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Intraspecific variability of popcorn S_7 lines for phosphorus efficiency in the soil

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ABSTRACT. The expansion of agriculture, coupled with the need for sustainable cropping, is one of the greatest challenges of the scientific community working on the generation of new cultivars adapted to abiotic stress conditions. The aim of this study was to evaluate the variability of popcorn lines as to responsiveness and efficiency in phosphorus use, as a first step towards the implementation of a breeding program interested in the practice of sustainable agriculture. Twenty-five popcorn lines were evaluated in two locations with different phosphorus levels in the soil, using a randomized block design. The following traits were measured: plant height, ear height, female flowering date, male flowering date, male-female flowering interval, ear diameter, ear length, 100-grain weight, grain yield, popping expansion, and expanded popcorn volume per hectare. A combined analysis of variance and test of means were performed, and the lines were classified as to their phosphorus use efficiency, according to their production performance in the different environments. The genetic diversity between the lines was estimated by Tocher's and UPGMA clustering methods, using generalized Mahalanobis distance. Lines L59, P7, P2, P3, P4, P8, P10, P9, L66, L70, L69, and P5 were efficient and responsive, whereas lines L75, L80, L61, L77, L63, L65, P1, L54, L53, L88, and L71 were inefficient and nonresponsive. Genetic variability was greater in the environments with low phosphorus in the soil, suggesting that the selection pressure exerted in the stressing environment is a decisive factor to obtain a higher expression of variability.

Key words: *Zea mays* L.; Genetic divergence; Abiotic stress

INTRODUCTION

In the pursuit of greater yields, today's agricultural activity has become highly dependent on chemical fertilizers (Cordell et al., 2009). In Brazil, this is especially true, since most soils have low nutrient availability, high acidity, and high phosphorus adsorption capacity (Cock et al., 2002; Fageria et al., 2011). Phosphorus is one of the nutrients of greatest agricultural importance for most cultivated species as it plays important functions in plant metabolism, energy transfer in the cell, respiration, and even photosynthesis (Baligar et al., 2001). It is also a structural component of nucleic acids and chromosomes, as well as of many coenzymes, phosphoproteins, and phospholipids (Fukuda et al., 2007).

There are many basic reasons for investing in research to discover more efficient and responsive cultivars in phosphorus use. One of them is the fact that chemical fertilizers are costly components for agriculture (Savary et al., 2014). Obtaining cultivars efficient in the use of phosphorus lowers production costs and reduces the indiscriminate use of fertilizers, which has adverse environmental impacts such as the contamination of water sources (Parentoni et al., 2011). Lastly, phosphorus is the second most widely used element in agriculture, and phosphate sources are non-renewable natural resources whose reserves may be largely depleted in the next 50 years (Cordell et al., 2009).

The knowledge of genetic variability in efficiency and responsiveness of phosphorus lines is a fundamental step for the study of inheritance and for the implementation of an appropriate breeding program to obtain superior genotypes (Cock et al., 2002). There are many studies investigating the variability within and between species for traits related to efficiency in the use of phosphorus and its genetic control (Whiteaker et al., 1976; Brasil et al., 2007; Boutraa, 2009; Zhang et al., 2009; Missaoui and Young, 2016). These genotypic variations clarify the adaptation of genotypes to several conditions of environmental stress and make up the genetic basis for the implementation of breeding programs.

To this end, the use of multivariate analysis is a feasible option in that it allows the breeder to predict the best combinations concerning the traits of importance (Hallauer et al., 2010; Cruz et al., 2012). Research has been carried out on genetic diversity in phosphorus use efficiency in many crops, using multivariate techniques, and yielded very encouraging results (Cock et al., 2002; Mundim et al., 2013; Reina et al., 2014); however, there are no literature studies addressing the mineral nutrition as it relates to the popcorn crop under field conditions.

Given the current agricultural scenario, the generation of cultivars better adapted to soils poor in phosphorus and the introduction and use of genotypes selected for certain environments bring an interesting perspective about the efficiency in the use of the phosphate fertilizer and the sustainability of the production system.

The objective of this study was to identify popcorn lines efficient and inefficient in the use of phosphorus, as well as to estimate their genetic diversity when grown in different environments regarding nutritional availability as a first step for an investigation of the inheritance of efficiency in phosphorus use and for the implementation of an adequate breeding program for the production of superior genotypes.

