



## Genetic Divergence in Corn Genotypes for High and Low Phosphorus in Pará, Brazil

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors WFS, LCM, BLDJ and JMP designed the study and performed the genetic analysis. Authors LFS, OJFJ, RMS and ASB managed the study and helped in the interpretation of the results. Author MO managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** The objective of this study was to estimate the genetic divergence between ten corn genotypes in the 2017/18 crop, under high and low phosphorus.

**Study Design:** The experimental design used in each test was randomized blocks, the treatments were: ten genotypes in two levels of phosphorus and three replications.

**Place and Duration of Study:** Sítio Vitória, municipality of Santa Maria das Barreiras, state of Pará, Brazil, in the 2017/18 crop.

**Methodology:** The experimental plot consisted of four lines five meters long, spaced with 0.90 m between the lines, with approximately 55,000 plants per hectare in the harvest. The genotypes used: 2B655, AG 1051, AG 8088, AL BANDEIRANTE, ANHEMBI, BR 206, BRS 3046,

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CATIVERDE, ORION e PR 27D28. The following agronomic characteristics were evaluated: plant height, ear height, ear diameter, ear length, number of grains per row, yield per plant and grain yield. Genetic divergence was estimated from Mahalanobis distances and genotypes were grouped by Tocher optimization methods.

**Results:** Genetic dissimilarity measurements, at each dose of phosphorus, showed a high magnitude ( $D^2= 9.72$  to  $1484.87$ ). Cluster analysis separated the genotypes into four groups at low phosphorus and two groups at high phosphorus. The characteristics that most contributed to genetic divergence, in the two levels of phosphorus, were yield per plant and grain yield.

**Conclusion:** There was a higher genetic divergence between genotypes AG 1051 and BANDEIRANTE in the low phosphorus assay and between the genotypes ANHEMBI and AL BANDEIRANTE in the high phosphorus assay. With this, these crosses are promising to obtain lineages in both tests.

*Keywords: Abiotic stress; maize breeding; phosphate fertilizer; Zea mays L.*

## 1. INTRODUCTION

Corn is a crop of importance in world agricultural production, its product is used for human food and for the production of feed. In the 2018/19 harvest, a worldwide production of 1.12 billion tons was achieved [1]. Its importance also lies in its ability to employ labor, since, due to its production characteristics, culture has great participation in the generation of employment in the rural sector. The importance of corn for animal production, and vice versa, can be inherited by the fact that almost 80% of all the corn produced in Brazil is consumed in the form of feed [2].

With this, there is a need to increase grain production, it is a crucial factor in meeting the global demand of the population. Brazil stands out in this scenario because it is a major producer and exporter of corn. In the country, corn production has reached 100.04 million tons, with grain yield estimated at  $5,719 \text{ kg ha}^{-1}$ . It stands out, because it is the second-highest value of production the grains in the country, being surpassed only by soybeans [3].

In the 2018/19 harvest, the mean grain yield of the state of Pará was  $3,067 \text{ kg ha}^{-1}$ , considered low when compared to other regions of the country: Midwest ( $6,197 \text{ kg ha}^{-1}$ ), southeast ( $5,995 \text{ kg ha}^{-1}$ ) and south ( $6,849 \text{ kg ha}^{-1}$ ). Thus, it is necessary to practice studies of breeding programs with different levels of cultivation to change this scenario through the great demand for cereal [3].

Seeking to aim for new scientific contributions to better explain the causes of low corn production in the state, work should be done focused on this region. In this region, most lands with crops have

characteristics of P deficiency, besides presenting acidity and toxicity by aluminum, characteristics that describe the region, in which it is part of the Brazilian Cerrado and such characteristics limit corn production, thus hindering cereal production growth.

P participates in a large number of compounds in plants, in several metabolic processes, and plays an important fundamental role in the transfer of energy in the cell, during breathing and photosynthesis [4]. Also, it is a structural component of nucleic acids, which constitute genes and chromosomes, as well as many coenzymes, phosphoproteins, and phospholipids [5]. Due to their high mobility within the plant, symptoms appear in older leaves, which have reddish coloration due to increased anthocyanin [6].

Plants respond to P deficiency with adaptations that allow them to maximize the likelihood of producing some viable seeds. Generally, P stress decreases the total number of seeds produced more than the seed size. In cereals, the reduction in the number of seeds occurs by reducing the number of fertile years and the number of grains per ear [6]. With a smaller number of seeds formed, the plant increases the supply of nutrients by seed, thus improving the viability of the plant. These breeding studies are important to know the genetic bank, thus aiming to know which genotypes can be used in future crosses in search of promising genotypes that change the current scenario.

