FUNCTIONAL PLANT BREEDING JOURNAL
Scientific Journal. ISSN 2595-9433
Volume 1, Number 1, Article n. 1, January/March
D.O.I. http:// dx.doi.org/10.35418/2526-4117/v1n1a1
Accepted: 12/07/2018 Published: 05/03/2019

CONTRASTING PHOSPHORUS ENVIRONMENTS AS INDICATORS FOR POPCORN BREEDING LINES

Thiago Rodrigues da Conceição Silva[1], Antônio Teixeira do Amaral Júnior[1], Janeo Eustáquio de Almeida Filho[1], Marta Simone Mendonça Freitas[2], Amanda Gonçalves Guimarães[1], Samuel Henrique Kamphorst[1]

[1]Laboratório de Melhoramento Genético Vegetal, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, 28013-602. [2] Laboratório de Fitotecnia, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, 28013-602.

Corresponding author: Antonio Teixeira do Amaral Junior (amaraljr@pq.cnpq.br).

Abstract-Phosphorus (P) is one of the most limiting factors in low investment agricultural systems. The aim of this study was to evaluate a panel of 29 popcorn maize lines to identify potential genotypes to be developed under P restriction conditions for integration into a breeding program. For this, these lines were phenotyped for complex traits of the shoot, root, and phosphorus use efficiency (PUE) at contrasting and controlled P environments (standard and low P contents of 22 and 4 mg dm^{-3}, respectively) and genotyped with 15 SSR–EST markers. The results indicated that through selection in shoot traits it is possible to obtain indirect gains in traits related to root development that are difficult to measure. In addition, the selection based on traits of shoot and root development at an environment promotes indirect gains in another environment. In the environment supplied with low P, the P uptake were more important for PUE than internal utilization of P. Lines P2, P4, P7, and P9 presented a better development of shoot and root system at the P-low environment, in addition to showing P content above the average in root and shoot. Based on genetic diversity, complementary and inheritance crosses were identified.

Keywords: *Zea mays* var. Everta, abiotic stress, mineral nutrition, PUE, diversity

Introduction

Phosphorus (P) is one of the mineral nutrients essential for plant development, being one of the most limiting minerals in grain production (Gaxiola et al., 2011). Due to the high demand for food to encounter the growing population, an increase in the use of this nutrient is expected, compromising the sustainability of agricultural production since phosphorus used in agriculture comes from rocks, which are natural resources (Van de Wiel et al., 2016). Moreover, a great use of this nutrient unbalances the environment due to eutrophication of rivers, lakes, and ocean coasts, thereby eliminating several life forms (Gaxiola et al., 2011). In addition, phosphate (Pi) rocks may contain heavy metals such as cadmium, which may accumulate in agricultural soils (Van de Wiel et al., 2016).

Historically, plant breeding has been selecting genotypes at environments without nutrient restriction, which leads to cultivars increasingly adapted to environments with an abundance of fertilizers (Wang et al., 2010). However, P is generally heterogeneous and immobile in the soil (Van de Wiel et al., 2016); in addition to being considered one of the most limiting factors in low investment agricultural systems (Ramaekers et al., 2010). Thus, with this increasingly demanding cultivars, agriculture tends to be more and more dependent on phosphate fertilization, damaging the environment and hampering the agricultural activity of low financial resource farmers (Good et al., 2004).

One of the way to mitigate the environmental impact promoted by fertilizer demand is the use of more efficient cultivars both in the available nutrient acquisition and in its conversion to fibers and grains (Wang et al., 2010). For this, breeding programs of the main crop species need to assess traits related to plant adaptation at environments with reduced nutrient demand (Van de Wiel et al., 2016). In this sense, some species have been studied, such as coffee (Neto et al., 2016), wheat (Gunes et al., 2006), rice (Wissuwa and Ae, 2001), bean (Ochoa et al., 2006), maize (Fritsche-Neto et al., 2012; DoVale and Fritsche-Neto, 2013), and popcorn (Mundim et al., 2013; Mundim et al., 2014).