MATERIAL AND METHODS

Experimental conditions and germplasm

The experiments were conducted between August and November 2015, in two locations and two environments that were different as to the availability of phosphorus, namely: Antonio Sarlo State College of Agriculture, located in the municipality of Campos dos Goytacazes, RJ (21°42'48"S latitude, 41°20'38"W longitude, 14 m asl), and the Experimental Station in the municipality of Itaocara, RJ (21°38'50"S latitude, 42°03'46"W longitude, 58 m asl), Brazil. The climate in these municipalities is classified as a tropical (Aw) type, with hot summers and mild winters and rains concentrated in the summer months.

Before the experiment was implemented, a chemical analysis was performed to characterize the environments as to phosphorus availability, based on soil samples collected from the 0-10- and 10-20-cm layers, forming a composite sample from 10 subsamples. The phosphorus availability levels in the soils from the municipalities of Campos dos Goytacazes and Itaocara were classified as low, with 8 and 11 mg/dm^3 P, and with clay contents of 305 and 140 g/dm^3, respectively.

Twenty-five popcorn S_7 lines from the State University of Northern Rio de Janeiro (UENF) were used as the treatments in two different environments regarding the phosphorus availability in the soil. The following lines were evaluated: L53, L54, and L59 (extracted from the 'Beija-Flor' population); L61, L63, L65, L66, L69, L70, and L71 (extracted from the 'BRS-Angela' population); L75, L76, L77, L80, and L88 (extracted from the 'Viçosa' population); P1 (extracted from the commercial hybrid 'Zélia'); P2 and P3 (extracted from the 'CMS-42' compound); P4 (extracted from South-American breeds); P5, P6, and P7 (extracted from the commercial hybrid 'Zaeli'); and P8, P9, and P10 (extracted from the commercial hybrid 'IAC-112').

A randomized block design with four replications was adopted. Each experimental plot consisted of a 5-m row with plants spaced 0.20 m apart and rows spaced 0.90 m from each other, totaling 25 plants per plot.

Phosphorus treatments

The fertilizer applied at planting for the environment under ideal phosphorus availability contained 30 kg/ha N, 70 kg/ha P_2O_5, and 40 kg/ha K_2O. For the environment under low phosphorus availability, the applied fertilizer contained 30 kg/ha N, 0 kg/ha P_2O_5, and 40 kg/ha K_2O. The soil in both environments was top dressed when the plants reached phonological stage V6, at the nitrogen dose of 100 kg/ha.

The primary macronutrients' supply capacity in the experimental areas was obtained according to the fertilization recommendations for the popcorn crop, considering some nutrients in the soil in the 0-20-cm layer revealed by the chemical analysis, except for the phosphorus level in the low P environment, which was zero.

The other cultivation practices were performed as recommended for the region. The experiments received supplemental irrigation whenever necessary to prevent water stress.

Phenotyping

The following traits were evaluated: plant height (PH) - expressed in centimeters, from the soil surface to the insertion point of the flag leaf; ear insertion height (EH) - expressed in centimeters, from the soil surface to the insertion point of the first ear; male flowering (MF), the number of days when 50% of the plants from the experimental unit started to open the tassel anthers; female flowering (FF), the number of days when 50% of the plants from the experimental unit started to release the style stigma from the ear; flowering interval (FI) - the difference between the number of days from male to female flowering; ear length (EL) - the average length of five husked ears, expressed in centimeters; ear diameter (ED) - the average diameter of five husked ears, expressed in millimeters; 100-grain weight (W100) - obtained as the average of the weight of two random 100-grain samples; grain yield (GY) - the average grain yield of the experimental unit in grams per plot, corrected for 13% moisture, and extrapolated to kg/ha; popping expansion (PE) - the ratio between the expanded popcorn volume and the mass of 30 g, expressed in mL/g, adopting the average of two samples per plot; and expanded popcorn volume per hectare (PV) - the product of grain yield and popping expansion, expressed in m^3/ha.

Classification of the lines as to phosphorus use efficiency

The lines were evaluated for efficiency and responsiveness to phosphorus based on the deviation of the grain yield means of each line relative to the average grain yield of each environment. These values were plotted in scatter graphs, where the x-axis represented the deviations under high phosphorus level (responsiveness in phosphorus use), and the y-axis represented the deviations under low phosphorus level (efficiency in phosphorus use).

The expression used to classify the lines for their efficiency in the use of phosphorus was $E_{use} = Y_{IL} - Y_{EL}$, where Y_{IL} represents the average grain yield of line 'i' in the environment with low phosphorus availability and Y_{EL} represents the average grain yield of the environment with low phosphorus availability. For the classification of the lines as to their responsiveness to phosphorus use, the following equation was adopted: $R_{use} = Y_{IH} - Y_{EH}$, where Y_{IH} represents the average grain yield of line "i" in the environment with high phosphorus availability, while Y_{EH} represents the average grain yield in the environment with high phosphorus availability. In this way, the lines were distributed into four quadrants in the scatter graph, depending on the production performance in the different environments regarding phosphorus availability: efficient and nonresponsive (ENR); efficient and responsive (ER); inefficient and responsive (IR); and inefficient and nonresponsive (INR).

