Mixed models and multivariate approach applied to maize breeding: A useful tool for biofortification

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Abstract

Biofortification and bioactive compounds enrichment of maize genotypes is a great alternative for mitigating micronutrients deficiency in human and animal diet, and also for improving the benefits of maize for human health. This work aimed to estimate variance components and genetic parameters of bioactive compounds and micronutrients to predict superior maize hybrids from different genetic bases, and to apply the RELM/BLUP methodology to multivariate techniques. The inbreed lines were crossed and the F1 hybrids were grown for evaluations in 2014/2015 and 2015/2016 growing seasons, respectively. Then, micronutrients and bioactive compounds related traits were evaluated. The variance components and genetic parameters were estimated by REML methodology. The BLUP methodology was employed to predict genetic values and to verify the percentages of genetic gain with selection. The predicted genetic values were applied to estimate genetic distances by the Mean Euclidean Distance. The relative contribution of each trait to genetic divergence was evaluated and the principal components analysis determined, proposing the genotypes that are potentially capable to increase a given trait. The presence of genetic variability was evidenced among genotypes, while some of them presented potential for increasing specific traits. The top cross hybrid L64XAS1590 showed the highest estimates for increasing antioxidant-responsible traits, and micronutrients contents such as manganese, cooper, iron and zinc. In general, there was the possibility of achieving genetic gains with selection under application of biofortified and bioactive compounds to enhance maize hybrids through conventional breeding. However, it does not applicable for iron content due to its low estimate of broad sense heritability.

Abbreviations: TP_ total phenols; TF_ total flavonoids; TC_ total carotenoids; DP_ antioxidant potential by DPPH; AB_ antioxidant potential by ABTS radical; SS_ soluble solids; pH_ hydrogenionic potential; SA_ seed acidity; SC_ seed color; Fe_ iron content; Cu_ copper content; Mn_ manganesecontent; Na_ sodium content; Zn_ zinc content; BLUP_ Best linear unbiased prediction; REML_ restricted maximum likelihood; PCA_ principal component analysis; UPGMA_ unweightedpair-group method; $^+\hat{\sigma}_g$_ Genotypic variance; $\hat{\sigma}_e$_ residual variance; $\hat{\sigma}_P$_ phenotypic variance; H^2_g_ broad sense heritability; H^2_{ml}_ heritability of the hybrid´s means; Aclinh_ selective accuracy; CVgi%_Coefficient of genetic variation; CVe%_Coefficient of residual variation; OM_ overall mean.

Introduction

Micronutrient deficiency affects many people around the world, especially in developing countries, where it is considered a public health problem (Bouis et al., 2011). The main source of vitamins and minerals in low-income populations are plant-based staple foods, which often present low levels or low availability of micronutrients (FAO, 2015). Biofortification aims to develop nutritionally enriched crops through conventional plant breeding methods (Ortiz-Monasterio et al., 2007), prioritizing the increase of iron, zinc and carotenoids (provitamin A), due to the prevalence of their deficiency among children up to five years old and women at childbearing age in developing areas of Africa, Asia and Latin America (Bouis and Welch, 2010).

Plant bioactive compounds are health supporting substances due to their antioxidant activity. They reduce the harmful effects of reactive oxygen species (ROS) and other free radicals, competing for active sites and receptors in diverse cellular structures. Furthermore, they modulate the expression of genes involved in intracellular defense mechanisms against degenerative oxidative processes of cellular structures (Bastos et al., 2009, Sikoraet al., 2008, Wen et al., 2012).

Maize (*Zea mays* L.) is a crop of major socioeconomic importance around the world. Its wide cultivation and

versatility of purposes make it one of the most produced cereals worldwide (FAO, 2015). However, its utilization dynamics is different due to economic and cultural issues (Awika, 2011). In developing countries that still face severe food shortages such as southern and eastern Africa, Central America and Mexico, maize is directly consumed in human diet, being an essential source of energy, minerals and other nutrients (Ranum et. al., 2014). In developed countries, where food shortages have been surpassed or the population's diet is based on other staple foods, maize is widely used for animal feed, ethanol production or by the industry (Paes, 2006). For all these reasons, maize is an interesting crop for biofortification.

An efficient and economically feasible alternative to achieve bioactive compounds and micronutrients enhanced (biofortified) maize genotypes is through conventional breeding programs, being necessary to benefit from different sources of genetic variability and different genetic bases. In this sense, single cross hybrids are potentially more productive than other types of hybrids due to the effects of specific combining ability and heterosis. However, it requires improved treats and growing conditions (Nardino et al, 2016). The three-way cross hybrids present uniform phenotype, intermediate yielding potential compared to single cross and double cross hybrids. However, they adapt better to environment variations due to their larger genetic base. The double cross hybrids are originated by crossing two single cross hybrids, and because of their broad genetic constitution, they present greater stability and lower cost of production (Emygdio et al., 2007).

