early-maturing (PROD, CP, Lys, Met, Leu and Cys variables) cultivars after the completion of the MANOVA using Wilk's criterion, the group mean vectors were found to differ. In the experiment with the early-maturing cultivars, considering the results from the comparison tests of the group means (Table 5) and the vectors of the means (Figure 3A), Group 2 was found to have higher PROD values and, in general, the smallest values of CP, Lys, Met, Val and Leu compared with the other groups. In addition, Groups 4 and 5 showed lower mean PROD and, generally, higher mean CP, Lys, Met, Val and Leu values.

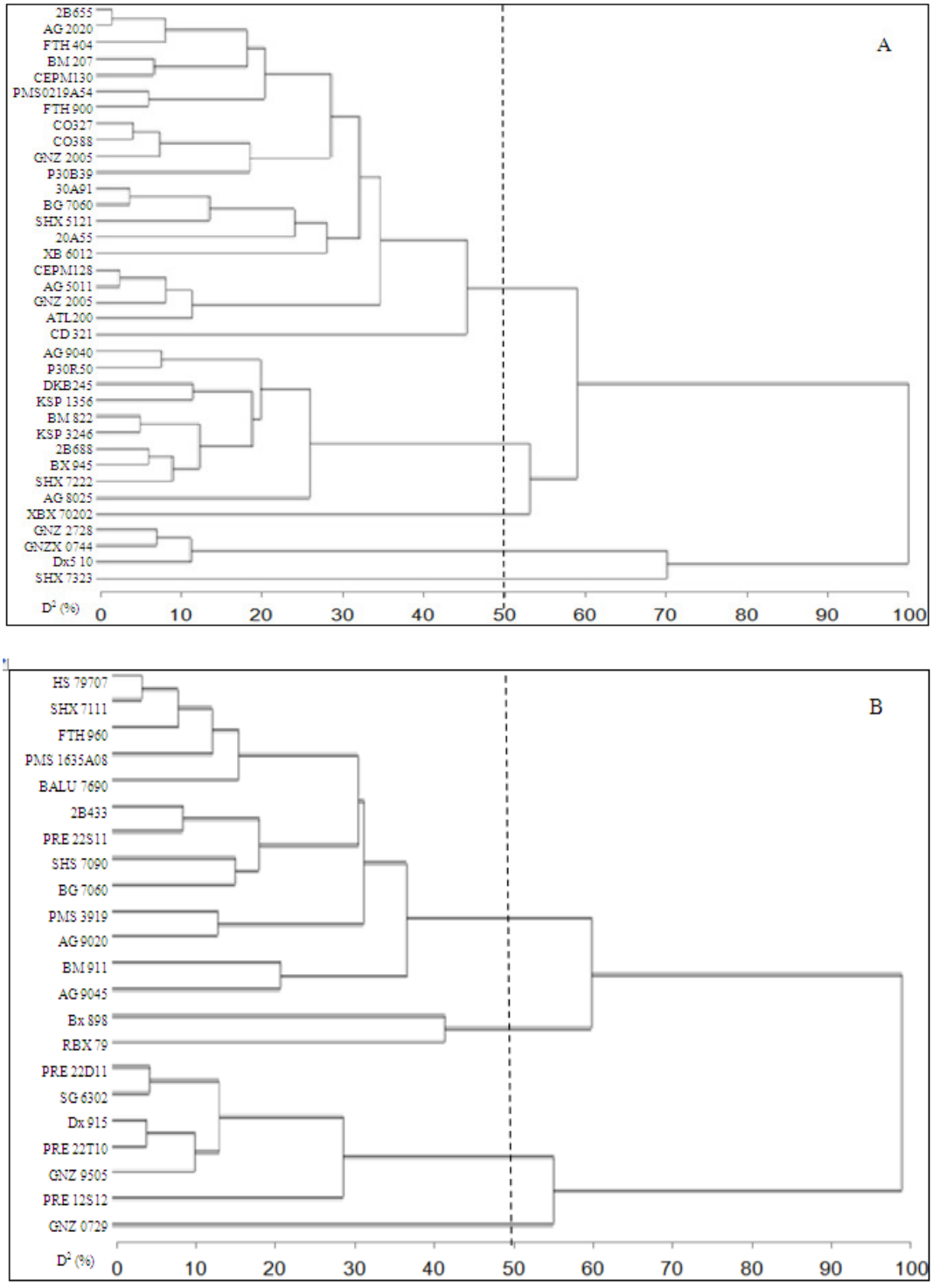

Figure 2. Dendrogram obtained by the average linkage between groups hierarchical clustering method (UPGMA) from the Mahalanobis generalized distance for 36 early-maturing corn cultivars. The variables used in the cluster were grain productivity, crude protein, lysine, methionine, valine and leucine. The cophenetic correlation coefficient was 0.67, which was significant at the 5% level of probability (A). Dendrogram obtained by the average linkage between groups hierarchical clustering method (UPGMA) from the Mahalanobis generalized distance for 22 extremely early-maturing corn cultivars. The variables used in the cluster were grain productivity, crude protein, lysine, methionine, cysteine and leucine. The cophenetic correlation coefficient was 0.58, which was significant at the 5% level of probability (B).