Studies on the genetic divergence between individuals or genotypes in plant species have been of great importance in breeding programs involving hybridizations because they provide parameters for the identification of parents that

allow greater heterotic effect on progeny and more likely to obtain superior genotypes in segregating generations [7].

However, there are few studies of genetic divergence under high and low phosphorus, as well as studies on phosphorus doses in this region, as a way to obtain better productive results.

Because of the above, the present study aimed to study genetic divergence in corn genotypes, under high and low P in southern Pará, Brazil.

## 2. MATERIALS AND METHODS

### 2.1 Study Location

The experiment was carried out on 23 December de 2017, 2017/2018 crop. Two tests of competition of corn genotypes were carried out at Sítio Vitória, Municipality of Santa Maria das Barreiras, State of Pará, Brazil (Fig. 1). The climate of the region is classified as Aw according to the classification of Köppen [8]. The soil of the experimental area is sandy (clay= 15%), and has phosphorus content classified as very low (P Mehlich-1= 4.9 mg dm<sup>-3</sup>) [9].

### 2.2 Experimental Design and Data Collection

Two tests installed under high P conditions (100 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>) and bass P (50 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>) applied in soil, during the sowing. The doses were defined according to Ribeiro et al. [10], considering the availability of the nutrient in the soil, and the need for the crop.

The experimental design used, in each test, was randomized blocks with 10 treatments and three

repetitions. The treatments were composed of ten genotypes (Table 1), seven hybrids: 2B655, AG 1051, AG 8088, BR 206, BRS 3046, PR 27D28 and ORION, and three populations of open pollination: AL BANDEIRANTE, ANHEMBI and CATIVERDE 02.

The experimental plot consisted of four lines five meters long, spaced with 0,90 m between the lines. In the harvest, the two central lines of each row were used, discarding 0,50 m from the ends of the rows.

The operations of plowing, grading, and sulcation were performed. Sowing was performed to obtain a population of 55,555 plants ha<sup>-1</sup>. The phosphate fertilization was performed in the planting furrow, the source of P using was simple superphosphate.

Nitrogen (N) and potassium (K) fertilization in cover were divided into stages V4 and V8 (four and eight completely open leaves), 50% of the dose in each. N was applied at a dose of 150 kg ha<sup>-1</sup>, with urea as its source (45% N). And k was applied at a dose of 90 kg ha<sup>-1</sup>, the source was potassium chloride (60% K<sub>2</sub>O).

Cultural tracts, such as phytosanitary control against diseases, pests, and weeds were carried out following the technical recommendations of the crop [2].

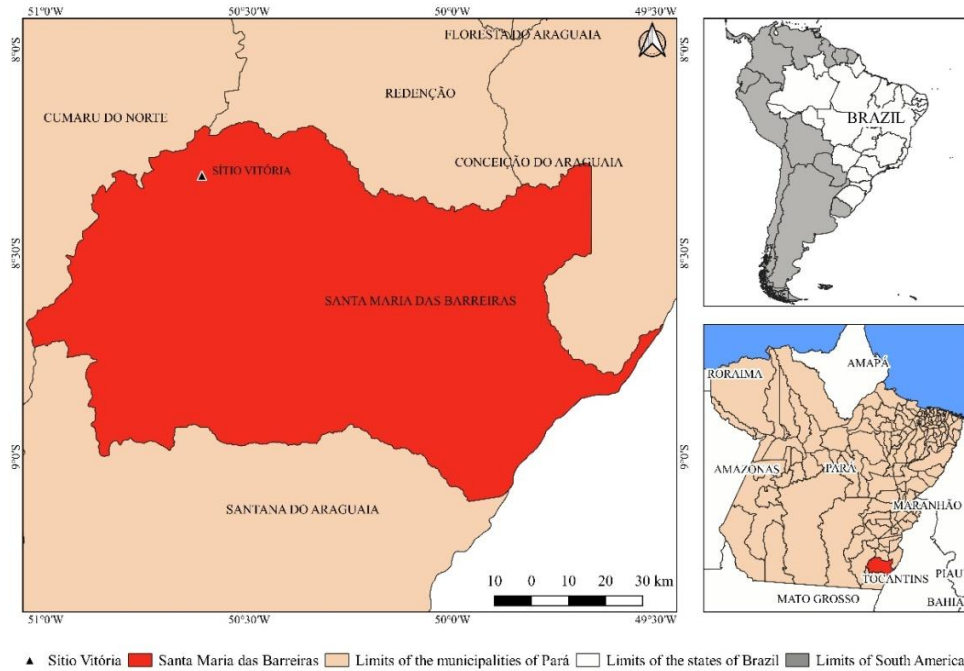
In the two central banks, of each plot, the ears were harvested at stage R6 (physiological maturity). The following characteristics of the: plant height, ear height, ear diameter, ear length, number of grains per row, grain yield per plant and grain yield: grain mass of each corrected portion for 13% moisture and transformed into kg ha<sup>-1</sup>.