The knowledge about the genotype´s genetic value for a particular trait is essential in breeding programs. In this view, statistical models employed to estimate variance components and genetic parameters aid at elaborating the most suitable strategy for selection of traits of interest. Genetic parameters of the traits of interest may be estimated through the restricted maximum likelihood method (REML), providing reliable and applicable estimates to maize breeding (Baretta et al., 2016). The best linear unbiased prediction (BLUP) enables estimation of genetic values and to predict the next generation´s new means (Resende and Duarte, 2007), allowing to rank and select the most promising genotypes for traits of interest (Borges et al., 2010).

The knowledge about genetic distances is fundamental in maize breeding, as this information allows improving the efficiency of lineage selection for crossings. Thus, multivariate analysis help breeders in the decision-making process since it allows evaluating a set of traits related to each other, resulting in the coefficients of genetic distance between genotypes (Cruz et al., 2012). For predicting genetic divergence, several multivariate approaches may be applied, standing out the agglomerative methods such as Euclidean distance and principal component analysis (Cruz et al., 2012). The multivariate approaches have been used for prediction of genetic values and to verify genetic distances that may greatly contribute to genetic breeding and biofortification of maize genotypes. These techniques can contribute to increase the accuracy and reliability of inferences by distinguishing genotypes that are phenotypically and genetically have higher values. This reduces the environmental deviations and provides greater gains with selection. Therefore, this work aimed to estimate variance components and genetic parameters of bioactive compounds and micronutrients, to predict the superior maize hybrids of different genetic bases, and to apply the RELM/BLUP methodology for multivariate techniques.

Results and discussion

The Deviance analysis revealed significance at 5% of probability trough the chi-square test for all evaluated traits (Table 2). Therefore, the estimates of variance components and genetic parameters present reliability and consistency.

The variances of the evaluated traits were compared by percentage, according to methodology proposed by Carvalho et al. (2017). Regarding the trait total phenols (TP), 86% of its phenotypic variation is caused by genetic causes (Table 2). It is interesting that the largest fraction of the phenotypic variance is originated from genetic causes, as this trait presents great importance for human health. Natural plant antioxidants, as phenolic compounds are involved in regulation of reactive oxygen species content, and they perform an important function as protective compatible osmolyte in scavenging free radicals, and facilitate a correction of altered redox potential (Harre et al., 1999).

The trait total flavonoids (TF) presented 96.5% of the phenotypic variance occurring due to genetic causes (Table 2). This elevated estimate may contribute to increase the genetic control of TP, as flavonoids and non-flavonoid compounds (Liu, 2004) form total phenols. It also suggests the possibility of incrementing both traits by genetic breeding. The trait total carotenoids (TC) evidenced that 99.1% of its phenotypic variance is attributed to genetic causes (Table 2), and heritability in the broad sense of 0.99. José Junior (2014), presented similar results (H^2: 98) evaluating VPAs, single cross and three-way cross maize hybrids, expressing the possibility of achieving genetic gains and superior genotypes (Kimura et al., 2007). These results are very promising for maize biofortification, as important traits for human health as they can be maintained along generations of selection. Carotenoids are a group of pigments responsible for the color of maize grains. They are located in the endosperm, more specifically in the aleurone layer and in the vitreous endosperm of the grains (Gallagher et al., 2004). Maize is one of the few sources of both xanthophilias (lutein and zeaxanthin), constituents of the so-called macular pigments involved in prevention of age-related macular degeneration (Gama and Silos, 2007). Alpha-carotene, beta-carotene and beta-kryptoxanthin are considered essential precursors of vitamin A for preventing hypovitaminosis A, which are still being considered a problem of public health in several countries (Butt, 2006).