Popcorn crop has a high international appeal due to its great popular acceptance, which generates income both in the grain production sector and in the final product commercialization, *i.e.* the popcorn burst. Acknowledging the importance of this crop, the State University of Northern Rio de Janeiro (UENF) has maintained a breeding program in popcorn for providing bred cultivars to farmers (Amaral Jr et al., 2013) and promoting the exchange of genetic materials among research institutions. However, to meet the socio-environmental demands of sustainable agricultural production, the characterization of available genetic resources is essential regarding the adaptation at P-low and standardP supply environments, aiming to select individuals that can integrate a breeding program with the purpose of obtaining cultivars that require the lowest possible supply of mineral fertilizers.

Considering the worldwide need for food production mitigating the environmental impact of both pollution and exacerbated use of non-renewable natural resources, the aim of this research was to assess a group of popcorn lines that represents the genetic diversity of the UENF breeding program. For this, these lines were assessed in a controlled environment for standardP content and another for low P , being measured complex traits related to plant adaptation at nutrient-restricted environments.

Materials and methods

Plant material

Twenty-nine popcorn inbreed lines from different genealogy belonging to the Germplasm Bank of UENF, Rio de Janeiro, RJ, Brazil (Table 1), were assessed at low and high phosphorus levels.

Table 1. Description of the 29 popcorn lines from the Germplasm Bank of UENF used for selecting the efficiency and responsiveness to P.

Genotype	Originating variety	Climatic adaptation	Development institution*
L51	Beija-flor UFV	Temperate/Tropical	UENF
L52	Beija-flor UFV	Temperate/Tropical	UENF
L53	Beija-flor UFV	Temperate/Tropical	UENF
L54	Beija-flor UFV	Temperate/Tropical	UENF
L55	Beija-flor UFV	Temperate/Tropical	UENF
L59	Beija-flor UFV	Temperate/Tropical	UENF
L61	BRS Angela Embrapa	Tropical	UENF
L63	BRS Angela Embrapa	Tropical	UENF
L65	BRS Angela Embrapa	Tropical	UENF
L66	BRS Angela Embrapa	Tropical	UENF
L69	BRS Angela Embrapa	Tropical	UENF
L70	BRS Angela Embrapa	Tropical	UENF
L71	BRS Angela Embrapa	Tropical	UENF
L74	BRS Angela Embrapa	Tropical	UENF
L75	Viçosa UFV	Temperate/Tropical	UENF
L76	Beija-flor UFV	Temperate/Tropical	UENF
L77	Viçosa UFV	Temperate/Tropical	UENF
L80	Viçosa UFV	Temperate/Tropical	UENF
L88	Viçosa UFV	Temperate/Tropical	UENF
P1	Hybrid Zélia	Temperate/Tropical	UEM
P2	Compound CMS–42	Temperate/Tropical	UEM
P3	Compound CMS–43	Temperate/Tropical	UEM
P4	South American races	Temperate/Tropical	UEM
P5	Hybrid Zélia	Temperate/Tropical	UEM
P6	Hybrid Zélia	Temperate/Tropical	UEM
P7	Hybrid Zélia	Temperate/Tropical	UEM
P8	Hybrid IAC112	Temperate/Tropical	UEM
P9	Hybrid IAC112	Temperate/Tropical	UEM
P10	Hybrid IAC112	Temperate/Tropical	UEM

Experimental design

For each low and high phosphorus levels, an experiment was conducted under greenhouse conditions at the Research Support Unit of the CCTA/UENF, Campos dos Goytacazes, RJ, Brazil (21°45'43.63" S and 41°17'15.65" W). Both experiments were installed in July 2015 in randomized block design with four replications. Each plot consisted of a polyethylene pot with a single plant, totaling 116 pots at each experiment.

Three seeds were sown per line in a polyethylene pot with a 5.0 dm^3 capacity, filled with a substrate composed of a proportion of three parts of soil (pH: 4.12; clay: 48%; P: 0.82 mg dm^{-3}) and a part of sand. After thinning, only one plant remained per pot. A complete substrate chemical analysis was performed (Table 2) and soil correction was carried out according to Sawazaki (2001). To correct the substrate pH, a dolomitic limestone (40.36% CaO; 10.01% MgO; 89.99% NP; 94.87% RR; 85.37% TRNP) was used in a dose of 3.125 g per pot based on the increasing soil base saturation method (Mundim et al., 2014). For limestone mixing with the substrate, plastic bags of 8 dm^3 were used, being moistened and incubated over a 30-day period.