**Table 1. Agronomic characterization of ten corn genotypes used in the experiment**

Genotype	GB*	Cycle	Grain type	Grain color	Use
AG 8088	SH	Early	Flint	Orange	G/WPS
2B655	TH	Early	Semi flint	Orange	G/WPS/WGS
BRS 3046	TH	Medium early	Dent	Yellow	MV
AG 1051	DH	Medium early	Dent	Yellow	G/MV/WPS
BR 206	DH	Early	Semi dent	Yellow/Orange	G/WPS
ORION	DH	Early	Semi dent	Red	G
PR 27D28	DH	Super early	Semi flint	Orange	G/WPS
AL BANDEIRANTE	OP	Medium early	Semi flint	Orange	G/WPS
ANHEMBI	OP	Early	Semi flint	Yellow/Orange	G/WPS
CATIVERDE 02	OP	Medium early	Dent	Yellow	MV/WPS

\*GB: genetic base, SH: simple hybrid, DH: double hybrid, TH: triple hybrid, OP: populations of open pollination, G: grain, MV: green corn, WPS: whole plant silage, WGS: wet grain silage, H: high, M: medium and L: low.

Adapted from Cruz et al. (2015) [11]



**Fig. 1. Map of Santa Maria das Barreiras, State of Pará, Brazil**

### 2.3 Genetic Analyses

For each of the low and high p-level assays, a study of genetic divergence was carried out, and the generalized distance of Mahalanobis ( $D^2$ ) was used as a measure of dissimilarity, taking into account the degree of dependence between characteristics studied.

For the establishment of similar groups, the hierarchical optimization agglomeration method indicated by Tocher [12], whose calculations were also based on the generalized Mahalanobis distance.

Singh criterion [13] was also used to quantify the relative contribution of the characteristics evaluated in genetic divergence. The analyses were performed using the Computational Genes program, version 2007 [14].

### 3. RESULTS AND DISCUSSION

Analysis of variance (Table 2) evidenced significant differences among the genotypes and doses of P for the measured characteristics. Coefficient of variation (CV) were generally classified as low (<10%), indicating good experimental precision [15]. These CV were next to those found by Freeman et al. [16], Silva et al. [17], Silva et al. [18] and Al-Naggar et al. [19].

The maximum characteristics values were generally found in high P. These values are similar to those found by Jumaa and Madab [20], Hassan et al. [21], Silva et al. [18], Ahmed et al. [22], Al-Naggar et al. [19] and Rocandio-Rodríguez et al. [23].

Genetic dissimilarity measurements, estimated from Mahalanobis distance, at each dose of P (Table 3), showed a high magnitude (9.72 a 1484.87), indicating the presence of genetic variability among genotypes. Similar approach was adopted by Jumaa and Madab [20], Santos et al. [24], Coelho et al. [25], Silva et al. [17] and Silva et al. [18]. The analysis of genetic distance can help in the choice of parents for future crossings, enabling time savings, labor and financial resources in future studies [17,18].

Below P, above the diagonal, the shortest distance was obtained between the ANHEMBI and BR 206 ( $D^2= 9.72$ ), followed by the combinations BRS 3046 and ORION ( $D^2= 10.29$ ), PR 27D28 and ORION ( $D^2= 10.73$ ), AG 8088 and ANHEMBI ( $D^2= 11.57$ ), AG 8088 and BRS 3046 ( $D^2= 15.38$ ). The combination of genotype AG 1051 and AL BANDEIRANTE was the most divergent ( $D^2= 189.89$ ), followed by the combinations CATIVERDE and AL BANDEIRANTE ( $D^2= 156.85$ ), ANHEMBI and AL

BANDEIRANTE ( $D^2= 142.25$ ), AG 8088 and AL BANDEIRANTE ( $D^2= 140.17$ ), AG8088 and 2B655 ( $D^2= 119.47$ ).

