**Heterosis and genetics parameters for yield and nutritional components in half- sibling maize progenies**

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**Abstract.** The growing demand for maize creates a challenge to breeders, requiring thedevelopment ofhigher yielding and higher quality genotypes. The objective of this work was to estimate the most relevant heterosis, variance components and genetic parameters, and to use a multivariate approach to define narrow sense heritability profiles for yield and nutritional components in half-sib maizeprogenies. The experimental design used was random blocks, with a male parent (hybrid tester), five inbred lines (S5) as maternal parents and the progenies (hybrid Top Cross), totalizing 11 maize genotypes arranged in six replicates. Agronomic and nutritional characters were evaluated. Half-sibling progenies reveal greater additive genetic contribution to phenotypic expression through grain width and thickness, iron content, total flavonoids and carotenoids, soluble solids, and methionine. Narrow sense heritability values between and within progenies are higher for manganese content, glycine, proline and tryptophan. Regardless of the inbreeding line S5 used, heterosis gains are obtained for insertion of spike height, number of grain rows per spike, stem diameter, zinc content, total carotenoids, soluble solids and pH. Specific heterosis is evidenced for grain yield, glycine, serine, threonine and phenylalanine. The multivariate approach used defines eight character profiles regarding their genetic trends andindicates narrow sense heritability of the progeny mean as the major cause for this distinction.

**Keywords**: *Zea mays,* aminoacids, nutritional profile of grains, breeding, biofortification.

**INTRODUCTION**

Maize (*Zea mays*) is a worldwide spread cereal with agricultural, economic and social impact, both used as food and feed, as well as raw material for industrialized products (Wen et al., 2016). Thus, the growing demand for maize puts a challenge to breeders, requiring the developmentof higher yielding and higher quality genotypes (UfazandCalili, 2008). Breeding is crucial to increase maize yield, where conventional breeding techniques make use of the available germplasm, directed hybridization and selection strategies to meet the agronomic ideotypes desired by the breeder. However, in order to find high potential genotypes, it is necessary to understand the additive gene fraction responsible for the phenotype expression and thus to determine the inheritable fraction of the character in the progenies.

When only phenotypic measures are available, genotypic inferences are obtained through the use of genetic designs and biometric techniques. When these tools are properly used, one can partition the total variation in phenotypic and genotypic variance components, to later obtain the genetic parameters necessary to explain the inheritable tendencies and to direct the selection (Falconer and Mackay, 1996).

In order to improve the target traits, maize breeding programs seek superior hybrid combinations, supported by the efficient selection of the parents and their ability to recombine alleles, due to the dominance deviations, the complementarity of the alleles in heterozygosity and the intergenic interactions result in heterosis or hybrid vigor gain. This biological phenomenon can be defined as the increase of a certain character in the progeny when compared to their parents (Falconer and Mackay, 1996).

Breeding studies using quantitative genetics to infer the variance components, genetic parameters and heterosis in progenies of maize half-sibs have been extensively performed (Hallauer and Miranda Filho, 1995; Heinz et al., 2012). However, biometric emphasis in grain yield components, micronutrients, bioactive compounds and aminoacids are not common. Therefore, the objective of this work was to estimate the most relevant heterosis, variance components and genetic parameters and to use a multivariate approach to define narrow sense heritability profiles for yield and nutritional components in half-sibmaizeprogenies.

**MATERIALS AND METHODS**

This experiment was performed in the harvest season 2015/2016 in the Plant Genomics and Breeding Center belonging to the Federal University of Pelotas (UFPel). The genotypes used were grown in the Agricultural Research Station in Capão do Leão – RS, Brazil, at latitude of 31º47’58’’ S and longitude of 52º31’02’’ W, with altitude of 13.2 meters (m). According toKöppen, the climate is described as Cfasubtropical and the soil it is characterized as Argissoil red yellow dystrophic. The experimental design used was random blocks, with a male parent (hybrid tester), five inbred lines (S5) as maternal parents and the progenies (hybrid Top Cross), totalizing 11 maize genotypes arranged in six replicates (Table 1).

**Table 1:** Organization of the Top Cross crossing system and the maize genotypes used.

|  |  |  |
| --- | --- | --- |
|  | Genotypes\* |  |
| Tester | Inbredlines (S5) | Hybrid (Top Cross) |
| CD 308  Double cross hybrid (DH)  Broad genetic basis | L1 (256) | HIB I (L1 x HD) |
| L2 (258) | HIB II (L2 x HD) |
| L3 (389) | HIB III (L3 x HD) |
| L4 (262) | HIB IV (L4 x HD) |
| L5 (225) | HIB V (L5 x HD) |

\*crosses carried out in harvest season of 2014/2015.

The seeding was performed manually in the first half of December 2015, where the population density of 80,000 plants per hectare. Fertilizer used was 350 kg ha-1 of NPK as base fertilizer in the formulation 10-20-20, applied in the phenological stage V4, 112 kg ha-1 of nitrogen-amine form. Weed and pest insect control were carried out according to the needs of the crop, in order to minimize the biotic effects in the results of the experiment. The experimental unit was composed of two sowing rows with five meters in length, and spacing of 0.5 m between rows. The harvest was carried out in the second half of April 2016.

The characters were measured by random sampling of ten plants per experimental unit, based on the methods proposed by Carvalho et al. (2016) and de Souza et al. (2015), as follows: plant height (PH, cm), spike insertion height (SH, cm), spike diameter (SD, mm), spike length (SL, cm), number of rows per spike (NR, unit), number of grains per row per spike(GR, unit) spike mass (SM, g), mass of grains per spike (GM, g), cob diameter (CD, mm), cob mass (CM, g), one hundred grains mass (HM, g), grain length (GL, mm), grain width (GW, mm), grain thickness (GT, mm) and grain yield (GY, kg ha-1).

The nutritional characters were measured, being the seeds crushed in Marconi® MA 020thousand equipped with a 0.053 mm sieve. In this way, the ground sample of each genotype was subdivided into six ground subsamples of 100 g (Carvalho et al., 2016). Subsequently, iron (Fe, mg kg -1), copper (Co, mg kg -1), zinc (Zn, mg kg -1), sodium (Na, mg kg -1) and manganese(Mn, mg kg-1) contents were measured in the seeds (Tedescoet al., 1995).

In the Laboratory of Secondary Metabolism also belonging to UFPel, the seed color (SC, Hue angle), acidity (AC, percentage), pH (pH, potential of hydrogen), soluble solids (SS, °Brix), total carotenoids (CA, μg g-1) (AOAC, 2005), total phenols (FO, μg g-1) (Singleton and Rossi, 1965), total flavonoids (FL, μg g-1) (Zhishean et al., 1999), antioxidant potential by the DPPH radical (DP, percentage of Inhibition) (Brand Williams et al., 1995), antioxidant potential by the ABTS radical (AB, percent inhibition) (Rufino et al. 2007).After, the LC-ESI-qToF-MSmethod of Mass spectrometry method (table 2) was used to quantify the intensities of the amino acids contained in maize seeds, being these, Alanine (AL), arginine (AR), asparagine (AS), cysteine (CI), glutamine (GU), glycine (GI), proline (PR), serine (SE), histidine (HI), threonine (TE) Tryptophan (TR), methionine (MT) and phenylalanine (PE) based on the methodology proposed by de Vos et al. (2007).