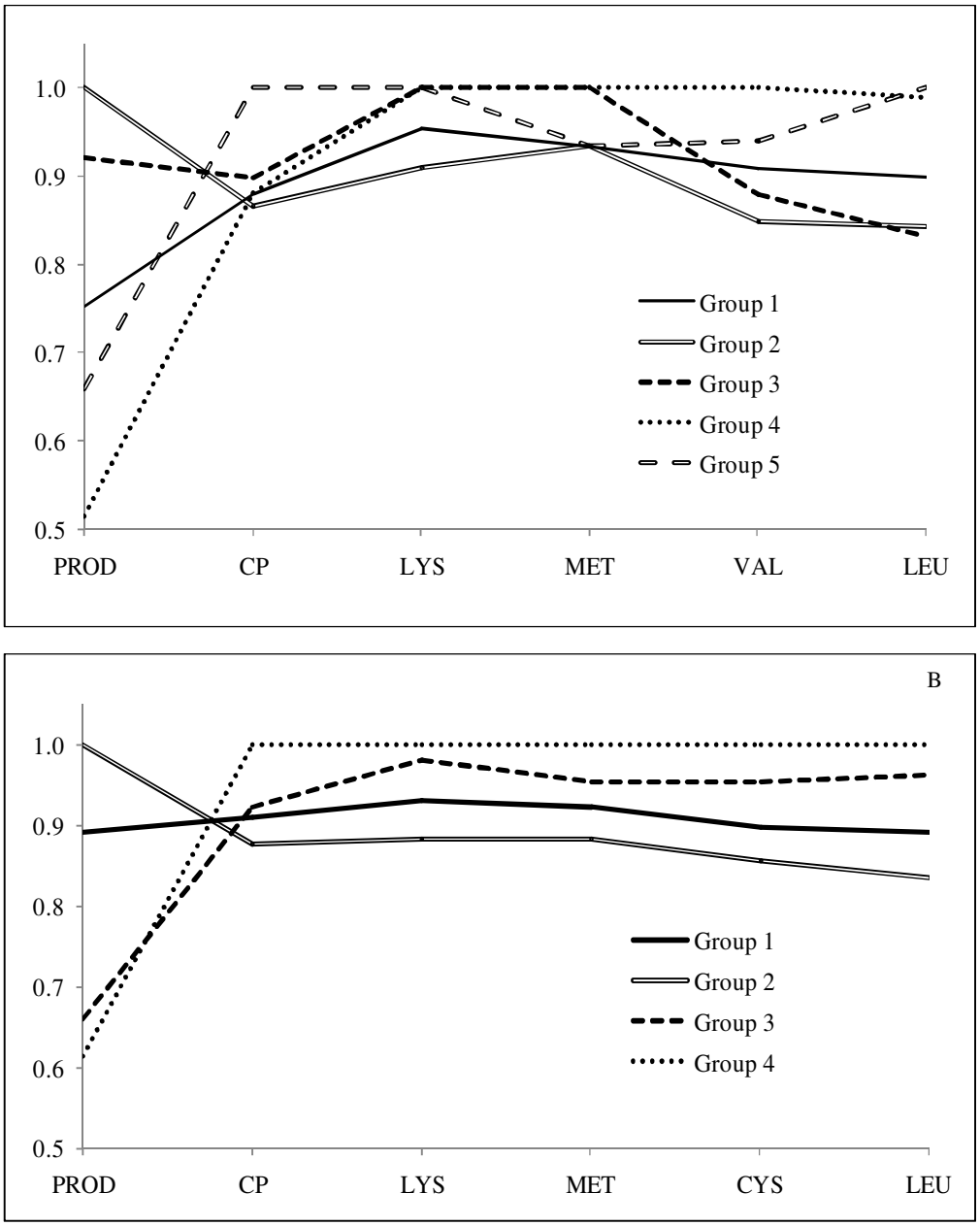

Figure 3. Profile of means for grain productivity (PROD), crude protein (CP), lysine (Lys), methionine (Met), valine (Val) and leucine (Leu) in early-maturing corn cultivars for each of the five groups (A). Profile of means for PROD, CP, Lys, Met, cysteine (Cys) and Leu in extremely early-maturing corn cultivars for each of the four groups (B).

For the experiment with the extremely early-maturing cultivars, Group 2, formed by the cultivars with higher PROD values, was found to have the lowest CP and amino acid levels (Figure 3B). By contrast, Group 4, formed by the cultivars with lower PROD values, showed higher CP, Lys, Met, Leu and Cys levels. Behavior similar to that of Group 4 was also observed for Group 3.

However, the cultivars of Group 1 showed intermediate values for all the variables measured. Thus, based on the results of the experiment with the early-maturing cultivars (Table 4 and Figure 3A) and, especially, the results of the experiment with the extremely early-maturing cultivars (Table 4 and Figure 3B), as the PROD of a cultivar group increased, the group protein and essential amino acid