The trait DPPH (DP) refers to the determination of antioxidant potential by the DPPH radical scavenging method. The antioxidant potential was also verified by the ABTS method (AB), using both to increase the results reliability. A similar behavior was found for DP and AB, which presented elevated genetic variance of 95.58% and 93.15% of the phenotypic variance, respectively (Table 2). These results affirm the elevated estimates of genetic variance verified for the antioxidant responsible traits,

Hybrids	GeneticBasis	Hybrids	GeneticBasis
P2530	Single-cross hybrid	L1 X 30F53	Top-cross hybrid
P30F53	Single-cross hybrid	L56 X 30F53	Top-cross hybrid
L210 X L272	Single-cross hybrid	L35 X CD308	Top-cross hybrid
L272 X L288	Single-cross hybrid	L48 X CD308	Top-cross hybrid
L288 X L272	Single-cross hybrid	L51 X CD308	Top-cross hybrid
L272 X L210	Single-cross hybrid	L22 X CD308	Top-cross hybrid
BM3061	Triple-cross hybrid	L42 X CD308	Top-cross hybrid
L258 X CD308	Top-cross hybrid	L43 X 30F53	Top-cross hybrid
L261 X CD308	Top-cross hybrid	BM207	Double-cross hybrid
L66 X AS1590	Top-cross hybrid	CD308	Double-cross hybrid
L64 X AS1590	Top-cross hybrid		
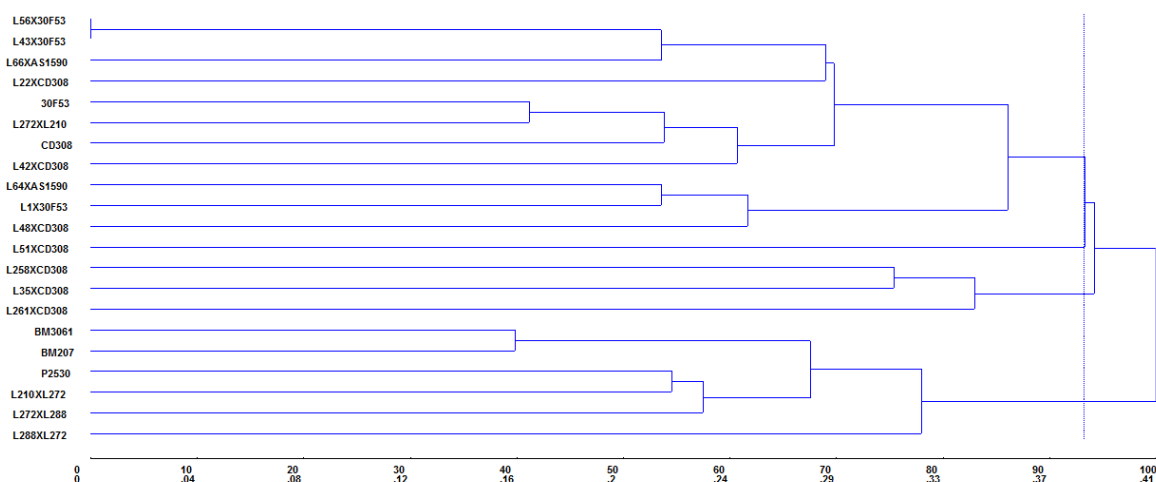
Fig 1. Dendrogram of genetic dissimilarity established by the Unweighted Pair-Group Method using the Arithmetic Average (UPGMA), based on the Euclidean distance for hybrids of different genetic bases.

Table 2: Variance components estimates and genetic parameters (REML) for the different genetic basis hybrids.

Comp. ofvariance[+]		TP[++]	TF	TC	DP	AB	SS	pH
Deviance[+++]		**	**	**	**	**	**	**
	$\hat{\sigma}_g$	38305.59	433505.53	4992.66	121.25	329.17	2.30	0.04
	$\hat{\sigma}_e$	6026.94	15680.85	44.89	5.59	24.37	0.73	0.00
	$\hat{\sigma}_P$	44332.53	449186.38	5037.54	126.85	353.55	3.03	0.04
H^2_g		0.86	0.97	0.99	0.96	0.93	0.76	0.84
H^2_{ml}		0.95	0.99	1.00	0.98	0.98	0.90	0.94
Aclinh		0.97	0.99	1.00	0.99	0.99	0.95	0.97
CVgi%		27.50	33.82	50.64	49.14	39.91	37.31	2.96
CVe%		10.91	6.43	4.80	10.55	10.86	21.02	1.31
OM		711.65	1946.55	139.53	22.41	45.46	4.07	6.34
Comp. ofvariance		SA	SC	Fe	Cu	Mn	Na	Zn
Deviance		**	**	**	**	**	**	**
	$\hat{\sigma}_g$	0.01	4.82	3.96	1.48	3.03	852.13	35.59
	$\hat{\sigma}_e$	0.00	0.07	15.31	1.13	1.53	96.02	1.57
	$\hat{\sigma}_P$	0.01	4.89	19.27	2.61	4.56	948.16	37.16
H^2_g		0.92	0.99	0.21	0.57	0.66	0.90	0.96
H^2_{ml}		0.97	1.00	0.44	0.80	0.86	0.96	0.99
Aclinh		0.99	1.00	0.66	0.89	0.93	0.98	0.99
CVgi%		23.77	2.34	7.11	32.96	14.73	22.49	20.35
CVe%		7.21	0.29	13.98	28.80	10.46	7.55	4.27
OM		0.32	93.22	28.00	3.69	11.82	129.77	29.32