**Table 2:** Especifications of the *LC-ESI-qToF-MS* mass spectrometry method used to quantify the maize seeds amino acids intensity.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Amino acid | Abreviation | Retention time | Experimental m/z | Theoretical m/z | Error (ppm) | Formula | Searchlocation |
| Alanine | ALβ | 1.8600 | 90,0550 | 90.5500 | 0 | C3H7NO2 | Metlin\* |
| Arginine | ARβ | 11.5400 | 454.2340 | 454.2384 | 9 | C6H12N4O2 | Metlin |
| Asparagine | ASβ | 8.0600 | 188.0100 | 188.0101 | 0 | C17H33N7O6 | Metlin |
| Cysteine | CIβ | 14.7300 | 565.2550 | 565.2551 | 0 | C24H36N8O6S | Metlin |
| Glutamine | GLβ | 1.5300 | 147.5000 | 147.0531 | 3 | C5H9NO4 | MassBank\*\* |
| Glycine | GIβ | 11.5400 | 454.2340 | 454.2384 | 9 | C17H33N7O6 | Metlin |
| Proline | PRβ | 1.9100 | 116.0600 | 115.0633 | 1 | C5H9NO2 | Metlin |
| Serine | SEβ | 11.5400 | 454.2340 | 454.2384 | 9 | C17H33N7O6 | Metlin |
| Histidine | HIα | 14.6800 | 441.2030 | 441.2027 | 0 | C17H25N7O4S | Metlin |
| Threonine | TEβ | 12.4500 | 468.2490 | 468.2463 | 7 | C12H33N5O7 | Metlin |
| Tryptophan | TR α | 14.8000 | 595.2760 | 595.2711 | 0 | C25H36N10O6 | Metlin |
| Methionine | MTα | 14.6800 | 441.2030 | 441.2027 | 0 | C17H25N7O4S | Metlin |
| Phenylalanine | PE α | 6.4100 | 166.0860 | 166.0863 | 1 | C9H11NO2 | Metlin |

\*https://metlin.scripps.edu; \*\*http://www.massbank.jp; αEssential amino acid for humans; β Non-essential amino acid for humans; m/z:masschargeratio

The data obtained were subjected to the normality test by Shapiro Wilk (1965), followed by the Deviance analysis (p ≤ 0.05) by the chi-square test (X²) in order to identify the significance of the character. For the estimate ofvariance components and genetics parameters (REML individual)for the half-sibling maize progenies and to meet the assumptions of the experiment, the model 01 proposed by Resende(2007) was used. Afterwards, the statistical model , where: y:is the data vector, r:are the effects of repetitions (fixed), a: are the individual additive genetics effects (random), p: are the portion effects, e: are the residual effects(random) was used.The additive genetic variance(Va), individual phenotypic variance (Vp), environment between progenies variance(Vep), residual variance(Ve), narrow sense heritability (h²a), narrow sense heritability between the progenies(h²ep), narrow sense heritability within the progenies(h²dp), heritability with restricted sense of the progenies average(h²m), coefficient of determination of progeny effects(c²), progeny accuracy(Ac), coefficient of genotypic variationof progenies (CVg), coefficient of residual variation(CVe)and overall average character(MG).

Theaverage results were used to determine the percentage of heterosis(Ramalho et al., 2012). Later, theh²a, h²ep, h²dpandh²mwere used to perform the genetic dissimilarity analysis of the characters using the mean Euclidean Distance andUnweighted Pair GroupingMethodwithArithmeticMean (UPGMA) clustering method. For the relative contribution of narrow sense heritabilities and character differentiation, Singh’s method was used (Singh, 1981). The analyses were carried out using the softwareSelegen (Resende, 2007) andGenes (CRUZ, 2013).

**RESULTS AND DISCUSSION**

Deviance analysis were performed for the 42 characters measured, where it revealed significance for 34 characters at P≤ 0.05 by the chi-square test.However, the spike insertion height (SH), cob diameter (CD), grains length (GL), total phenols (FO), cysteine (CI), glutamine (GU), histidine (HI) and phenylalanine (PE) were not significant, so the estimates of variance components and genetic parameters did not obtain the necessary consistency for these characters.

The phenotypicratios(Vp)for the plant height of maize half-sibling progenies were 9.4% due to additive genetic effects(Va). However, the differentiations expressed between progenies (Vep)werecertain por26.7% Va (Table3). The narrow sense heritability between (h²ep: 0.15) and within (h²dp: 0.11) progenies were low, and reports of half-sibling maizeinbreedsindicated low narrow sense heritability(h²:0.18) inMinasGerais (Faluba et al., 2010). Sincethis character is highly influenced by the environment, 70.5% of the ratio between the coefficients of genotypic (CVg) and residual (CVe)variation were due to environmental effects.