Statistical analysis

An individual analysis of variance was performed for the environments with high and low levels of phosphorus, in addition to a combined analysis of the two environments. For the GY and PE variables, means were grouped by the Scott-Knott algorithm (Scott and Knott, 1974) at the 5% probability level.

The genetic dissimilarity between the lines was estimated by the generalized Mahalanobis distance (D^2), based on the means of the ten traits evaluated in the popcorn lines and of the matrix of residual covariance (Cruz et al., 2014). Tocher's (Rao, 1952) and group-link (UPGMA) methods were employed in the clustering of the popcorn lines, using the Genes computer program (Cruz, 2013). The cutoff point of the dendrograms and definition of the number of groups were established by Mojena's (1977) method, based on the relative size of the levels of fusions (distances) in the dendrogram. The PV trait was not included in the dissimilarity and clustering analyses because it was redundant with grain yield and popping expansion, given the high correlations that associate them.

RESULTS AND DISCUSSION

There was a significant difference between the lines ($P < 0.01$) for all the evaluated traits, indicating the existence of genetic variability. Genetic variation is indispensable for the identification of a different germplasm in efficiency and responsiveness to phosphorus use when aiming to implement a breeding program for the practice of sustainable agriculture. The significant effect of the phosphorus level on grain yield demonstrates that the phosphorus levels used were sufficient to discriminate between efficient and inefficient lines (Table 1).

The non-significance for the genotype x phosphorus level interaction (G x P) for grain yield and popping expansion reveals that the classification of the lines was not changed, in the comparison between the experiments under high and low phosphorus availability. This indicates that the selection of popcorn lines for efficiency in phosphorus use based on yield or popping expansion can be practiced both in environments with low and high phosphorus availability. The opposite occurs for the PV trait, which, despite the redundancy for grain yield and popping expansion, revealed significance in the interaction, demonstrating that the popcorn volume means are different, and the classification of lines was probably altered in the comparison between the two experiments. These findings indicate that the selection of popcorn lines efficient in phosphorus use based on the PV trait should be specific for each nutritional availability environment (Table 1).

Except for the FI trait, the coefficients of variation were of low magnitude, ranging from 2.21 to 15.11% for MF and GY, respectively, indicating good experimental precision (Table 1). In plant breeding studies for abiotic stresses, coefficients of variation of higher magnitudes are acceptable when compared with the environments without nutritional stress, since the means of traits under stress are usually lower and the mean residual squares are higher. The studies of Bänziger et al. (1997) were examples in which coefficient of variation of higher magnitudes was obtained in environments under nutritional (nitrogen) stress when the authors evaluated the genotypic variability of corn in environments with low and high nitrogen soils.

A 7.05% decrease was observed in the estimate of the overall mean for grain yield in the low phosphorus environment as compared to the environment with high phosphorus contents (Table 2). This decreased yield might have stemmed from the reduction in respiration or photosynthetic rate that the phosphorus deficiency causes in plants. Phosphorus deficiency can also reduce the synthesis of nucleic acids and protein, inducing accumulation of soluble nitrogen compounds in the tissue, slowing cellular growth and consequently causing a delay in leaf emergence and a reduction in the sprouting and development of secondary roots, as well as in dry matter production and yield (Grant et al., 2001). Barreto and Fernandes (2002) evaluated corn yield as a function of increasing levels of phosphorus applied to the soil and observed increases of over 50% in grain yield.

Table 1. Combined analysis of variance for eleven traits evaluated in 25 popcorn S_7 lines in different environments regarding the phosphorus level in the soil (Campos dos Goytacazes and Itaocara, RJ, Brazil).

SV	Mean square						
	d.f.	PH	EH	FF	MF	FI	ED
(B/P)/E	12	409.69	198.84	9.80	9.32	2.07	4.96
Genot (G)	24	4862.86**	1967.32**	176.77**	175.41**	4.06**	179.33**
Env (E)	1	152302.86**	73630.82**	260.02**	215.35**	2.10ns	4040.08**
Level (P)	1	91.77ns	342.62ns	502.88**	59.68*	216.09**	50.03**
G x E	24	233.39**	125.78**	1.64ns	1.57ns	1.05ns	11.66**
G x P	24	95.89ns	92.54ns	5.38**	5.83**	2.56**	4.48*
E x P	1	4558.95**	5439.06**	6.89ns	0.11ns	5.29ns	3.34ns
G x E x P	24	68.28ns	52.82ns	1.49ns	1.68ns	0.89ns	3.29ns
Error	288	95.65	60.43	2.42	1.75	0.91	2.46
Average		147.81	76.04	61,22	59.83	1.40	26.31
CV (%)		6.62	10.22	2.54	2.21	68.25	5.96