Already at high P, below the diagonal, the shortest distance was obtained between the pairs BRS 3046 and AG 1051 ( $D^2= 21.82$ ), followed by the combinations PR 27D28 and ORION ( $D^2= 25.47$ ), CATIVERDE and 2B655 ( $D^2= 30.03$ ), 2B655 and ORION ( $D^2= 33.29$ ), BR 206 and ORION ( $D^2= 40.51$ ). The combination of genotype ANHEMBI and AL BANDEIRANTE was the most divergent ( $D^2= 1484.87$ ), followed by the combinations AG 1051 and AL BANDEIRANTE ( $D^2= 1156.52$ ), BRS 3046 and AL BANDEIRANTE ( $D^2= 1071.26$ ), AG 8088 and AL BANDEIRANTE ( $D^2= 656.14$ ), 2B655 and AL BANDEIRANTE ( $D^2= 569.25$ ).

The shorter the distance between individuals, the greater the genetic similarity between them,

which would reduce the probability of success to obtain hybrids with a high degree of heterosis [25,26].

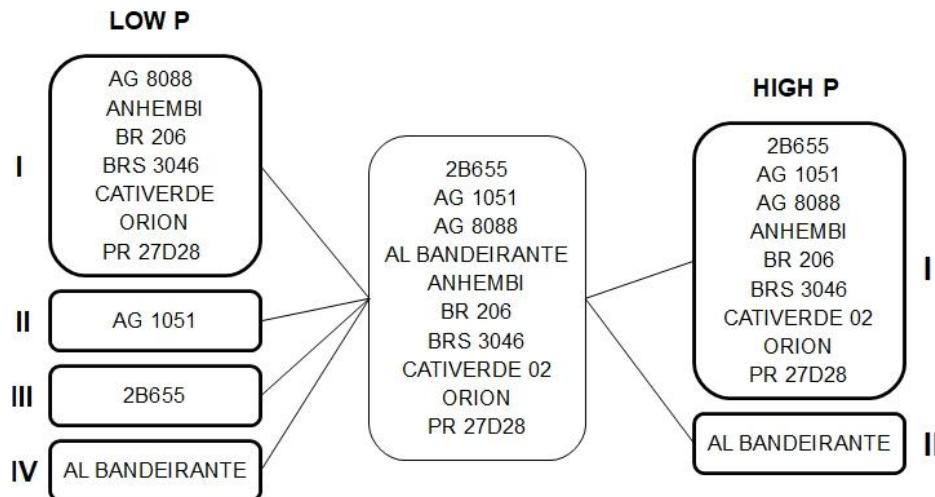
The cluster analysis by the Tocher method separated the ten genotypes into four groups down P and two groups for high P (Fig. 2). In breeding programs, the formation of groups is essential, since success depends on the choice of genitors with greater dissimilarity and their potential "per se" [17].

Below P, it has four groups, where groups II, III and IV have only one genotype and group I has seven genotypes. In high P, it has two groups, where the group I has nine genotypes and group II has a genotype. The AL BANDEIRANTE genotype composed the group alone in both trials. Groups formed by only one genotype point in the direction that such genotypes are more divergent than the others [11].

**Table 2. Summary statistics for characteristics of ten corn genotypes evaluated in high and low P**

Characteristic	Minimum	Maximum	Mean	CV	MS G	MS P	MS GxP
Ear height (cm)	83.2 (LP)	147.7 (HP)	105.4	6.3	*	*	*
Plant height (cm)	181.8 (LP)	240.0 (HP)	211.3	1.4	*	*	*
Ear diameter (mm)	45.6 (LP)	55.2 (HP)	50.5	3.5	*	*	ns
Ear length (mm)	145.8 (LP)	210.0 (LP)	17.7	12.5	*	ns	ns
Number of grains per row	27.6 (LP)	40.2(HP)	32.2	4.8	*	*	ns
Grain yield per plant (kg plant <sup>-1</sup> )	99.0 (LP)	208.8 (HP)	146.2	5.9	*	*	*
Grain yield (kg ha <sup>-1</sup> )	5445.6 (LP)	11486.4 (HP)	8043.1	3.5	*	*	*

Values are followed by level of phosphorus (P) in parenthesis, LP: low P, HP: high P, CV: coefficient of variation, MS: Mean squares from ANOVA, G: genotype, GxP: genotype-level of P interaction, \* indicate significance at 0.05 probability level, ns: non significance



**Fig. 2. Grouping of the ten corn genotypes by the Tocher method, low (left) and high P (right)**

**Table 3. The dissimilarity between ten corn genotypes to the characteristics, based on the generalized distance of Mahalanobis ( $D^2$ ), under high P (below the diagonal) and low P (above the diagonal)**