**Table3:** Estimates of variance componentsand genetic parameters (individual REML) for half-sibling maize progenies.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Characters | D(x²5%) | Va+ | Vp | Vep | Ve | h²a | h²ep | h²dp | h²m | c² | Ac | CVg | CVe | MG |
| PH++ | \* | 110.07 | 1168.01 | 411.01 | 646.92 | 0.09 | 0.15 | 0.11 | 0.08 | 0.35 | 0.28 | 2.42 | 11.81 | 216.54 |
| SH | ns | 45.48 | 447.61 | 49.99 | 352.14 | 0.10 | 0.11 | 0.09 | 0.11 | 0.11 | 0.34 | 2.99 | 11.86 | 112.71 |
| SD | \* | 5.48 | 58.96 | 21.59 | 31.89 | 0.09 | 0.15 | 0.11 | 0.08 | 0.37 | 0.27 | 2.74 | 13.56 | 42.73 |
| SL | \* | 1.20 | 12.28 | 3.78 | 7.23 | 0.10 | 0.14 | 0.11 | 0.08 | 0.31 | 0.29 | 4.33 | 20.17 | 12.65 |
| NR | \* | 0.847 | 8.86 | 2.97 | 5.04 | 0.10 | 0.14 | 0.11 | 0.08 | 0.34 | 0.28 | 3.42 | 16.41 | 13.44 |
| GR | \* | 7.91 | 86.44 | 33.06 | 45.47 | 0.09 | 0.15 | 0.12 | 0.07 | 0.38 | 0.27 | 6.14 | 30.91 | 22.92 |
| SM | \* | 392.76 | 5543.21 | 3190.86 | 1959.58 | 0.07 | 0.17 | 0.13 | 0.05 | 0.58 | 0.22 | 7.95 | 50.35 | 124.71 |
| GM | \* | 389.18 | 4438.71 | 1875.07 | 2174.45 | 0.09 | 0.15 | 0.12 | 0.07 | 0.42 | 0.26 | 9.29 | 48.91 | 106.18 |
| CD | ns | 1.32 | 12.77 | 1.61 | 9.84 | 0.10 | 0.12 | 0.09 | 0.11 | 0.13 | 0.33 | 2.31 | 9.20 | 24.83 |
| CM | \* | 16.32 | 165.62 | 49.37 | 99.92 | 0.10 | 0.14 | 0.11 | 0.09 | 0.30 | 0.29 | 8.74 | 40.28 | 23.12 |
| HM | \* | 3.97 | 39.73 | 11.05 | 24.71 | 0.10 | 0.14 | 0.11 | 0.09 | 0.28 | 0.30 | 3.27 | 14.76 | 30.50 |
| GL | ns | 0.30 | 2.89 | 0.45 | 2.13 | 0.10 | 0.12 | 0.10 | 0.11 | 0.16 | 0.33 | 2.35 | 9.53 | 11.68 |
| GW | \* | 0.05 | 0.46 | 0.11 | 0.31 | 0.10 | 0.13 | 0.10 | 0.10 | 0.24 | 0.31 | 1.31 | 5.70 | 8.32 |
| GT | \* | 0.01 | 0.13 | 0.02 | 0.09 | 0.10 | 0.12 | 0.10 | 0.11 | 0.16 | 0.33 | 1.27 | 5.20 | 4.54 |
| GY | \* | 1914697.87 | 21818829.39 | 9200378.65 | 10703752.86 | 0.09 | 0.15 | 0.12 | 0.07 | 0.42 | 0.26 | 9.32 | 49.01 | 7425.65 |
| Fe | \* | 2.25 | 21.92 | 5.05 | 14.61 | 0.10 | 0.13 | 0.10 | 0.10 | 0.23 | 0.31 | 3.05 | 13.16 | 24.61 |
| Cu | \* | 0.34 | 3.55 | 1.15 | 2.06 | 0.10 | 0.14 | 0.11 | 0.08 | 0.32 | 0.29 | 8.97 | 42.53 | 3.26 |
| Zn | \* | 4.21 | 56.24 | 30.43 | 21.60 | 0.07 | 0.16 | 0.13 | 0.05 | 0.54 | 0.23 | 3.18 | 19.28 | 32.25 |
| Na | \* | 61.29 | 617.47 | 178.10 | 378.08 | 0.10 | 0.14 | 0.11 | 0.09 | 0.29 | 0.30 | 2.18 | 9.96 | 179.39 |
| Mn | \* | 2.88 | 51.39 | 35.45 | 13.06 | 0.06 | 0.18 | 0.14 | 0.03 | 0.69 | 0.19 | 5.36 | 40.21 | 15.83 |

+D: Deviance per X² a 5% of probability; Va: additive genetic variance; Vp: phenotypic individual variance; Vep: environmental variance between the progenies; Ve: residual variance; h²a: Narrow sense heritability of additive effects; h²ep: Narrow sense heritability between the progenies; h²dp: Narrow sense heritability within the progenies; h²m: Narrow sense heritability, mean between the progenies; c²: Coefficient of determination of progenies effects; Ac: Accuracy of the progenies; CVg: Coefficient of genotypic variation between the progenies; CVe: Coefficient of residual variation; MG: General mean of the character.

++PH: Plant Height; SH: spikeinsertion height; SD: spike diameter; SL: spikelenght; NR: number of rows of grains per spike; GR: number of grains per row per spike; SM: spike mass; GM: mass of grains per spike; CD: cob diameter; CM: cob mass; HM: one hundred grains mass; GL: grain length; GW: grain width; GT: grain thickness; GY: grain yield; Fe: iron content; Cu: copper content; Zn: zinc content; Na: sodium content; Mn: manganese content in the grain.

Regardingmaizespikecharacters, higher contributions of additive gene fractions (Va) to the phenotypic expression (Vp) was observed for stem mass (9.8%), spike length (9.7%) and number of grain rows per spike (9.5%).However, the additive genetic features contributed with 33.0% for cob mass and 31.7% for spike length and phenotypically differentiated progenies(Vep). The additive effects of narrow sense heritabilityrevealed similar magnitudes (h²a:0.10) for spikelength, number of grain rows per spike and cob mass.On the other hand, narrow senseheritabilitybetween the progenies were larger for spikediameter (h²ep: 0.15) and mass (h²ep: 0.17). Research carried out on 27 maize genotypes grown in five environments of the Southern Region of Brazil showed broad sense (H²)heritabilitiesof 0.70, 0.65, 0.62 and 0.70 for spike length, spike mass, grains mass per spike and stem mass, respectively(Nardino et al., 2016).

The coefficient of determination for progeny effects was higher for spike mass (c²: 0.58).The low accuracy (Ac: 0.22) obtained indicated a largeenvironmentaleffecton this character.Accuracywas divided in high (0.70 <Ac), moderate (0.50 <Ac <0.65), and low (0.10<Ac<0.40), according to Resende and Duarte (2007).The spikeand cob mass evidenced the highest coefficients of genotypic variation (CVg) for these characters, which demonstrated variability for the progenies, but with marked residual effects.

Regarding the dimensional properties of maize grains, the width was determined by 10.8% of additive genetic effects (Va), when compared to the one hundred grains mass (9.9%) and grain thickness (7,6%). However, between the progenies (Vep) the grain thickness was 50.0% of the additive gene fraction (Va), being greater than mass of one hundred grains (35.9%) and grain width (45.4%). The narrow sense heritability for additive effects (h²a) and within the progenies (h²dp) was similar (0.10) for mass of one hundred grains, grain width and thickness. However, the highest narrow sense heritability between the progenies (h²ep: 0.14) was observed for mass of one hundred grains. Reports on intervarietal crosses in maize define that narrow sense heritability (h²) was low for the mass of one hundred grains (0.06), grain length (0.11) and width (0.19) (Carvalho et al., 2016).

Thecoefficientofdeterminationforthe progeny effects was higher for mass of one hundred grains (c²: 0.28), between the grain size properties this was the most influenced by the environment effects, but the accuracy was low (Ac: 0.30) for these characters. The ratio of the coefficient genotypic variation (CVg) to the residual (CVe) indicated a contribution of 24.4% of the genetic fraction in the total variation of grain thickness.

Grain yield was the result of 8.7% of additive gene effects (Va).Between the progenies (Vep), these effects (Va) were even higher, reaching 20.8% of the phenotypic variation. Thenarrow sense heritability between (h²ep: 0.15) and within (h²dp: 0.12) progenies was low. In this way, understanding the additive genetic variation becomes essential to the breeder even if it does not reveal which gene actions are involved. However, it can be determined by the average effects of several alleles together and will allow inheritable effects, genotypic differentiations and selection responses (Falconer and Mackay, 1996). Reports on half-sibling maize lines indicate narrow sense heritability of h²: 0.49 for grain yield. However, the magnitude of this parameter could be influenced by the number of plants measured (Palomino et al., 2000).

The coefficient of determination for progeny effects was high (c²= 0.42) and low accuracy (Ac= 0.26). Reports regarding single cross hybrids cultivated in five environments indicated coefficient of determination estimates (c²= 0.66) and moderate accuracies for grain yield. The magnitude of these parameters could be due to the nature of the genotype, the number of observations and the variations imposed by the environment (Souza et al., 2015).