SV	Mean square					
	d.f.	EL	W100	GY	PE	PV
(B/P)/E	12	0.76	0.70	150381.77	10.69	159.92
Genot (G)	24	42.15**	26.90**	10947166.74**	322.79**	7308.90**
Env (E)	1	169.60**	309.61**	13569969.03**	2.58ns	8671.00**
Level (P)	1	0.61ns	0.69ns	764743.05*	162.35**	1501.76**
G x E	24	2.05**	5.99**	331994.65**	19.73**	335.61**
G x P	24	0.93ns	0.62ns	53541.69ns	8.09ns	83.46*
E x P	1	4.05*	0.15ns	166869.88ns	212.91**	946.22*
G x E x P	24	0.88ns	0.59ns	106709.84**	15.57**	125.13**
Error	288	0.78	0.71	37649.67	8.02	48.02
Average		12.28	11.25	1284.13	26.18	33.54
CV (%)		7.18	7.51	15.11	10.82	20.66

^{ns}Not significant at the 5% probability level; *significant at the 5% probability level by the F-test; **significant at the 1% probability level by the F-test. PH: plant height; EH: ear height; FF: female flowering; MF: male flowering; FI: flowering interval; ED: ear diameter; EL: ear length; W100: 100-grain weight; GY: grain yield; PE: popping expansion; PV: expanded popcorn volume per hectare. SV: source of variation; d.f.: degrees of freedom; CV: coefficient of variation.

In the environment with high phosphorus, the estimated yield was 1,327.86 kg/ha (Table 2), with 10 groups formed with distinct means, whereas in the environment with low phosphorus, in which the overall mean was 1,240.41 kg/ha, eight groups were formed by the Scott-Knott algorithm, which reveals a significant variability to be exploited in breeding programs. Even if the yields are lower in the environment with low phosphorus, the lower production cost in this system may compensate for the disadvantage, making it viable, mainly for small producers with low purchasing power.

For popping expansion, the mean estimates were similar between the environments with high and low phosphorus (26.82 and 25.54 mL/g, respectively) (Table 2). The lower formation of line groups for popping expansion regarding yield is probably related to the fact that PE is an oligogenic qualitative trait, as described by Dofing et al. (1991). Despite being oligogenic, the heritability of popping expansion ranges from 70 to 90% (Pereira and Amaral Júnior, 2001; Arnhold et al., 2009), in which the additive effects are the main components of genetic variance (Larish and Brewbaker, 1999; Pereira and Amaral Júnior, 2001; Moterle et al., 2012). According to Lu et al. (2003), popping expansion is little influenced by the environment, determining that performance *per se* is imperative in the expression of this trait. These same authors identified four QTL on chromosomes 1S, 3S, 5S, and 5L, which, together, explained 45% of the phenotypic variation in popping expansion.

Table 2. Estimates of grain yield (GY), popping expansion (PE), and popping volume (PV) means of 25 popcorn lines grown in environments with high and low phosphorus availability.