[+]$\hat{\sigma}_g$: Genotypic variance; $\hat{\sigma}_e$: residual variance (environment); $\hat{\sigma}_P$: phenotypic variance; H^2_g: broad sense heritability; H^2_{ml}: heritability of the hybrid´s means; Aclinh: selective accuracy; CVgi%: Coefficient of genetic variation; CVe%: Coefficient of residual variation; OM: overall mean. [++]TP: Total Phenols; TF:TotalFlavonoids;TC: Total Carotenoids; DP: antioxidant potential by DPPH radical; AB: antioxidant potential by ABTS radical; SS:soluble solids; pH:hydrogenionic potential; SA: seed acidity; Cu: copper; Fe:iron; SC: seed color; Mn: manganese; Na: sodium; Zn: zinc. [+++]*Deviance* at 5% of probability by the chi-square test.
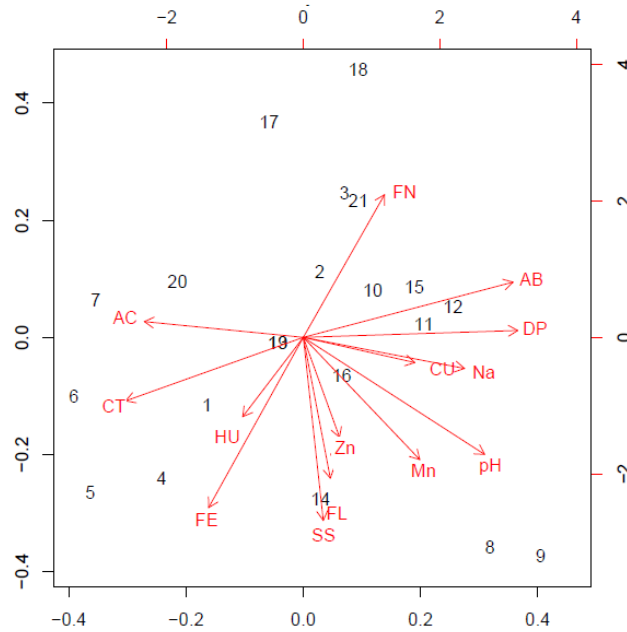
Fig 2. Graphic representation of the Principal Component Analysis (PCA). CP1: 28.86%; CP2: 17.87%; CP3: 14.50%; 1: L56X30F53;2: L43X30F53; 3:L66XAS1590; 4: L22XCD308; 5: 30F53; 6: L272XL210; 7:CD308; 8:L42XCD308; 9: L64XAS1590; 10: L1X30F53; 11: L48XCD308; 12: L51XCD308; 13: L258XCD308; 14: L35XCD308; 15: L261XCD308; 16: BM3061; 17: BM207; 18: P2530; 19: L210XL272; 20: L272XL288; 21: L288XL272. TP: Total Phenols; TF: Total Flavonoids; TC: Total Carotenoids; DP: antioxidant potential by DPPH radical; AB: antioxidant potential by ABTS radical; SS: soluble solids; pH: hydrogenionic potential; SA: seed acidity; Cu: copper; Fe: iron; SC: seed color; Mn: manganese; Na: sodium; Zn: zinc.

Table 3. Ranking of the single cross, double cross, three-way cross and top cross maize hybrids evaluated through individual BLUP for the traits antioxidant potential by ABTS radical (AB); antioxidant potential by DPPH radical (DP); total phenols (TP); total flavonoids (TF); total carotenoids (TC); seed color (SC) and soluble solids (SS).