Iron, copper and sodium micronutrients revealed the highest contributions of the additive genetic variance (Va) to the phenotypic expression, indicating 10.2%, 9.5% and 9.9%, respectively. Between the progenies (Vep),larger effects were obtained for iron content, being 44.5% of the phenotypic variation resultant from the additive gene fraction (Va). Narrow sense heritability was similar (h²a: 0.10) for iron, copper and sodium content. In contrast, narrow sense heritability between and within progenies was higher for zinc (h²ep: 0.16; H²dp: 0.13) and manganese (h²ep:0.18; h²dp: 0.14). The coefficient of determination of progeny effects (c²: 0.69) was high for manganese. However, for all micronutrients, low accuracy was obtained and this can be attributed to environmental effects. Iron content revealed that 23.1% of total variance (CVe) originated from genetic causes (CVg). In maize intervarietal genotypes, the highest contribution of additive genetic variance and narrow sense heritability (h²: 0.34) was obtained forsodiumcontent, since for micronutrients the low magnitudes of the genetic parameters were due to the great environmentaleffecton the dynamics of these characters and non-additive deviations (Carvalho et al., 2016).

Bioactive compounds such as total flavonoids, soluble solids and seed color (Table 4) displayed 10.0%, 10.0% and 10.4%, respectively,of the phenotypic expression (Vp) explained by additive genetic variance (Va). Betweenprogenies (Vep) character trends are maintained and proportions are increased to 38.4%, 36.6% and 50.0% of the contribution of the additive gene fraction (Va) to the phenotype. Low genetic contributions were presented for the acid and pH of the seeds in the general scope and between half-sibling progenies, the differentiations revealed for these characters are in its majority caused by non-additive deviations and environmental effects. In maize, the total flavonoids are abundant and essential for the defense of stresses arising from temperature, water deficits, salinity, ultraviolet radiation, diseases and insectpests (Wen et al., 2014).

**Table4:** Estimates of variance components and genetics parameters (individual REML) for the half-sibling maize progenies.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Character | D(x²5%) | Va+ | Vp | Vep | Ve | h²a | h²ep | h²dp | h²m | c² | Ac | CVg | Cve | MG |
| FO++ | ns | 6488.84 | 62318.12 | 9287.00 | 46542.27 | 0.10 | 0.12 | 0.09 | 0.11 | 0.15 | 0.33 | 4.06 | 16.38 | 992.53 |
| FL | \* | 44893.77 | 444533.24 | 116721.41 | 282918.06 | 0.10 | 0.14 | 0.11 | 0.09 | 0.26 | 0.30 | 5.57 | 24.78 | 1902.59 |
| CA | \* | 111.61 | 1334.37 | 616.64 | 606.13 | 0.08 | 0.16 | 0.12 | 0.06 | 0.46 | 0.25 | 4.72 | 25.98 | 112.01 |
| DP | \* | 13.61 | 177.46 | 93.32 | 70.53 | 0.08 | 0.16 | 0.13 | 0.05 | 0.53 | 0.23 | 6.11 | 36.31 | 30.20 |
| AB | \* | 5.31 | 74.39 | 42.51 | 26.57 | 0.07 | 0.17 | 0.13 | 0.05 | 0.57 | 0.22 | 2.07 | 13.03 | 55.73 |
| SS | \* | 0.37 | 3.70 | 1.01 | 2.32 | 0.10 | 0.14 | 0.11 | 0.09 | 0.27 | 0.30 | 8.38 | 37.68 | 3.64 |
| AC | \* | 0.00 | 0.02 | 0.01 | 0.01 | 0.10 | 0.14 | 0.11 | 0.09 | 0.27 | 0.30 | 7.43 | 33.34 | 0.32 |
| pH | \* | 0.00 | 0.05 | 0.02 | 0.03 | 0.09 | 0.15 | 0.12 | 0.07 | 0.40 | 0.27 | 0.53 | 2.72 | 6.49 |
| SC | \* | 0.07 | 0.69 | 0.14 | 0.48 | 0.10 | 0.13 | 0.10 | 0.10 | 0.20 | 0.32 | 0.14 | 0.59 | 95.52 |
| AL | \* | 218015.35 | 2200276.92 | 640192.97 | 1342068.60 | 0.10 | 0.14 | 0.11 | 0.09 | 0.29 | 0.30 | 6.29 | 28.78 | 3712.84 |
| AR | \* | 5233121.83 | 60037972.78 | 25674033.92 | 29130817.02 | 0.09 | 0.15 | 0.12 | 0.07 | 0.43 | 0.26 | 9.85 | 52.15 | 11615.43 |
| AS | \* | 259676.77 | 2614672.92 | 752330.56 | 1602665.59 | 0.10 | 0.14 | 0.11 | 0.09 | 0.29 | 0.30 | 8.12 | 37.07 | 3136.40 |
| CI | ns | 32130.48 | 832592.43 | 10733.77 | 789728.19 | 0.04 | 0.04 | 0.03 | 0.05 | 0.01 | 0.23 | 1.01 | 5.97 | 8892.94 |
| GU | ns | 102468.54 | 1012833.92 | 109493.00 | 800872.38 | 0.10 | 0.11 | 0.09 | 0.11 | 0.11 | 0.34 | 5.28 | 20.93 | 3029.52 |
| GI | \* | 912504.89 | 15142032.79 | 9982214.80 | 4247313.10 | 0.06 | 0.18 | 0.14 | 0.04 | 0.66 | 0.19 | 8.69 | 62.05 | 5495.02 |
| PR | \* | 7083504.20 | 115622119.00 | 82803986.70 | 32456965.78 | 0.06 | 0.18 | 0.14 | 0.04 | 0.68 | 0.19 | 5.43 | 39.89 | 24487.44 |
| SE | \* | 74798.25 | 1029818.92 | 577482.82 | 377537.86 | 0.07 | 0.17 | 0.13 | 0.05 | 0.56 | 0.22 | 3.47 | 21.56 | 3940.63 |
| HI | ns | 4286022.58 | 42472496.11 | 4508988.22 | 33677485.31 | 0.10 | 0.11 | 0.09 | 0.11 | 0.11 | 0.34 | 5.45 | 21.58 | 18996.29 |
| TE | \* | 1499322.74 | 25656358.33 | 17262293.34 | 6894742.24 | 0.06 | 0.18 | 0.14 | 0.04 | 0.67 | 0.19 | 5.57 | 40.62 | 10992.42 |
| TR | \* | 19349.95 | 226458.99 | 100696.10 | 106412.93 | 0.09 | 0.15 | 0.12 | 0.06 | 0.44 | 0.25 | 1.84 | 9.93 | 3782.83 |
| MT | \* | 84415.78 | 821344.13 | 189121.84 | 547806.51 | 0.10 | 0.13 | 0.10 | 0.10 | 0.23 | 0.31 | 1.11 | 4.80 | 13065.54 |
| PE | ns | 25999.11 | 6121892.81 | 6663.53 | 6089230.17 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.08 | 0.41 | 7.19 | 19870.72 |

+D: Deviance per X² a 5% of probability; Va: additive genetic variance; Vp: phenotypic individual variance; Vep: environmental variance between the progenies; Ve: residual variance; h²a: Narrow sense heritability of additive effects; h²ep: Narrow sense heritability, between the progenies; h²dp: Narrow sense heritability within the progenies; h²m: Narrow sense heritability, mean between the progenies; c²: Coefficient of determination of progenies effects; Ac: Accuracy of the progenies;CVg: Coefficient of genotypic variationbetween the progenies; CVe: Coefficient of residual variation; MG: General mean of the character.