Lines	GY (High P)	GY (Low P)	PE (High P)	PE (Low P)	PV (High P)	PV (Low P)
	kg/ha		mL/g		m^3/ha	
L53	620.20^{i}	585.58^{g}	27.12^{c}	23.83^{c}	17.16^{f}	13.90^{f}
L54	615.83^{i}	538.05^{g}	21.83^{e}	21.91^{c}	13.11^{g}	11.84^{g}
L59	2940.26^{a}	2774.07^{a}	21.50^{e}	20.79^{d}	63.06^{b}	57.70^{b}
L61	407.5^{j}	347.91^{h}	32.25^{b}	30.33^{a}	13.16^{g}	10.51^{g}
L63	500.64^{j}	405.69^{h}	25.16^{d}	23.75^{c}	12.61^{g}	9.57^{g}
L65	466.90^{j}	385.81^{h}	20.54^{e}	19.79^{d}	9.73^{g}	7.69^{g}
L66	1598.61^{e}	1650.55^{d}	31.83^{b}	29.50^{a}	51.46^{c}	48.98^{c}
L69	1645.55^{e}	1542.36^{d}	29.00^{c}	28.83^{a}	48.00^{c}	44.48^{d}
L70	1591.11^{e}	1652.22^{d}	27.29^{c}	25.04^{b}	42.41^{d}	41.43^{d}
L71	905.23^{h}	573.49^{g}	33.62^{a}	31.29^{a}	30.55^{e}	17.39^{f}
L75	388.78^{j}	258.90^{h}	18.41^{f}	18.66^{d}	7.19^{g}	4.91^{g}
L76	1191.11^{g}	1274.76^{f}	25.08^{d}	26.20^{b}	28.66^{e}	33.02^{e}
L77	441.66^{j}	383.74^{h}	26.83^{c}	22.91^{c}	12.08^{g}	8.83^{g}
L80	361.66^{j}	267.14^{h}	28.54^{c}	27.20^{b}	10.45^{g}	7.53^{g}
L88	460.00^{j}	660.55^{g}	26.04^{c}	26.20^{b}	12.13^{g}	18.23^{f}
P1	626.94^{i}	386.38^{h}	30.12^{b}	29.16^{a}	19.22^{f}	11.66^{g}
P2	2611.94^{b}	2460.70^{b}	20.62^{e}	20.29^{d}	53.21^{c}	49.98^{c}
P3	2231.38^{c}	2304.44^{b}	22.00^{e}	18.66^{d}	48.06^{c}	43.10^{d}
P4	2075.79^{c}	1930.55^{c}	18.50^{f}	18.87^{d}	38.33^{d}	36.43^{e}
P5	1562.50^{e}	1476.94^{e}	29.70^{b}	30.27^{a}	46.31^{c}	46.22^{d}
P6	1329.89^{f}	1224.44^{f}	26.79^{c}	26.20^{b}	35.97^{d}	32.10^{e}
P7	2917.18^{a}	2733.88^{a}	30.33^{b}	27.37^{b}	88.83^{a}	75.44^{a}
P8	1961.66^{d}	1861.70^{c}	31.45^{b}	31.16^{a}	62.29^{b}	57.56^{b}
P9	1800.94^{d}	1586.94^{d}	34.37^{a}	31.58^{a}	61.74^{b}	50.86^{c}
P10	1943.11^{d}	1743.33^{d}	31.41^{b}	28.66^{a}	61.05^{b}	50.53^{c}
Average	1327.86	1240.41	26.82	25.54	35.47	31.60
CV (%)	14.34	15.93	9.93	11.71	18.84	22.68

Means followed by the same letter in the column belong to the same group by the Scott-Knott algorithm at the 5% probability level. CV: coefficient of variation.

Considering the PV as a super trait that gathers the traits GY and PE, the use of PV is an advantageous option for a reliable production of simultaneous gains in the two main traits of economic interest in the crop, GY and PE. Based on PV, lines P7, L59, P8, P9, P10, P2, and L66 stood out in the environment with low phosphorus, as they showed the most expressive means of 75.44, 57.70, 57.56, 50.86, 50.53, 49.98, and 48.98 m^3/ha, respectively. Nevertheless, despite the genotype x phosphorus level interaction, the lines that most stood out in the classification by the Scott-Knott algorithm in the environment with high phosphorus availability were the same as those of the environment with low phosphorus, but with means higher by up to 12% in PV (Table 2), which allows us to conclude that: i) PV outdid the negative correlation between GY and PE; and ii) PV benefits from the concomitant selection of efficient and responsive genotypes. It is, therefore, a trait of interest to be introduced in evaluations for the selection of superior genotypes in popcorn breeding programs.

The scatter graph of the lines for efficiency and responsiveness revealed that lines L59, P7, P2, P3, P4, P8, P10, P9, L66, L70, L69, and P5 were distributed in the quadrant classified as ER, i.e., these genotypes expressed higher average yields than averages found in the environments with high and low phosphorus (Figure 1). These ER lines displayed yields ranging from 200.00 to 1,600.00 kg/ha higher than the average found in the environments with high and low phosphorus.

Lines L75, L80, L61, L77, L63, L65, P1, L54, L53, L88, and L71, in turn, were classified as INR; in other words, they had lower average yields in the environments with low and high phosphorus, whose negative estimates varied, respectively, from 500.00 to 1,000.00 kg/ha (Figure 1). A specific, worth-mentioning case was line L76, whose yield was slightly above the overall mean of the environments under nutritional stress. Line P6 was not different between the different environments, not expressing inferiority or superiority for the estimated average yield. These genotypes probably have allele symmetry for genes responsible for efficiency and responsiveness to phosphorus and are thus of interest in studies of inheritance.