Ranking	AB	DP	TP	TF	TC	SC	SS
1st	L64XAS1590	L1X30F53	BM207	L272XL288	P30F53	L48XCD308	L42XCD308
2nd	P2530	L48XCD308	L66XAS1590	L48XCD308	L22XCD308	L261XCD308	L35XCD308
3rd	L288XL272	L64XAS1590	P2530	L56X30F53	CD308	L42XCD308	L22XCD308
4th	L42XCD308	L51XCD308	BM3061	L64XAS1590	L258XCD308	L51XCD308	L43X30F53
5th	L1X30F53	L42XCD308	L288XL272	L1X30F53	L210XL272	L64XAS1590	L64XAS1590
6th	BM207	P2530	L43X30F53	L35XCD308	BM207	L272XL210	L258XCD308
7th	L51XCD308	L66XAS1590	L64XAS1590	L42XCD308	L272XL288	P2530	L210XL272
8th	L258XCD308	L258XCD308	L1X30F53	30F53	L272XL210	L66XAS1590	L56X30F53
9th	L210XL272	L210XL272	L258XCD308	L258XCD308	L56X30F53	L288XL272	30F53
10th	L66XAS1590	L261XCD308	L210XL272	L210XL272	L35XCD308	L35XCD308	L272XL210
11th	L35XCD308	L288XL272	L35XCD308	L22XCD308	L42XCD308	L272XL288	L51XCD308
12th	L43X30F53	BM207	L48XCD308	L51XCD308	P2530	L43X30F53	L66XAS1590
13th	L261XCD308	L43X30F53	30F53	L261XCD308	BM3061	CD308	CD308
14th	BM3061	BM3061	L51XCD308	L288XL272	L64XAS1590	L56X30F53	L272XL288
15th	L48XCD308	L35XCD308	L56X30F53	L66XAS1590	L1X30F53	L1X30F53	L48XCD308
16th	L22XCD308	L22XCD308	L261XCD308	L43X30F53	L43X30F53	30F53	L1X30F53
17th	CD308	L272XL210	L22XCD308	P2530	L288XL272	BM3061	BM3061
18th	L272XL288	30F53	L42XCD308	BM3061	L66XAS1590	L258XCD308	BM207
19th	L56X30F53	L272XL288	L272XL210	BM207	L261XCD308	L210XL272	L288XL272
20th	L272XL210	L56X30F53	CD308	CD308	L51XCD308	L22XCD308	L261XCD308
21th	30F53	CD308	L272XL288	L272XL288	L48XCD308	BM207	P2530

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Table 4. Ranking of the single cross, double cross, three-way cross and top cross maize hybrids evaluated through individual BLUP for the traits seed acidity (SA); hydrogenionic potential (pH); manganese content (Mn);cupper content (Cu); iron content (Fe); sodium content (Na); and zinc content (Zn).

Ranking	SA	pH	Mn	Cu	Fe	Na	Zn
1st	L272XL210	L64XAS1590	BM3061	BM3061	30F53	L288XL272	L64XAS1590
2nd	L22XCD308	L51XCD308	L64XAS1590	L64XAS1590	L35XCD308	L42XCD308	BM3061
3rd	L35XCD308	L35XCD308	L261XCD308	30F53	L43X30F53	L64XAS1590	L261XCD308
4th	L288XL272	L42XCD308	L51XCD308	L258XCD308	CD308	L43X30F53	L35XCD308
5th	30F53	BM3061	L22XCD308	L210XL272	L42XCD308	L1X30F53	30F53
6th	BM207	L22XCD308	L272XL210	L272XL210	L64XAS1590	L35XCD308	L56X30F53
7th	P2530	L48XCD308	L35XCD308	L48XCD308	L272XL210	BM3061	L22XCD308
8th	CD308	L258XCD308	30F53	L22XCD308	L22XCD308	L66XAS1590	L42XCD308
9th	L51XCD308	L210XL272	CD308	CD308	L1X30F53	BM207	L258XCD308
10th	L258XCD308	L261XCD308	L288XL272	L272XL288	BM3061	L51XCD308	L210XL272
11th	L210XL272	L272XL288	L272XL288	L1X30F53	L56X30F53	L258XCD308	BM207
12th	L66XAS1590	L1X30F53	L66XAS1590	L43X30F53	L288XL272	L210XL272	L48XCD308
13th	BM3061	L56X30F53	L56X30F53	L66XAS1590	L272XL288	L48XCD308	L66XAS1590
14th	L56X30F53	L43X30F53	BM207	L51XCD308	L258XCD308	P2530	L51XCD308
15th	L43X30F53	BM207	L42XCD308	L288XL272	L210XL272	L261XCD308	L1X30F53
16th	L1X30F53	L288XL272	L258XCD308	BM207	L66XAS1590	L22XCD308	P2530
17th	L48XCD308	P2530	L210XL272	L56X30F53	L48XCD308	L56X30F53	L43X30F53
18th	L272XL288	L66XAS1590	P2530	L35XCD308	L261XCD308	L272XL210	CD308
19th	L261XCD308	30F53	L48XCD308	L261XCD308	P2530	30F53	L272XL288
20th	L64XAS1590	CD308	L43X30F53	L42XCD308	BM207	CD308	L288XL272
21th	L42XCD308	L272XL210	L1X30F53	P2530	L51XCD308	L272XL288	L272XL210

Table 5. Relative contribution to genetic diversity based on predicted genotypic values for traits evaluated in maize hybrids through Singh´s methodology (1981).