++FO: phenols; FL: flavonoids; CA: carotenoids; DP: DPPH antioxidant radical; AB: ABTS antioxidant radical; SS: soluble solids; AC: acid; pH: hydrogenation potential; SC: seed color; AL: alanine; AR: arginine; AS: asparagine; CI: cysteine; GU: glutamine; GI: glycine; PR: proline; SE: serine; HI: histidine; TE: threonine; TR: tryptophan; MT: methionine; PE: phenylalanine.

The narrow sense heritability is similar (h²: 0.10) for total flavonoids, soluble solids and seed color. Differential behavior was expressed by the narrow sense heritability between and within the progenies, with higher magnitudes for total carotenoids (h²ep: 0.16; h²dp: 0.12), antioxidant potential by the DPPH radical (h²ep: 0.16; h²dp: 0.13) and ABTS radical (h²ep: 0.17; h²dp: 0.13). Reportshavedescribed that the main carotenoids in maize are lutein, zeaxanthin, α and β carotene, which are highly dependent on inbred lines, environmental and harvest season (Azmach et al., 2013). In progenies of half-sibling maize narrow sense heritability was obtained (h²: 0.19) for the total carotenoids (Halilu et al., 2016). With genitor-progeny regression in maize, heritability was obtained with restricted sense for soluble solids (h²:0.25), total flavonoids (h²:0.08), total carotenoids (h²:0.48), antioxidant potential for the radical DPPH radical and ABTS radical (h2:0.26 and h²:0.07), respectively (Carvalho et al., 2016). The narrow sense heritability of the progeny mean (h²m: 0.10) is pronounced only for seed color, because this is a character with the greatest contribution of the additive gene fraction to the phenotype and to the indirect effects of the total carotenoids. Pigmentation from carotenoids result in differentiations in the color of maize seeds (Bóremand Rios, 2011).

The coefficient of determination of progeny effects showed to be higher for the antioxidant potential by the DPPH radical (c²: 0.53) and ABTS radical (c²: 0.57). These high results are due to the indirect estimation method of these characters, and can be bypassed through the increment of observations in the experimental conditions, in general, low accuracies were revealed for the bioactive compounds.When comparing proportionality between the coefficients of variation, it was observed that the total flavonoids, soluble solids, and the seed color showed 22.4%, 22.2% and 23.7% of the contribution of the genetic fraction to the total variation of the bioactive compounds.

Essential amino acids are constituents of some structural proteins present in plants (Ufaz and Calili, 2008). Essential amino acids are called those that human and animal organisms do not have the ability to synthesize naturally, thus, these molecules must be supplemented through diet (Wen et al., 2016). These being, lysine, methionine, tyrosine, phenylalanine, tryptophan, valine, isoleucine, leucine and histidine (Galili et al., 2016). In this study, using half-sibling maize progeny it was possible to identify the presence of 13 amino acids, including alanine, arginine, asparagine, cysteine, glutamine, glycine, proline, serine, histidine, threonine, tryptophan, methionine and phenylalanine.Reportsindicated that in 100g of maize protein, it was possible to detect7.8% alanine, 3.6% arginine, 2.9% glycine, 10.0% proline, 4.2% serine, 3.1% threonine, 0.31% tryptophan and 1.9% methionine. Studies using 15 singlecross maize hybrids grown in four environments of Rio Grande do Sul obtained 6.5% crude protein in the grains (Carvalho et al., 2016).

Among the amino acids identified, alanine, asparagine, tryptophan and methionine show a contribution of 9.9%, 9.9%, 8.5% and 10.2% of the phenotypic expression (Vp) by additive genetic variance (Va). Betweenthe progenies (Vep) character trends were maintained and proportions were increased, where alanine, asparagine and methionine were determined by 34.0%, 34.5% and 44.6% of the additive gene fraction (Va), respectively. Thenarrow sense heritability of additive effects was low (h2a: 0.10) for alanine, asparagine and methionine. For narrow sense heritability within and between the progenies, prominent results were expressed for arginine and tryptophan (h²ep: 0.15; h²dp: 0.12), glycine, proline and threonine (h²ep: 0.18; h²dp: 0.14), and serine (h²ep: 0.17; h²dp: 0.13). The highest narrow sense heritability of the progeny mean (h²m: 0.10) was evidenced for methionine. Maize breeding may increase the total proportion of amino acids by increasing the protein fraction of the grains. However, lysine and tryptophan may accumulate at lower amounts than other amino acids, while higher phenylalanine may lead to oil content increases (Wen et al., 2016).

The coefficient of determination of progeny effects was high for glycine (c²: 0.60), proline (c²: 0.68), serine (c²: 0.56) and threonine (c²: 0.67), being these results of genetic differentiation, environmental effects and the peculiarities involved in the measurement of these. For all the amino acid studied low accuracy was obtained. However, when analyzing the ratios of coefficients of variation, a contribution of 21.8%, 21.9%, and 23.1% of the genetic fraction to the total variation of the amino acids alanine, asparagine and methionine, respectively, was found.

Heterosis can be defined as the increase of a character measured in progeny when compared to their parents (Falconer and Mackay, 1996). It is expressed through the deviations of dominance, the complementarity of allelic forms in heterozygosis called overdominance, and the intergenic interactions arising from the epistatic events.The heterosis was estimated for the 42 characters measured in half-sibling maize progenies (Table 5), where the estimation of this parameter was obtained for each inbred line S5 (L1: 256, L2: 258, L3: 389, L4: 262 and L5: 225) cross-linked with a broad genetic based tester (HD: CD308) pollen donor.