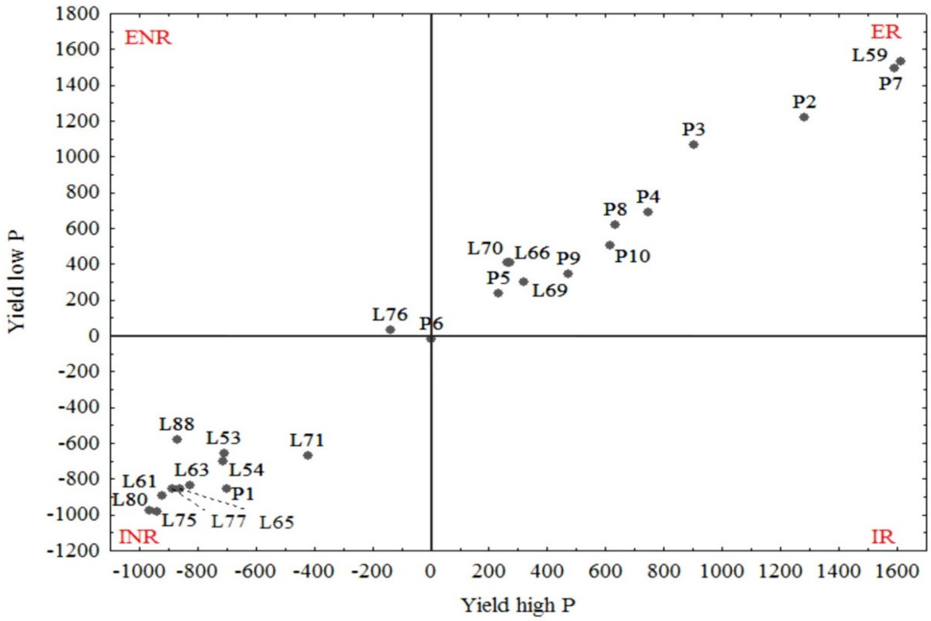

Figure 1. Distribution of 25 popcorn lines as to phosphorus use efficiency. ER = efficient and responsive; ENR = efficient and nonresponsive; IR = inefficient and responsive; INR = inefficient and nonresponsive.

This identification of different lines as to efficiency and responsiveness to phosphorus use is crucial to studies of genetic control and for knowing the predominant type of genetic action for the choice of the most suitable breeding method that can allow for the production of superior popcorn genotypes. This is especially true when the target is sustainable farming in agricultural frontiers with low phosphorus availability, as well as increased production in environments where the nutrient is present.

Some studies have been conducted aiming to understand and exploit the variability of traits related to phosphorus use efficiency in plants; however, this has shown to be a rather complex task, because the genetic control of these traits is usually polygenic and thus strongly influenced by the environment. Most studies of genetic control for phosphorus use efficiency are based on the evaluation of absorption and accumulation of phosphorus in the seedling phase (Gorz et al., 1987; Furlani et al., 1998). These studies, conducted on nutrient solutions, indicate that both additive and dominant effects are important in the control of efficiency in phosphorus use. However, it must be stressed that results obtained in nutrient solutions might not correlate with results obtained in field conditions. In this regard, for grass crops, experiments carried out in field conditions evaluating their “end product” - grain yield - are required for a better understanding of the genetic effects of phosphorus use efficiency-related traits.

Of the lines classified as efficient in phosphorus use, P9, P8, P10, P5, and P7 had the highest means for PE, with estimates ranging from 33 and 29 mL/g, which makes them interesting options for parents to obtain desirable segregant-derived populations not only for efficiency in phosphorus use but also for popping expansion, which is the quality trait of greatest agronomic and commercial interest in popcorn. These lines were extracted from hybrids “Zaeli” and “IAC-112”, which shows that these populations have genes of interest not only for efficiency and responsiveness to phosphorus regarding grain yield but also for a high expression of popping expansion. Efficiency in the use of phosphorus is directly related to production performance, a trait negatively correlated with popping expansion; this makes it difficult to obtain cultivars that integrate these two traits simultaneously, as reported by many authors (Dofing et al., 1991; Daros et al., 2004; Amaral Júnior et al., 2010; Scapim et al., 2010). In this way, based on an analysis of genealogic context, lines P9, P8, P10, P5, and P7, which have favorable genes for efficiency in phosphorus use regarding grain yield and popping expansion, can be options for obtaining superior hybrids for crops grown in environments where the nutrient is not highly available in the soil, thereby enabling the combined expression of the two main traits of economic interest for the crop: GY and PE.

The measures of genetic dissimilarity between the popcorn lines estimated by the generalized Mahalanobis distance revealed that lines L70 and L69 were the most similar genetically, in both the environments with high and low phosphorus, whereas L61 and L59 were the most divergent for the high phosphorus environment and L63 and P7 for the environment under phosphorus stress.