Traits+	Relative Contribution (%)
TP	11.19
DP	10.13
TC	9.00
AB	8.23
Cu	7.82
TF	7.37
pH	7.20
Zn	6.75
SA	5.98
Na	5.53
Mn	5.51
SS	5.46
Fe	5.36
SC	4.46

+TP: total phenols; DP: antioxidant potential by DPPH radical; TC: total carotenoids; AB: antioxidant potential by ABTS radical; Cu: cupper; TF: total flavonoids; pH: hydrogenionic potential; Zn: zinc; SA: seed acidity; Na: sodium; Mn; manganese; SS: soluble solids; Fe: iron; SC: seed color.

evidencing the possibility of breeding bioactive compounds and nutrients enhanced maize.

The micronutrients copper (Cu), zinc (Zn) and manganese (Mn) constitute the superoxide dismutaseenzymes (SODs), which take part in the body's defense against reactive oxygen species (Stipanuk, 2000). There is an agreement that mineral content in edible parts of plants is dependent on their availability for absorption from the soil, followed by their reallocation in the tissues that will serve as food (Welch and Graham, 2005). However, several authors affirm that mineral contents vary in function of genotype (Ahmadi et al, 1993; Wardyn, 2004). Regarding the trait Cu, 56.7 % of the phenotypic variance may be explained by genetic causes (Table 2). A similar pattern was verified for Mn, which presented 66.44% of genotypic variance (Table 2). Regarding the content of Zn, 95.77% of the phenotypic variance occurred due to genetic causes (Table 2). A distinct behavior

was evidenced by Fe, which presented only 25.86% of phenotypic variance attributed to genetic causes, while the other 74.14% was due to residual variance (Table 2), which made the selection of genotypes for increasing this trait difficult. The Na content presented 89.88% of genotypic variance (Table 2), being possible to achieve genetic gains with selection.

Soluble solids content (SS) (Table 2) is used as an indirect measure of the grains sugar content. It also gathers other dissolved substances, such as organic aciditys, vitamins, phenolic compounds, pectins. However, sugars may constitute 85-90% of the soluble solids (Chitarra, 2000). Thus, the increment of soluble solids in the grains result in a higher production of functional components such as phenolic compounds, vitamins and organic aciditys. From the phenotypic variance verified for this trait, it is estimated that approximately 75.9% occur as a function of the genetic

variance. The pH is proportional to the fraction of soluble sugars, and inversely proportional to seed acidity (SA) (Carvalho, 2016). For both traits, the phenotypic variances were explained totally, by their respective genotypic variances (Table 2), allowing to achieve gains with selection.

The heritability estimated in the broad sense corresponds to the proportion of phenotypic variability caused by genetic causes, considering effects of dominance, additivity and epistasis. According to classification described by Resende (2002) heritability is considered of low magnitude when $\hat{H}_b^2 <$ 0.15, intermediate magnitude between 0.15 $<\hat{H}_b^2<$ 0.50 and of high magnitude when $\hat{H}_b^2 >$ 0.50. The evaluated traits presented high broad sense heritabilities, except Fe ($\hat{H}_b^2 =$ 0.21), evidencing the great influence of environment in this trait´s phenotypic expression. These results reaffirm those previously stated by the genotypic variances.

The selective accuracy (Aclinh) reflects the quality of procedures and results used to predict genetic values. This parameter is associated with precision of selection and refers to the correlation between predicted genetic values and true genetic values of the genotypes (Pimentel et al., 2014). Aclinh values range from 0 to 1, and are classified as very high (Aclinh ≥ 0.90), high (0.70 ≤ Aclinh <0.90), moderate (0.50 ≤ Aclinh < 0.70) and low (Aclinh <0.50) (Resende and Duarte, 2007). Accuracy values higher than 0.70 are sufficient to provide a precise and efficient inference about genotypes genetic value, and because it is a measure associated with precision in selection, accuracy is the main element of genetic progress that may be altered by the breeder, maximizing genetic gains (Resende, 2002). Most of the evaluated traits presented very high aclinh estimates (Table 2).