**Table5:** Estimates of heterosis (%) obtained in Top Cross crosses between inbred lines S5 (L1:256, L2:258, L3:389, L4:262, L5:225) and a tester (HD: CD 308) with broad genetic basis.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Heterosis (H+) | | | | | | | | | | | |
| Character | L1xHD | L2xHD | L3xHD | L4xHD | L5xHD |  | L1xHD | L2xHD | L3xHD | L4xHD | L5xHD |
| HIB (I) | HIB (II) | HIB (III) | HIB (IV) | HIB (V) | HIB (I) | HIB (II) | HIB (III) | HIB (IV) | HIB (V) |
| PH++ | -6.88 | 16.65 | -0.24 | 26.57 | 20.36 | FL | -20.18 | 15.40 | -59.71 | -15.84 | -15.88 |
| SH | 3.09 | 18.53 | 7.07 | 50.46 | 24.78 | CA | 51.97 | 8.65 | 35.98 | 60.55 | 24.38 |
| SD | -4.81 | 16.46 | 12.76 | 7.83 | 10.20 | DP | 113.27 | 82.13 | -15.16 | 91.97 | 182.51 |
| SL | -8.74 | 19.25 | -2.15 | -3.04 | 1.27 | AB | 40.06 | 21.85 | 8.37 | 36.37 | -15.04 |
| NR | 13.93 | 5.29 | 30.23 | 29.90 | 50.54 | SS | 33.33 | 200.00 | 100.00 | 7.69 | 33.33 |
| GR | -13.45 | -2.35 | 24.84 | 23.08 | 5.76 | AC | -52.99 | -46.01 | -36.15 | -47.49 | -59.35 |
| SM | -26.17 | 54.81 | 10.86 | -6.39 | 12.70 | pH | 6.32 | 6.06 | 6.24 | 7.11 | 8.78 |
| GM | -24.40 | 77.30 | 18.89 | 0.69 | 24.68 | HU | -0.27 | 2.06 | -0.39 | 1.51 | 0.68 |
| CD | 3.59 | 1.02 | 10.49 | 13.26 | 14.50 | AL | -48.05 | -2.54 | -24.73 | -29.66 | -48.15 |
| CM | -35.31 | 46.51 | -21.16 | 25.55 | 57.67 | AR | -77.27 | -32.70 | -25.91 | -79.76 | -60.08 |
| HM | -10.56 | 5.18 | 2.33 | 1.24 | 2.22 | AS | -44.91 | 5.24 | -30.90 | -41.48 | -35.50 |
| GL | 5.46 | 8.54 | 18.35 | 14.16 | -5.71 | CI | 1.97 | 2.68 | 16.12 | -18.37 | -3.72 |
| GW | -5.15 | -0.96 | -3.69 | -0.47 | -8.16 | GL | -8.50 | 33.11 | -11.82 | 6.24 | 9.40 |
| GT | 2.80 | -11.66 | 0.13 | -8.68 | -3.12 | GI | 41.44 | 193.21 | -26.42 | -40.09 | -54.59 |
| GY | -24.40 | 77.30 | 18.89 | 1.22 | 24.68 | PR | 20.20 | 10.62 | -25.54 | 13.95 | 91.44 |
| Fe | 2.57 | 22.18 | 36.21 | -12.12 | -9.34 | SE | -46.17 | 26.01 | 18.88 | -17.86 | -6.55 |
| Cu | 160.78 | -73.37 | 31.35 | -6.90 | -57.40 | HI | -3.25 | -38.57 | -44.98 | -21.05 | -16.09 |
| Zn | 55.02 | 32.46 | 30.97 | 30.95 | 6.12 | TE | -9.81 | 84.07 | -36.96 | -27.87 | -37.58 |
| Na | -17.17 | 6.15 | -13.64 | -2.58 | -28.16 | TR | 5.63 | -11.77 | -11.42 | -25.21 | -14.96 |
| Mn | 15.13 | 0.75 | 49.70 | 45.34 | -22.19 | MT | 7.84 | -9.02 | -3.77 | -11.76 | -5.04 |
| FO | 32.14 | -48.43 | 38.67 | -39.74 | 13.27 | PE | 5.04 | 22.34 | 12.14 | -5.63 | 3.42 |

++PH: Plant Height; SH: spike insertion height; SD: spike diameter; SL: spike length; NR: number of rows of grains per spike; GR: number of grains per row per spike; SM: spike mass; GM: mass of grains per spike; CD: cob diameter; CM: cob mass; HM: one hundred grains mass; GL: grain length; GW: grain width; GT: grain thickness; GY: grain yield; Fe: iron content; Cu: copper content; Zn: zinc content; Na: sodium content; Mn: manganese content in the grain. FO: phenols; FL: flavonoids; CA: carotenoids; DP: DPPH antioxidant radical; AB: ABTS antioxidant radical; SS: soluble solids; AC: acidity; pH: hydrogenation potential; SC: seed color; AL: alanine; AR: arginine; AS: asparagine; CI: cysteine; GU: glutamine; GI: glycine; PR: proline; SE: serine; HI: histidine; TE: threonine; TR: tryptophan; MT: methionine; PE: phenylalanine

Regardless of the inbreeding line S5 used, the crosses showed heterosis for insertion of spike height, number of grain rows per spike, stem diameter, zinc content, total carotenoids, soluble solids and pH, with a mean increase of 20.8% 26.0%, 8.6%, 31.1%, 36.3%, 74.9% and 6.9% in the half sibling progenies, respectively. Using the inbred line L2: 258 as the female parent, it was possible to specifically increase sodium content, total flavonoids and threonine amino acid by 6.1%, 15.4% and 84.1%, respectively. Therefore, the efficient choice of the line to be used in the crossing makes it possible to increase a given micronutrient, bioactive compound or amino acid in the progeny by means of heterosis. In this way, heterosis is dependent on the genetic distance, allelic frequency and hybrid combination performed between certain parents, these conformations are complementary and provide evidence of non-additive effects from dominance and overdominance (Paterniani et al., 2008).

In general, it was possible to define that among all the characters measured the L2:258 and L3:389 lines were the most efficient, where 73.8% and 54.7% of the characters measured in the progenies were increased by heterosis, respectively. By stratifying the heterosis contributions by groups of characters, it was possible to emphasize that the grain yield showed increases of 77.3% when using the lineage L2: 258 as maternal parent. Genetic improvement studies for intervarietalmaize hybrids obtained a mean heterosis of 37.3% for grain yield (Bernini et al., 2013).

When it is desired to increase the micronutrients in the progeny through crossbreeding gains by heterosis, the lineage L3: 389 can be used as maternal parent, which resulted in increases of 36.2%, 31.3%, 30.9% and 49.7% for iron content, copper, zinc and manganese, respectively.Reportsdefine that biofortified maize genotypes with micronutrients can be achieved by genetic engineering and/or conventional breeding, through recombination of parents and progenies that are more efficient in absorbing, transporting and accumulating in the grains higher iron, zinc, copper and manganese contents. Copper, zinc and manganese can be potentiated by increasing the proportions of the amino acids methionine and cysteine, these micronutrients make up enzymes that fight the actions of reactive oxygen species and can minimize the presence of free radicals, in contrast, iron makes up the enzyme catalase that reduces the effects of hydrogen peroxide precursor of oxidative stress in plants (Rios et al., 2015).

By showing the heterosis effects on the bioactive compounds and amino acids in the half sibling maize progenies, it was possible to show that the line L2: 258 was the best maternal parent, since it increased the largest fraction of the nutritional characters measured. This lineage allowed the heterosis for total flavonoids (15.4%), total carotenoids (8.6%), antioxidant potential by the DPPH radical (82.1%) and ABTS (21.8%), soluble solids (200.0%), pH (6.0%), seed color (2.0%), asparagine (5.2%), cysteine (2.6%), glutamine (33.1%), glycine (193.2%), proline (10.6%), serine (26.0%), threonine (84.0%) and phenylalanine (22.3%).

Heterosis values for bioactive compound in half-sib progenies were lower than those found for progenies from intervarietal crosses. Parental genotype and allele complementarity in heterozygous loci could explain the amplitude of the results for this parameter (Carvalho et al., 2016). The increases in progenies from heterosis due to the use of the L2: 258 line for the eight amino acids were important. However, it is essential to direct maize breeding under these conditions to essential amino acids for humans and animals (Galili et al., 2016), emphasis to phenylalanine where the progenies were 22.3% higher for this amino acid when compared to their parents.