The amplitude of the genetic distances between the popcorn lines evidenced the existence of genetic variability, increasing the possibility of success in studies of inheritance of the efficiency trait as well as the chances of obtaining superior hybrids; in this case, using the estimate of genetic distance, together with the dominance deviations, for the estimate of heterosis (Falconer, 1987; Hallauer et al., 2010; Cruz et al., 2012).

Tocher’s optimization method had six distinct groups formed for the environment with high levels of phosphorus in the soil, and eight groups for the environment in which nutritional stress was induced (Table 3). Group I from the environment with high phosphorus had the highest number of lines - 12 -, representing 48% of the lines. Groups II, III, and IV were represented by five, two, and four lines, respectively. Groups V and VI contained only one line each - L65 and L77 -, respectively. According to Cruz et al. (2012), the groups with only one member represent genotypes with a marked divergence relative to the others. In the low phosphorus environment, however, there was a greater distribution of the lines across the eight groups formed, revealing that the environment under nutritional stress provided a greater expression of the genetic variability between the popcorn lines. Several authors, in studies investigating the genetic diversity of several crops under nutritional stress conditions, have shown that in stressing environments, genotypes tend to express higher genetic variability between individuals (Moura et al., 1999; Sávio et al., 2008; Silva et al., 2015).

It is believed that one of the reasons why there was a higher expression of genetic variability in the environment with low phosphorus resulting from the larger number of groups formed is due to the fact that most of these lines had already been obtained and adapted from the beginning of their breeding process exclusively for environments with proper nutritional conditions. Being grown in stressing environments, there is a tendency for these lines to express their variability in a different manner, because the genes controlling the efficiency and responsiveness to phosphorus are not present in all lines, or are in a lower number, causing this discrimination of performance between the lines in the environments with low phosphorus, i.e., genetically identical individuals can develop unequally in different environments.

Table 3. Clustering of 25 popcorn lines according to Tocher's optimization method in the environments with low and high phosphorus contents in the soil.

Group	Low P	Group	High P
	Lines		Lines
I	L70, L69, L66, L76	I	L70, L69, L66, L76, P6, P5,
II	P10, P9, P5, P8, P6, P3, P4, P2, P7		P8, P9, P10, P3, P4, P2
III	L80, P1, L71, L75, L65	II	L80, P1, L53, L75, L54
IV	L63, L61	III	L59, P7
V	L53, L54	IV	L63, L61, L88, L71
VI	L77	V	L65
VII	L59	VI	L77
VIII	L88	-	-

Just as Tocher's method, UPGMA revealed a larger number of groups in the environment with low phosphorus, with four groups formed, while three groups were formed for the high phosphorus level, with estimated cophenetic correlation coefficients of 0.68 and 0.69, respectively (Figures 2 and 3). There was, thus, similar consonance in the reliability of group formation, both for the high and low levels of the nutrients. In the environment with high phosphorus contents in the soil, group I was formed by lines L66, L69, L70, P10, P9, P8, P5, P6, P7, L59, P2, P3, and P4; group II, only by L77; and group III, by L76, L71, L54, L75, L65, L88, L61, L63, P1, L80, and L53. In the environment with low phosphorus contents in the soil, group I was formed by lines P1, L80, L71, L75, L65, L88, L61, and L63, coinciding with the same lines that were placed in group III of the environment with high phosphorus levels. The second group for the environment with low phosphorus in the soil was composed of lines P8, P9, P10, P5, P6, L59, P7, P3, P2, and P4, whose totality was present in group I for the environment with high phosphorus. The third group included lines L76, L66, L69, and L70; and group IV was composed of L77, L54, and L53.

In the grouping of lines in the environment with high levels of phosphorus in the soil by the UPGMA method (Figure 2), there was an agreement between the 13 lines that were allocated in the first group and those distributed in the quadrant corresponding to the ER lines, except for P6, which was exactly at the intermediate point of the graph (Figure 1). Nevertheless, except for L76, all the other lines that made up the second and third groups were placed in the quadrant of the INR (Figure 1). In the dendrogram grouping the lines in the environment with low phosphorus (Figure 3), the first and fourth groups coincided with the constitution of the lines classified in the quadrant of the INR (Figure 1). Lastly, the second and third groups were distributed in the quadrant of the ER (Figure 1), except for L76 and P6.