Table 2. Results of chemical analysis of substrate soil used (soil: sand).

Chemical analysis*		
pH	4.1	H_2O > 1:2 and 5
P	2	mg dm^{-3}
K	1	$mmol_c$ dm^{-3}
Ca	1.2	$mmol_c$ dm^{-3}
Mg	3.6	$mmol_c$ dm^{-3}
Al	6	$mmol_c$ dm^{-3}
H+Al	19.0	$mmol_c$ dm^{-3}
Na	0.8	$mmol_c$ dm^{-3}
C	1.8	g dm^{-3}
OM	3.10	g dm^{-3}
CEC	25.60	$mmol_c$ dm^{-3}
SB	6.60	$mmol_c$ dm^{-3}
BS	26	%
ASI	48	%
NaSI	3	%
Fe	7.46	mg dm^{-3}
Cu	0.15	mg dm^{-3}
Zn	1.20	mg dm^{-3}
Mn	2.27	mg dm^{-3}
B	0.92	mg dm^{-3}

* pH in water, KCl and CaCl – relation: 1:2.5; P, Na, K, Fe, Zn, Mn, and Cu – extractor: Mehlich 1; Ca, Mg, and Al – extractor: KCl 1 mol L^{-1}; H+Al – extractor: calcium acetate mol L^{-1} at a pH 7.0; B – hot extractor; S – extractor: monocalcium phosphate; SB = sum of bases and exchangeable; CEC – cation exchange capacity at a pH 7.0; BS = base saturation; ASI – aluminum saturation index; NaSI – sodium saturation index; OM – organic matter, OM = organic carbon × 1.724, Walkley-Black.

Single superphosphate was the P fertilizer used at doses that provided availability levels of 4 and 22 mg dm^{-3} in the substrate, called as low and standardP, respectively. For this, soil's ability to fix phosphorus was determined using 25 and 150 mg dm^{-3} P to the respective levels. After incubation, phosphorus fertilization was carried out by means of adding 1.59 and 12.72 g single superphosphate per bag for low and high P level experiments, respectively. Potassium (K) fertilization was applied together with P fertilization in a dose of 0.5645 g KCl per plot, which is equivalent to 70 kg ha^{-1} K. Nitrogen (N) was applied in a dose of 20 mg mL^{-1} NH_4NO_3, divided between the fifth, thirteenth, and twentieth day after seedling emergence.

Assessed traits

Plants were harvested in the vegetative stage V6 (six fully expanded leaves), approximately 30 days after emergence, making a cut close to the substrate, separating root system from the shoot. At that moment, the following traits were assessed: i) plant height (PH) and ii) length of the last leaf (LL), both in cm; iii) stem diameter (SD, mm), measured at the plant base using a digital caliper.

After harvest, roots were washed with running water, removing all soil. The following traits were assessed: i) root length (RL), ii) root surface area (RS), and (iii) root volume (RV), quantified by means of root image processing of each plant by using an Scanner

Epson Perfection 10000XL and estimated by using the software WinRhizo 2009 PRO (Regent Instruments, Quebec). Results were expressed in cm, cm^2, and cm^3, respectively.

Shoot (SDM) and root (RDM) dry mass were determined by weighing after drying in a forced air oven at 70 °C for 36 hours, with results expressed in mg. Subsequently, samples were milled in order to quantify shoot (SPC) and root (RPC) P content by means of digestion with HNO_3 and H_2O_2 and reading in ICP–OESi in an open digestion system (plasma gas: 8.0 L min^{-1}; auxiliary gas: 0.70 L min^{-1}; carrier gas: 0.55 L min^{-1}), with results expressed in mg g^{-1}.