A multivariate approach was applied considering the heritability of the additive effects (h²a) between the progenies (h²ep), within the progenies (h²dp) and the mean progenies (h²m) for the 42 characters measured in maize genotypes. The mean Euclidean distance was analyzed in order to show the profile of the characters as to their inherent tendency, the UPGMA grouping was used to express the distances through a dendrogram (Figure 1), and the mean (0.0553) of the matrix of the distances was evidenced as the criterion of separation of the profiles of restricted heritability.

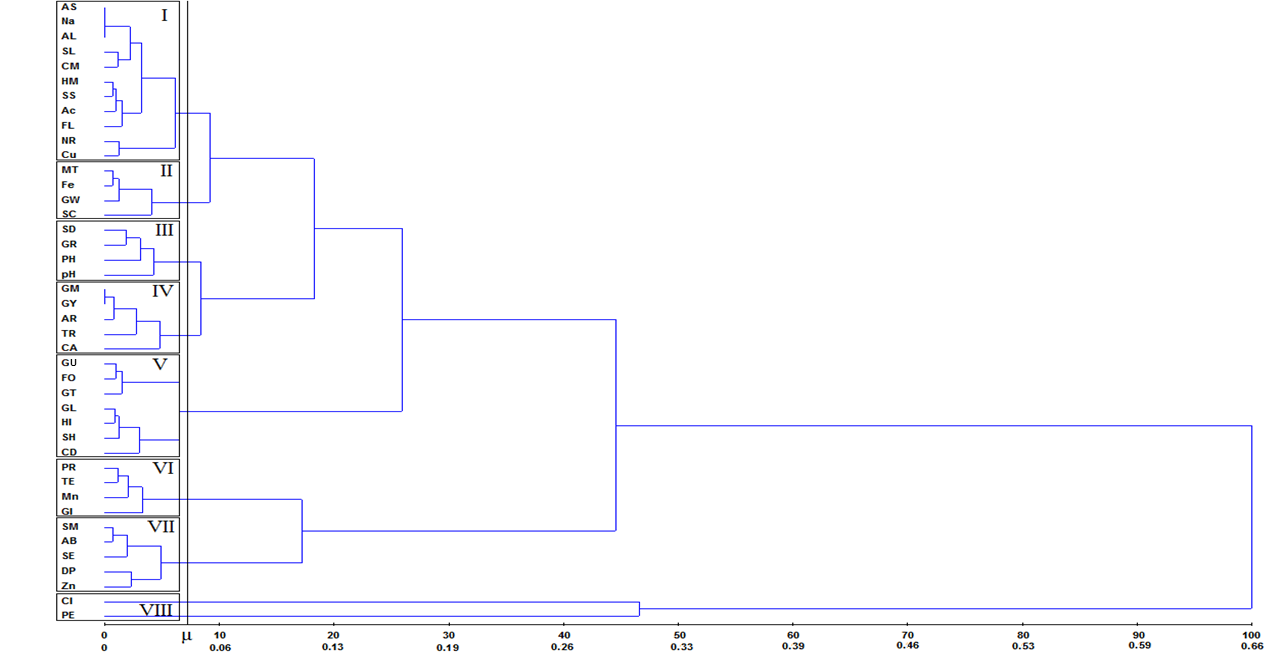


Figure 1: Dendrogram with the genetic dissimilarity obtained through the narrow sense heritability of the additive effects (h²a), narrow sense heritability between the progenies (h²ep), narrow sense heritability within the progenies(h²dp), and narrow sense heritability of the mean between the progenies(h²m) of the 42 characters measured in half-sibling of maize progenies, using the Euclidean distance mean, and UPGMA grouping method. ++PH:Plant Height; SH: spike insertion height; SD: spike diameter; SL: spike length; NR: number of rows of grains per spike; GR: number of grains per row per spike; SM: spike mass; GM: mass of grains per spike; CD: cob diameter; CM: cob mass; HM: one hundred grains mass; GL: grain length; GW: grain width; GT: grain thickness; GY: grain yield; Fe: iron content; Cu: copper content; Zn: zinc content; Na: sodium content; Mn: manganese content in the grain. FO: phenols; FL: flavonoids; CA: carotenoids; DP: DPPH antioxidant radical; AB: ABTS antioxidant radical; SS: soluble solids; AC: acidity; pH: hydrogenation potential; SC: seed color; AL: alanine; AR: arginine; AS: asparagine; CI: cysteine; GU: glutamine; GI: glycine; PR: proline; SE: serine; HI: histidine; TE: threonine; TR: tryptophan; MT: methionine; PE: phenylalanine.

Roman numerals represent the narrow sense heritability profiles of the evaluated traits; μ: mean of the narrow sense heritability distances matrix.

The profile I (h²a: 0.10, h²ep: 0.14, h²dp: 0.11, h²m: 0.09) clustered spike length, spike mass, mass of one hundred grains, number of grain rows per spike, copper and sodium content, soluble solids, acidity, total flavonoids, asparagine and alanine. Profile II (h²a: 0.10, h²ep: 0.13, h²dp: 0.10, h²m: 0.10) clustered grain width, iron content, seed color and methionine. Profile III (h²a: 0.09, h²ep: 0.15, h²dp: 0.12, h²m: 0.08) associated plant height, spike diameter, number of grains per spike row and ph. Profile IV (h²a: 0.09, h²ep: 0.15, h²dp: 0.12, h²m: 0.07) clustered grain mass per spike, grain yield, total carotenoids, arginine and tryptophan. Profile V (h²a: 0.10, h²ep: 0.12, h²dp: 0.09, h²m: 0.11) included the spike insertion height, stem diameter, grain length and thickness, total phenols, glutamine and histidine. Profile VI (h²a: 0.06, h²ep: 0.18, h²dp: 0.14, h²m: 0.04) associated manganese content, glycine, proline and threonine. Profile VII (h²a: 0.07, h²ep: 0.16, h²dp: 0.13, h²m: 0.05) included spike mass, zinc content, antioxidant potential by DPPH and ABTS radicals, and serine. Profile VIII (h²a: 0.02, h²ep:0.02, h²dp: 0.02, h²m: 0.03) clustered cysteine and phenylalanine. Thus, the use of Singh's statistical method (1981) indicated that the narrow sense heritability of progeny means is the parameter that contributed the most (34.8%) to distinguish the inheritable genetic parameter profiles.

Considering the results obtained in this quantitative genetics study, it was possible to understand which yield and nutritional components are important for maize breeding, to minimize the lack of information on the contribution of variance components, genetic parameters and heterosis for the half-siblings progenies, to gather univariate genetic parameters and to define multivariate profiles for narrow sense heritability. Therefore, it was possible to obtain relevant and applicable information for the genetic improvement of maize regarding selection for grain yield and nutritional quality.

**CONCLUSIONS**

Half-sibling progenies reveal greater additive genetic contribution to phenotypic expression through grain width and thickness, iron content, total flavonoids and carotenoids, soluble solids, and methionine.

Narrow sense heritabilityvaluesbetween and within progenies are higher for manganese content, glycine, proline and tryptophan.

Regardless of the inbreeding line S5 used, heterosis gains are obtained for insertion of spike height, number of grain rows per spike, stem diameter, zinc content, total carotenoids, soluble solids and pH. Specific heterosis is evidenced for grain yield, glycine, serine, threonine and phenylalanine.