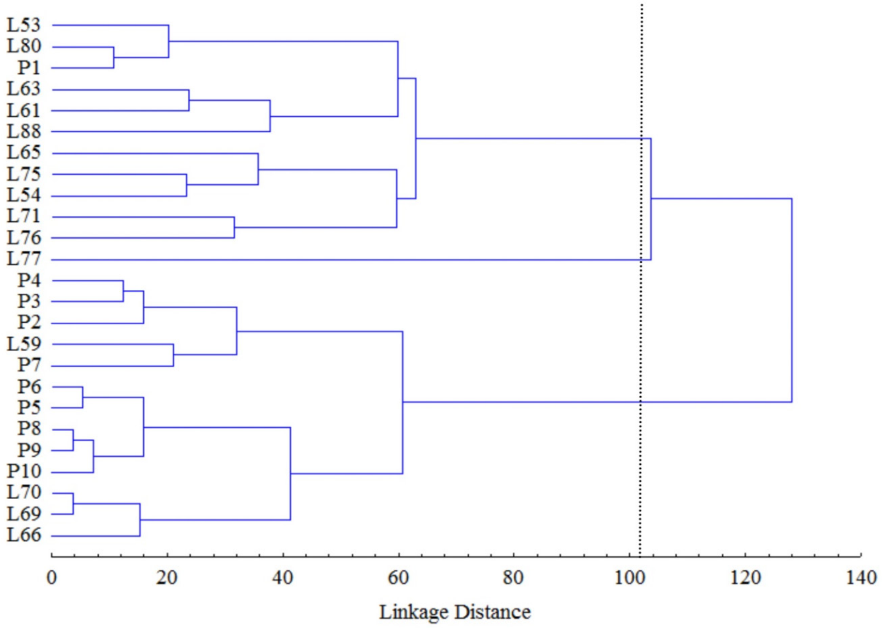

Figure 2. Dendrogram illustrating the UPGMA method for 25 popcorn lines evaluated in an environment with high phosphorus contents in the soil.

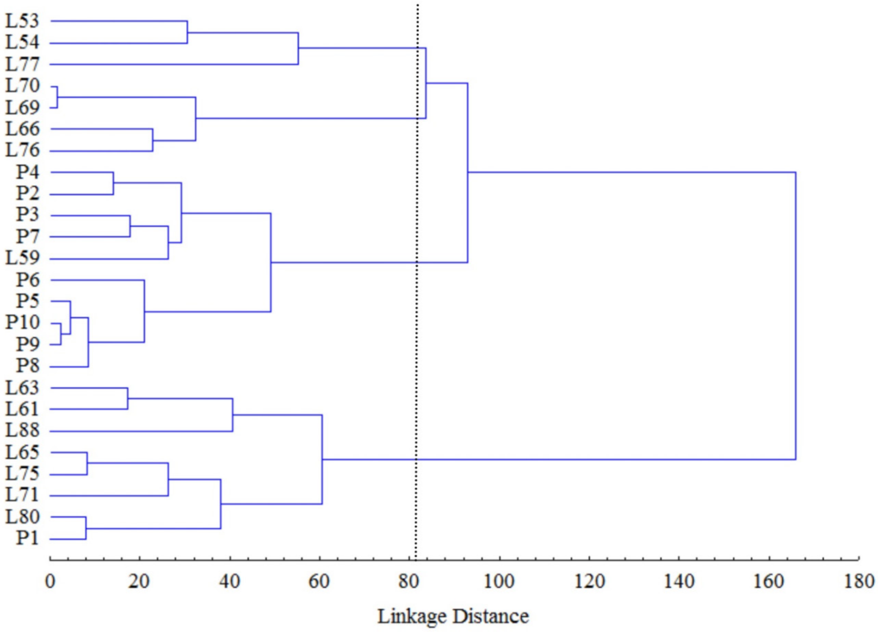

Figure 3. Dendrogram illustrating the UPGMA method for 25 popcorn lines evaluated in an environment with low phosphorus contents in the soil.

Among the populations that originated the 25 popcorn lines evaluated here, a genetic similarity was observed between the lines from the populations of compound CMS-42, South-American breeds, hybrid Zaeli, and hybrid IAC-112, because the majority of lines from these populations was classified in the same group, in both the environments with high and low phosphorus, by Tocher's and UPGMA methods. The lines originating from the other populations were ordered randomly in the other groups formed.

CONCLUSIONS

There is sufficient genetic variability across the evaluated popcorn lines to be exploited in breeding programs.

Lines L59, P7, P2, P3, P4, P8, P10, P9, L66, L70, L69, and P5 were efficient and responsive to phosphorus use.

Lines L75, L80, L61, L77, L63, L65, P1, L54, L53, L88, and L71 were inefficient and nonresponsive to phosphorus use.

The phosphorus-stressed environment allows for a greater opportunity for expression of the variability of the genomes.

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