Nutrient Use and Efficiency indices

The traits SDM and root RDM were evaluated based in the Moll et al (1982) indices: PUE=PUtE x PUpE; PUE is the P Use Efficiency (mg mg^{-1}); PUtE is the P Utilization Efficiency (mg mg^{-1}) and PUpE is the P Uptake Efficiency (mg mg^{-1}). For SDM these indices were: PUtE_SDM=SDM/Pt_SDM; PUpE_SDM=Pt_SDM/Ps; PUE_SDM=PUtE_SDM x PUpE_SDM=SDM/Ps, where Pt_SDM is total P in the shoot and Ps is the total P supplied, both Pt_SDM and Ps were given in mg/plant. For RDM these indices were: PUE_RDM=RDM/Ps; PUtE_RDM=RDM/Pt_RDM; PUpE_RDM=Pt_RDM/Ps, where Pt_RDM is total P in the root and the others terms were already defined.

Statistical analyses

For the statistical analysis, uni-trait models were used to predict genetic merit and estimate variance and heritability components. Bi-trait and bi-environment models were also adjusted to estimate correlation components between traits and between environments. The following general bivariate linear model was adopted: + , where , , , is the phenotype vector for a trait in a given environment, is the phenotype vector for another trait measured in the same environment (bi-trait models) or the phenotype vector of the same trait, but at another environment (bi-environment models), is the fixed-effects vector of blocks, is the random-effects vector of genotypes, is the random errors vector, the subscript *i* refers to the vector ($i = 1$ or 2), *I* is an identity matrix of order equal to the length of the vector , is the Kronecker product, is a matrix with all null elements, and and are incidence matrices of the vectors , and respectively.

For estimating genetic and environmental correlation components, a matrix structure of heterogeneous variance and correlated effects were adopted: and .

The inference on genetic correlation components was obtained by means of the likelihood ratio test (LRT) using a reduced model with for inference on , a model with for inference on , and an approximate test for with both G0 and R0 adjusted as diagonals. The estimation of was carried out as reported in Falconer and Mackay (1996): . Wherein: is the heritability estimated by the vector .

For the uni-trait model, a similar model to the general was adopted. However, considering only the terms with subscript 1, and for inference on variance components, a reduced model was adjusted without the respective effect. All analyses were performed using the software R (R Core Team, 2016). For model adjustments, the package Asreml (Butler, 2009) was used, being the LRT tests performed with the package AsremlPlus (Brien, 2016).

Genotyping

DNA extraction

For the assessment of genetic diversity of 29 popcorn lines from the Germplasm Bank of UENF at a level of molecular markers, two seeds of each line were sown in a polystyrene tray containing commercial substrate. Young leaves were collected for DNA extraction by the standard CTAB method, with modifications suggested by Daher et al. (2002). After extraction, DNA samples were quantified using a Qubit® 2.0 fluorometer (Invitrogen) and diluted to working concentration (5 ng μL^{-1}), being subsequently used in amplification reactions (PCR).

Microsatellite markers and polymerase chain reaction

Genic microsatellite markers (SSR–ESTs) were identified based on sequences developed and mapped by Sharopova et al. (2002) for corn (*Zea mays*) and located in the database of the National Center of Biotechnology Information (NCBI) database. One hundred ninety-three SSR–EST primers were selected to check which would present polymorphic bands and optimal temperature of polymerase chain reaction (PCR).

PCR reactions were performed in Applied Biosystems/Veriti 96-well thermocyclers in a 35 cycle program, under the following conditions: 94 °C for 4 minutes (initial denaturation); 94 °C for 1 minute (cyclic denaturation); at a specific temperature (in °C) of each primer for 1 minute (annealing); 72 °C for 2 minutes (cyclic extension); 72 °C for 7 minutes (final extension); and 4 °C forever. The final volume was 13 µL per reaction, as follows: 2 µL DNA (5 ng μL^{-1}), 1.5 µL 10X buffer ($(NH_4)SO_4$), 1 µL $MgCl_2$ (25 nM), 1.5 µL dNTPs (2 mM), 1 µL primer (R+F) (5 µM), and 0.12 µL Taq-DNA polymerase (5 U μL^{-1}).