The coefficient of genetic variation ($CVgi$) is a parameter commonly used to compare the genetic variability evidenced for a trait (Resende, 2002). The coefficients of genetic variation ranged from low to very high in the set of evaluated traits, varying from 2.34 % (SC) to 50.64% (TC), which expresses the high genetic variability among genotypes. The coefficient of residual variation (CV_e) were considered low to intermediate for majority of the evaluated traits, demonstrating precision in conducting the experiment and expressing reliability of the data. According to Vencovsky (1987), a relation between CV_{gi} and CV_e of 1 or more reflects a favorable situation to achieve gains with selection. For all traits except Fe, the CV_{gi} was higher than CV_e, which indicates the possibility of achieving gains with selection.

The top cross hybrid L64XAS1590 was among the highest ranked genotypes through the best linear unbiased prediction (individual BLUP). This high ranking was based on predicted genotypic effects of genotypes for antioxidant potential of ABTS (AB), DPPH (DH), total phenols (TP) and total flavonoids (TF) (Table 3). It evidences the potential of this hybrid for maize breeding programs aiming at increasing antioxidant potential related traits. Regarding the traits such as total carotenoids (TC) and seed color (SC), the occurrence of hybrids in high ranking positions was not verified (Table 3). This result suggests the interference of another pigment influencing seed color, possibly anthocyanins, which should be considered in future studies. The single cross hybrid P30F53 was the first ranked for TC, followed by the top cross hybrid L22XCD308, while the top cross hybrids L48XCD308 and L261XCD308 were the highest ranked for SC. The top cross hybrid L35XCD308 was ranked at the three first

positions, considering soluble solid (SS) (Table 3), seed acidity (SA) and pH (Table 4). The top cross hybrid L22XCD308 also ranked in the top positions for these traits. The top cross hybrid L64XAS1590 was ranked in the top positions for manganese (Mn), copper (Cu), iron (Fe), sodium (Na) and zinc (Zn) contents (Table 4), demonstrating its potential to be used in breeding programs aimed at incrementing these minerals.

The predicted genetic distance verified through the mean Euclidean distance for the set of analyzed hybrids presented an average of 0.27. The cut-off point (0.38) enabled to visualize four groups of genotypes (Figure 1). Group one was composed by six hybrids (BM3061; BM207; P2530; L210XL272; L272XL288; L288XL272) with the presence of single cross, double cross and three-way cross maize hybrids. Group two was composed by three top-cross maize hybrids (L261XCD308; L35XCD308; L258XCD308), where the double cross hybrid CD308 was used as tester. The male genitor was observed in all genotypes of this group. Group three was composed of only one top-cross hybrid (L51XCD308), and group four by eleven hybrids (L48XCD308; L1X30F53; L64XAS1590; L42XCD308; CD308; L272XL210; 30F53; L22XCD308; L66XAS1590; L43X30F53; L56X30F53), evidencing the genetic variability among genotypes and different genetic basis.

The relative contribution revealed that total phenols (TP) (11.19%), antioxidant potential by DPPH method (DP) (10.13), total carotenoids (TC) (9.00%), antioxidant potential by ABTS method (AB) (8.23%) and copper content (Cu) (7.82%) were the traits that most contributed to explain the differences expressed by variation in the predicted genetic values of the tested hybrids (Table 5). The interest in evaluating traits' relative contribution is the possibility of discarding those with little participation in genotypes discrimination, reducing labor, time and financial resources spent on experimentation. However, since all evaluated traits are important for maize biofortification, and becauuse no low contributing trait was observed, the exclusion of characters is not recommended.

The principal component analysis (PCA) allows to condense the largest amount of original information contained in p variables (p = 14) into two orthogonal latent variables called principal components, which are linear combinations of the original variables created with the two largest eigenvalues of the covariance matrix (Hair et al., 2005). Therefore, the initial set of fourteen variables is expressed by two new latent variables, which are plotted in a two-dimensional figure (Figure 2). The PCA allows visualizing the possible affinity of genotypes to express certain trait(s), where it is verified that genotypes L66XAS1590 and L288XL272 presented proximity to the trait total phenols (TP). The genotype L35XCD308 presented proximity to the total flavonoids (TF) and soluble solids (SS). In addition, the genotype L56X30F53 showed proximity to seed color (SC), genotype CD308 with seed acidity (SA), and genotype L272XL210 with total carotenoids (TC). Thereby, these hybrids should be considered in breeding programs that aim to increase these specific traits.