Table 5. Comparison of means between groups for grain productivity (PROD) in t·ha^{-1}, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), valine (Val) as and leucine (Leu) in 36 early-maturing corn cultivars allocated to five groups formed by the UPGMA. Comparison of means between groups for PROD, CP, Lys, Met, cysteine (Cys) and Leu 22 extremely early-maturing corn cultivars allocated to five groups formed by the UPGMA.

Experiment early-maturing							
Numbers cultivars	**Group***	**PROD**	**CP**	**Lys**	**Met**	**Val**	**Leu**
21	1	4.68462^{b}	7.53587^{b}	0.21190^{b}	0.14270^{b}	0.30286^{c}	0.80127^{b}
10	2	6.21951^{a}	7.41133^{b}	0.20567^{c}	0.14000^{c}	0.28033^{d}	0.74800^{c}
1	3	5.73301^{a}	7.69333^{b}	0.22000^{a}	0.15000^{a}	0.29333^{c}	0.74333^{c}
3	4	3.20584^{b}	7.54444^{b}	0.22444^{a}	0.15222^{a}	0.33222^{a}	0.88556^{a}
1	5	4.10773^{b}	8.56667^{a}	0.22000^{a}	0.14000^{c}	0.31667^{b}	0.89667^{a}
Experiment extremely early-maturing							
Numbers cultivars	**Group***	**PROD**	**CP**	**Lys**	**Met**	**Cys**	**Leu**
13	1	6.39209^{b}	7.27692^{b}	0.21436^{b}	0.14462^{c}	0.15564^{c}	0.82564^{c}
2	2	7.17089^{a}	7.01000^{c}	0.20333^{c}	0.13833^{d}	0.14833^{d}	0.77333^{d}
6	3	4.72558^{c}	7.37889^{b}	0.22556^{a}	0.14944^{b}	0.16556^{b}	0.89278^{b}
1	4	4.40876^{c}	7.99333^{a}	0.23000^{a}	0.15667^{a}	0.17333^{a}	0.92667^{a}

*For each variable, group means within columns followed by different letters differed using a t test at the 5% probability level.

levels declined.