The multivariate approach used defines eight character profiles regarding their genetic trendsandindicatesnarrow sense heritability of the progeny mean as the major cause for this distinction.

The obtained genetic parameters are essential and applicable to plant breeding, where they can aid in the selection strategies of maize yield and nutritional components.

**REFERENCES**

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 2005. Official methods of analysis of the AOAC, 18th ed. Gaithersburg, M. D, USA. Method 970. 64.

AZMACH, G.; GEDIL, M.; MENKIR, A.; SPILLANE, C. 2013. Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. *BMC Plant Biology* 13: 227.

BERNINI, C.S.; PATERNIANI, M.E.A.G.Z.; DUARTE, A.P.; GALLO, P.B.; SOUZA GUIMARÃES, P.; ROVARIS, S.R.S. 2013. Inbreeding depression and heterosis of hybrids in F2 populations of maize in the Sao Paulo State, Brazil. *Bragantia* 72: 217-223.

BRAND-WILLIAMS, W.; CUVELIER, M.; BERSET, C. 1995. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT – *Food Science and Technology*, 28: 25–30.

CARVALHO, I.R.; NARDINO, M.; PELEGRIN, A. J.; FERRARI, M.; DEMARI, G.; SZARESKI, V. J.; BARBOSA, M. H.; SOUZA, V. Q. 2016. Path analysis and Annicchiarico method applied in relation to protein in corn (Zea mays L.) grains. *Australian Journal of Basic and Applied Sciences*, 10: 300-306.

CARVALHO, I.R.; NARDINO, M.; DE PELEGRIN, A.J.; HOFFMANN, J.F., POLETO, S.M.; FERRARI, M.; SZARESKI, V.J.; MEIRA, D.; CHAVES, F.C.; DE SOUZA, V.Q.; OLIVEIRA, A.C. 2016. Estimate of genetic parameters in bioactive and micronutrients compounds of maize. *African Journal of Agricultural Research*, 11: 3123-3133.

CRUZ, C. D. 2013. GENES: a software package for analysis in experimental statistics and quantitative genetics. *Acta ScientiarumAgronomy*, 35: 271-276.

DE VOS, R.C.; MOCO, S.; LOMMEN, A.; KEURENTJES, J.J.; BINO, R.J.; HALL, R.D. 2007. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nature Protocols* 2: 778–791.

FALCONER, D.S.; MACKAY, T.F.C. (1996) Introduction to quantitative genetics. Longmans green, London, NY, USA.

FALUBA, J. S.; MIRANDA, G. V.; LIMA, R. O.; SOUZA, L. V.; DEBEM, E.A.; OLIVEIRA, A. M. C. 2010. Genetic potential of maize population UFV 7 for breeding in Minas Gerais. *Ciência Rural*, 40: 1250-1256.

GALILI, G. A. D.; AMIR, R.; AND ALISDAIR, R. F. 2016. The regulation of essential amino acid synthesis and accumulation in plants. *Annual review of plant biology,* 67:153-178.

HALLAUER, A.R.; MIRANDA FILHO, J.B (1995) Quantitative genetics in maize breeding. Iowa State University Press. Ames, Iowa, USA.

HALILU, A.D.; ADO, S.G.; ABA, D.A; USMAN, I.S. 2016. Genetics of carotenoids for provitamin a biofortification in tropical-adapted maize. *The Crop Journal*, 4: 313-322.

HEINZ, R.; DE SOUSA MOTA, L.H.; GONÇALVES, M.C.; NETO, A.L.V.; CARLESSO, A. 2012. Selection of half-sib of maize for nitrogen use efficiency. *Revista Ciência Agronômica,* 43: 731-739.

NARDINO, M.; BARETTA, D.; CARVALHO, I. R.; FOLLMANN, D. N.; KONFLANZ, V. A.; DE SOUZA, V. Q.; OLIVEIRA, A. C. 2016. Phenotypic, genetic and environment correlation between traits of hybrid maize*. Biometric brazilian Journal*, 34:379-394.

PALOMINO, E.C.; RAMALHO, M.A.P.; FERREIRA, D.F. 2000. Sample size for half-sib family evaluation in maize. *Pesquisa Agropecuária Brasileira,* 35:1433-1439.

PATERNIANI, M.E.A.G.Z.; GUIMARÃES, P.D.S.; LÜDERS, R.R.; GALLO, P.B.; SOUZA, A.D.; LABORDA, P.R.; OLIVEIRA, K.M. 2008. Combining ability, genetic divergence among maize lines and correlation with heterosis. *Bragantia*, 67: 639-648.

RAMALHO, M.; SANTOS, J. B.; PINTO, C. B.; SOUZA, E. A.; GONÇALVES, F. M. A.; SOUZA, J. C. (2012) *Genetics in the Agriculture*, UFLA, Lavras, MG, Brazil.

RESENDE M.D.V. (2007) Software Selegem – REML/BLUP: Statistic system and computerized genetic selection via mixed linear models. *Embrapa Florestas*, Colombo, PR, Brazil.

RESENDE, M.D.V.; DUARTE, J.B. 2007. Precision and quality control in variety trials. *Pesquisa Agropecuária Tropical*, 37:182-194.

RIOS, S.D.A.; ALVES, K.R.; COSTA, N.M.B.; MARTINO, H.S.D. 2009. Biofortification: micronutrient enriched crops by genetic improvement. *Ceres,* 56: 713-718.

RUFINO, M. S. M. (2007) Determination of the total antioxidant activity in fuits by the ABTS free radical capture. Comunicado Técnico, 128 *Embrapa*, p.3–6.

SHAPIRO, S.S.; WILK, M.B. 1965. An Analysis of Variance Test for Normality. *Biometrika* 52: 3-4.

SINGLETON, V. L.; ROSSI, J. A. JR. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16: 144-158.

SINGH, D. 1981. The relative importance of characters affecting genetic divergence. *The Indian Journal of Genetics and Plant Breeding*, 41:237-245.

SOUZA, V.Q.; CARVALHO, I.R.; FOLLMANN, D.N.; NARDINO, M.; BELLÉ, R.; BARETTA, D.; SCHMIDT, D. 2015. Defoliation and its effects on morphological and productive traits in maize hybrids. *Revista brasileira de milho e sorgo*, 14: 61-74.

TEDESCO, M.J.; GIANELLO, C.; BISSANI, C.A.; BOHNEN, H.; VOLKWEISS, S.J. (1995) Analysis of soil, plants and other materials. *UFRGS Prees*, Porto Alegre, RS, Brazil.

UFAZ, S.; GALILI, G. 2008. Improving the content of essential amino acids in crop plants: goals and opportunities. *Plant Physiology*, 147: 954-961.

WEN, W.; LI, D.; LI, X.; GAO, Y.; LI, W.; LI, H.; LIU J. 2014. Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights. *Nature communications*, 5: 3438.

WEN, W.; BROTMAN, Y.; WILLMITZER, L.; YAN, J.; ALISDAIR, R. F. 2016. Broadening Our Portfolio in the Genetic Improvement of Maize Chemical Composition. *Trends in Genetics*, 32: 459-469.