<b>Genotype</b>	<b>Ag 8088</b>	<b>Brs 3046</b>	<b>Pr 27d28</b>	<b>Anhembi</b>	<b>Cativerde 02</b>	<b>2b655</b>	<b>Br 206</b>	<b>Ag 1051</b>	<b>Al bandeirante</b>	<b>Orion</b>
Ag 8088		15.38	48.58	11.57	55.45	119.47	20.65	52.07	140.17	34.97
Brs3046	88.48		23.10	18.25	25.43	65.19	16.12	52.07	100.58	10.29
Pr 27d28	77.43	175.31		47.70	65.59	48.69	19.64	104.72	33.29	10.73
Anhembi	183.21	56.18	373.67		58.07	117.88	9.72	65.06	142.25	38.74
Cativerde	77.49	117.73	118.39	197.94		56.80	53.05	60.28	156.85	27.01
2B655	45.13	111.69	52.45	236.97	30.03		80.21	89.57	76.73	35.79
Br 206	77.10	280.56	49.08	482.46	140.89	99.08		77.98	87.11	119.02
Ag 1051	100.03	21.82	224.23	48.20	170.43	141.67	327.32		189.89	73.05
Al band.	656.14	1071.26	419.00	1484.87	701.22	569.25	308.24	1156.52		71.05
Orion	66.22	202.44	25.47	378.70	78.80	33.29	40.51	243.11	393.13	

The most contrasting combinations and groupings of genotypes were different in low and high P. This change in genetic divergence was also observed for nitrogen and potassium levels [26,27]. This difference occurs due to the availability of the nutrient, which allowed or not the expression of alleles linked to the acquisition, translocation, assimilation and remobilization of the nutrient [17,28].

Intergroup dissimilarity (Table 4), also obtained by the Tocher optimization method, allows distinguishing between the formed groups, which are more genetically divergent. The highest intergroup distances were observed between groups II and IV ( $D^2=189.89$ ), I and IV ( $D^2=104.47$ ), for high P only one group I and II ( $D^2=751.07$ ).

The combinations AG 1051 and AL BANDEIRANTE, 2B655 and AL BANDEIRANTE, and AG 8088 and AL BANDEIRANTE, are promising to obtain lineages in both tests, because they are more divergent among themselves, being quite promising for future crosses between this genotype.

These findings are corresponding with what obtained by Santos et al. [29], Jumaa and Madab [20], Santos et al. [24], Silva et al. [17] and Sigh et al. [30] about intergroup distances of corn genotypes.

The great interest in assessing the relative importance of the characters (Table 5) lies in the possibility of using only characteristics that contributed the most to discriminating the

genotypes, thus reducing, labor, time and costs spent in experimentation [11].

The characteristics that most contributed to genetic divergence, both in low and high phosphorus were grain yield per plant and grain yield. On the other hand, the ones that contributed the least to the explanation of genetic divergence between genotypes in high and low phosphorus were ear length, number of grains per row and ear diameter.

Although the number of grains is heavily influenced by P stress [6]. The genotypes presented low variability for this characteristic, so it is not among the most contributing for divergence.

Changes in the contributions of the characteristics to genetic divergence of corn at nutrient levels, were also reported by Santos et al. [24] in study with nitrogen.

Santos et al. [29], Coelho et al. [25], and Freeman et al. [16] also use these characteristics among which more and less contributed to the divergence. For Silva et al. [17], grain yield per plant presented low contribution. For Silva et al. [18] and Sigh et al. [30] the length and diameter ear were among the largest contributions, respectively.

With the relative contribution analysis, we can identify which of the characteristics evaluated, it best contributes to the genetic divergence of the genotypes evaluated.

**Table 4. Intermediate intergroup distances estimated by the tocher optimization method, involving corn genotypes, low P (above diagonal) and high P (below diagonal)**

Groups	I	II	III	IV
I		69.36	74.86	104.47
II	751.07		89.57	189.89
III				76.73
IV				

**Table 5. The relative contribution of genetic divergence (in %) in the analysis of variance of the evaluated characteristics of corn genotypes**

Characteristic	Low P	High P
Ear height	0.95	22.27
Plant height	10.37	3.10
Ear diameter	1.31	0.63
Ear length	0.23	0.36
Number of grains per row	0.83	0.30
Grain yield per plant	49.10	34.82
Grain yield	37.21	38.51

#### 4. CONCLUSION

Genetic variability was observed between genotypes for both assays. The combinations AG 1051 and AL BANDEIRANTE, 2B655 and AL BANDEIRANTE, and AG 8088 and AL BANDEIRANTE, are promising to obtain strains in both tests. The grain yield and grain yield per plant characteristics contributed the most to genetic divergence.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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