Materials and methods

The experiment was conducted at the Genomic and Plant Breeding Center of the Federal University of Pelotas. The genotypes were crossed and cultivated during the 2014/2015 and 2015/2016 growing seasons, respectively, at

the Palma Agricultural Center, in the city of Capão do Leão – RS, Brazil, at latitude 31º47'58''S and longitude 52º31'02''W, with altitude of 13.2 meters (m). According to Köppen, the climate is classified as subtropical Cfa, and the soil is characterized as Dystrophic Yellow Red Argisol (EMBRAPA, 2006). The experimental design was randomized blocks containing 21 maize hybrids of different genetic basis (Table 1) arranged in three replicates.

Seeding occurred in the first half of December 2015, with population density of 80,000 plants per hectare. The base fertilization consisted of 350 kg ha^{-1} of NPK in the formulation 10-20-20. For topdressing, 110 kg ha^{-1} of nitrogen in the amidic form was applied at the V_4 phenological stage. The control of weeds and pests were preventively carried out to reduce biotic effects in the experiment results. The experimental unit consisted of two lines of five meters (m) length, spaced 0.50 m. The harvest occurred in the second half of April 2016, when all genotypes presented foliar senescence.

The evaluated traits were: total phenols (TP) in µg g^{-1} (Singleton and Rossi, 1965); total flavonoids (TF) in mg g^{-1} (Zhishean et al., 1999); total carotenoids (TC) in mg g^{-1} (AOAC, 2005); antioxidant potential by DPPH (DP), in inhibition percentage (Brand-Williams et al., 1995); antioxidant potential by ABTS radical (AB), in inhibition percentage (Rufino et al., 2007); soluble solids (SS) in °Brix (AOAC, 2005); Hydrogenionic potential (pH); seed acidity (SA), in percentage of citric acid; seed color (SC) in hue angle; and the contents of iron (Fe); copper (Cu);manganese (Mn);sodium (Na) and zinc (Zn), which were expressed in mg Kg^{-1} (Tedesco et al., 1995).

The data were submitted to normality test by Shapiro and Wilk (1965), and the deviance analysis was performed at 5% of probability by the chi-square test (X^2) to identify the significance of the traits. For estimating variance components and genetic parameters of the hybrids by REML, the model 21 (Resende, 2016) was used. It was followed the statistical model $y = Xr + Zg + e$, where: y: is the data vector, r: are the effects of repetitions (fixed) added to the overall mean; e: are the residue effects (random). The variance components that verified were: genetic variance ($\sigma^2 g$), residual variance ($\sigma^2 e$), phenotypic variance ($\sigma^2 p$), broad sense heritability ($H^2 g$), broad sense heritability of the hybrids average ($H^2 ml$), accuracy of hybrids selection (Aclinh), coefficient of residual variation (CVe) and overall mean of the experiment (OM).

Single Best Linear Unbiased Prediction (BLUP) were estimated to rank the genotypes (R), to evidence the predicted genetic effects (G), to predict the genetic value (U+G), and to achieve the percentage of genetic gain with selection (Gain%) and new mean (NM). The analyzes were performed using the Selegen® statistical software (Resende, 2016).

Based on genetic values (U+G) predicted by the BLUB methodology, the Mean Euclidean Distance was obtained, and the dendrogram was assembled through the UPGMA (Unweighted Pair-Group Method using the Arithmetic Average) method. The cut-off point was established by adding one standard deviation to the mean, dividing the dendrogram and displaying groups of genotypes. The trait´s relative contribution for genetic divergence was performed according to methodology proposed by Singh (1981), and the principal components analysis (PCA) with biplot representation (Johnson andWichern, 1982) to determine which genotypes have potential to increase a given trait. For statistical analysis the software Genes® (Cruz, 2013) and R®(R Core Team, 2015) was used.

Conclusions

There is the possibility of achieving genetic gains with selection and to develop biofortified and bioactive compounds enhanced maize hybrids through conventional breeding. However, it does not apply for iron content due to its low estimate of broad sense heritability. Among the evaluated genotypes, the most promising for antioxidant potential was the top cross hybrid L64XAS1590. The top cross hybrid L35XCD308 presented potential to be used by breeding programs aimed at increasing soluble solids, seed acidity and pH potential. The top cross hybrid L64XAS1590 presents potential to be employed by breeding programs aimed at increasing mineral contents such as manganese, copper, iron, sodium and zinc. The evaluated genotypes present genetic variability for the set of evaluated traits. The closest relation verified through Principal Component Analysis were between the hybrids L66XAS1590 and L288XL272 with total phenols, and the hybrid L35XCD308 with total flavonoids and soluble solids.

Acknowledgements

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