DISCUSSION

Assumptions and analysis of variance

In both experiments, the high proportion of variables with normally distributed errors and the homogeneity observed among the residual variances for all the variables indicated that the database was suitable for performing the analysis of variance and complementary studies. The analysis of variance based on the 13 measured variables showed that the early and extremely early-maturing cultivars were genetically variable. The existence of genetic variability is essential in a breeding program, as highlighted by Vilela et al. (2008), and is a requirement for performing clustering analysis. Furthermore, the experimental precision based on the SA values was found to be high or extremely high in the present study. Accordingly, Cargnelutti Filho and Storck (2009) noted that SA is more suitable than the coefficient of variation (CV) for the verification of experimental precision in genetic studies. In addition, according to Resende and Duarte (2007), an SA value exceeding 0.90 (exceptionally high experimental precision) would be ideal for safer statistical inference.

The Scott-Knott means comparison test confirmed the existence of genetic variability, forming two or more distinct groupings of cultivars for all the variables measured (Tables 3 and 4). The mean PROD of the early and extremely early-maturing cultivars was 5.00 and 5.92 t·ha^{-1}, respectively, which surpassed the 4.46 t·ha^{-1} mean productivity of the state of Rio Grande do Sul, Brazil for the 2009/2010 crop (CONAB, 2010). However, some of the 36 early maturing and 22 extremely early-maturing cultivars showed PROD values that were below the state average. The variations occurred for each variable in both early and extremely early maturing cultivars are related to the genetic characteristics of each cultivar, once all the genotypes were exposed to similar growing conditions, indicating the existence of variability. According to Zhu and Khan (2001), the genotype, the environment and the interaction between these factors influence the protein and amino acid composition in grain. Although, the protein content of corn is less expressive than its carbohydrates content, variations in the content and amino acid composition of this nutrient can cause significant effects on animal metabolism, which uses this cereal as a major ingredient of the diet.

CP is the major variable in the chemical composition of food to be determined to adequately balance animal feed (Vieira et al., 2007). In the experiments with the early-maturing and extremely early-maturing corn cultivars, the grain CP content averaged 7.54 and 7.31% RM, respectively. These values were lower than those observed by Has et al. (2010), which ranged from 11.2 to 15.6%, and also lower than those described by Prasanna et al. (2001), which ranged from 8.9 to 10.2%. Aliu et al. (2012) found CP values between 11.02 and 13.02%. However, Vieira et al. (2007) reported levels between 6.73% and 10.04%, similar to those obtained in the present study. Moore et al. (2008) evaluated six maize hybrids, found an average value PB of 5.16%, lower than

those observed in this study. Although these results suggested that the cultivars used in the present study, which are available for cultivation in Rio Grande do Sul, have low CP contents, some studies have shown that the grain CP content varies with the level of N fertilizer, making it difficult to obtain convergent values (Shewry, 2007). Furthermore, Singh et al. (2005) determined the variability in corn protein by near-infrared transmission and observed CP values between 5.7 and 11.0%, with the highest protein content in plots who received 202 kg ha^{-1} of N, close to those used in this study, of 200 $kg \cdot ha^{-1}$ of N. The protein quality of corn grains depends on their amino acid profile. In this sense, the corn grains were deficient in essential amino acids, such as Lys, Met and Trp (Sofi et al., 2009). The mean values of amino acids obtained in the present study were lower than the levels determined by Abou-Deif et al. (2012), except for Ile, Cys and Trp, which were not quantified by the authors. In a study by Moore et al. (2008), were obtained lower amino acid values that in the present study, with the exception of TRP, ILE and FEN which presented similar values in early genotypes and TRP and ILE in superprecoce genotypes. Values higher than in the present study were reported by Lovatto et al. (2006) for the amino acids LIS, MET, THR, TRP, VAL, IT, READ, FEN, HIS and ARG. Although the corn kernels are use essentially as energetic ingredients in feed, variations in its protein content as well as in its composition in its amino acid profile, can cause significant effects on animal metabolism, which have this cereal as the majority of their diet.

Diagnosis of multicollinearity and elimination of variables

In clustering analysis, the diagnosis of multicollinearity is a basic assumption to be met because the variables will be weighted more heavily in the presence of multicollinearity (Barroso and Artes, 2003; Hair et al., 2009). The degree of multicollinearity of the matrix can be established based on the condition number, which is the ratio between the higher and the smallest eigenvalue of the matrix. Thus, when the number of conditions is less than 100, multicollinearity is weak; between 100 and 1000, multicollinearity is moderate to heavy, and when it is larger than 1000, multicollinearity is severe (Montgomery and Peck, 1982). In the case of the moderately or severe multicollinearity, proceeded to elimination of highly correlated variables.

In the study developed by Alves et al. (2014), was also observed severe multicollinearity among the ethereal extract and nitrogen-corrected apparent metabolizable energy variables in the extremely early-maturing cultivars. In a clustering analysis of corn crops, Cargnelutti Filho and Guadagnin (2011) performed a diagnosis of multicollinearity. These authors found that it was not necessary to discard variables because those used showed a low degree of multicollinearity, whereas certain variables were discarded in the present study because they created multicollinearity.

Analysis and validation of clusters

After variability among the cultivars was verified, the study of genetic diversity in corn was initiated based on a hierarchical method, enabling the classification of cultivars into groups. The formation of groups using hierarchical methods was also utilized by Osorno and Carena (2008), Silva et al. (2009), Subramanian and Subbaraman (2010), Oliboni et al. (2012) and Alves et al. (2014). In clustering analysis, the average linkage between groups hierarchical method (UPGMA), which was considered to be usual and appropriate by Mohammadi et al. (2003) and Cargnelutti Filho and Guadagnin (2011), was used. In the early and extremely early-maturing corn cultivars examined in the present study, five and four groupings, respectively were formed. According to Osorno and Carena (2008), the clustering analysis by UPGMA method showed the presence of five diversity groups based on their breeding origin. Osorno and Carena (2008), used ten early maturity maize improved populations, in a diallel mating design without reciprocals, based on grain quality traits (percentage of protein, oil, and starch) and agronomic traits. They also highlighted that a preliminary classification of the genotypes into groups of genetic diversity could help decide which combinations are the best.

The high and significant values of the CCCs indicated that the dendrograms of the two experiments reported here reliably expressed the similarity of the cultivars within each group and the genetic differences between the groups.

In each experiment, the analysis of variance, the tests to compare means and the cluster analysis indicated genetic variability, in early and extremely early maturing corn cultivars. In the study developed by Kil et al. (2014), significant differences in the coefficients of ileal apparent digestibility of CP and AA (arginine, histidine, lysine, tryptophan, aspartic acid, cysteine, glycine, and proline) among corn sources from different origins were observed. Already Alves et al. (2014), using cluster analysis to assess the variability of grain productivity and energy profile of maize cultivars, observed that groups with more productive genotypes presented lower ethereal extract and higher amylase in grain from early and extremely early-maturing corn cultivars.

Finally, the present study found that the higher the PROD of a particular group of cultivars, the lower the protein and amino acid levels were in these cultivars. This inverse relationship has been described by Duvick

(2005), Idikut et al. (2009) and Aliu et al. (2012). Therefore, it is necessary for researchers involved in corn genetic breeding programs to be attentive to the relationship between PROD and the nutritional composition of the grains. It is important to minimize the reduction of nutrient levels in cultivars of high productive potential or seek the development of cultivars with highly nutritious grain without significantly compromising the productivity of the cultivar.

Conclusions

There was genetic variability for PROD and for grain CP and amino acid levels in the early and extremely early maturing corn cultivars. For the early and extremely early maturing corn cultivars, respectively, five and four groupings were formed based on the PROD, CP content and amino acid profile, indicating the possibility of crossbreeding these cultivars to improve grain protein content and amino acid profiles. Groupings of cultivars with higher grain yields generally exhibited lower CP and amino acid levels.

Conflict of Interest

The authors have not declared any conflict of interest.

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