Amplification products were separated by means of 4% MetaPhor agarose gel electrophoresis immersed in TAE buffer (90 mM Tris-Acetate (pH 8.0) + 10 mM EDTA), stained with Gel Red™ and Blue Juice (1:1), visualized by the MiniBis Pro (Bio-Imaging Systems) photo-documentation system, and compared with a 100 bp (0.5 ng μL^{-1}) High DNA Mass Ladder marker (Invitrogen). Fifteen pairs of polymorphic primers

were selected (Table 3) for further analysis in the Fragment Analyzer (Advanced Analytical) capillary electrophoresis system. Samples were prepared with a 24-µL volume, using 4 µL amplified material and 20 µL buffer, distributed in 96-well plates for capillary electrophoresis. To determine the amplified fragment sizes, a DNA Ladder marker containing a variation from 35 to 500 bp was used and images were analyzed for allele determination. The data obtained by SSR–EST were used for calculate genetic distances through Gower (1971) algorithm and from this distance matrix were realized the Principal Components Analysis (PCA) using the software R (R Core Team, 2016).

Table 3. Sequence of 15 pairs of SSR–EST primers, chromosome location (Cr), position in the genetic map in centimorgan (cM), annealing temperature (T °C), and number of alleles (Na).

Locus	Sequence (5'®3')	Cr	cM	T °C	Na
umc1073	CACCAACGCCAATTAGCATCC GTGGGCGTGTTCTCCTACTACTCA	1	206.7	64.0	2
umc2112	AGCTCTACCAAACACGAGCTTCAT CAAATGCAGAAAGATAACGCGAAT	1	360.4	63.0	3
umc1515	AGAGAGGCTGCTTCAATAAGTTGC TTAGTAGTTTCGGTGTCCGTTTCC	1	393.0	62.0	3
glb1	GCACACACACAGGACGACAGT TGTTGCTCGGTCACCATACC	1	748.7	62.0	3
umc1118	ATCAGATTCCGAAGGGTCCATAAT GTAGTGAAATGAATCGTGAGAGCG	1	902.2	62.0	2
phi96100	AGGAGGACCCCAACTCCTG TTGCACGAGCCATCGTAT	2	24.0	59.0	5
ole1	AGTAAAAGAGGCAAGGACTACGGC GCGGCGATATATACGAGGTTGT	2	202.7	62.0	3
umc1252	CTTCTGCATCATCATCATCGTCTT GCGTCGGAGAAGTACATCAAGTTT	2	516.7	63.0	3
bip2	AGCAAGCAGTTCGAAACAAGGAT GACACCAGCACCACTTGAACG	4	669.1	64.0	3
umc2319	GCTCTCACTAGCCTCGCATTCC GATCCACGCGAGGTTCACTG	6	244.9	64.0	3
y1	CAAGAAGAGGAGAGGCCGGA TTGAGCAGGGTGGAGCACTG	6	99.4	65.0	3
umc1241	TGAAGCAAGTCACTGGTAAGAGCA TGACACACCCATACTTCCAACAAG	7	13.8	63.0	2
umc1139	TTTGTAATATGGCGCTCGAAAACT GAAGACGCCTCCAAGATGGATAC	8	26.8	63.0	2
umc1130	TTGGGACTCATTACTTCCGGACT GCTAGGGGAAAGCTCGTACTATGG	8	293.2	63.0	2
umc1982	AATCGTACTTGGAGGAGGCGTT TTCATCTTCCTAGTCTCGTCTCCG	9	581.6	63.0	3

Results

Genetic parameters under standard and low P conditions

Divergent estimates were observed for the genetic parameters heritability (h^2) and accuracy (Acc) for all assessed traits at both P levels (Table 4). Estimates of h^2 varied from 0 to 0.74 at standardP environment and from 0 to 0.67 at the P-low environment. For accuracy, estimates ranged from 0 to 0.94 and from 0 to 0.93 for high and low P levels, respectively. Most of the traits showed higher h^2 and accuracies at the standardP environment. In traits related to shoot growth (LL, PH and SD) presenting average h^2 values of 0.65 (s.d.=0.02) at standardP environment and 0.60 (s.d.=0.07) at the low P- environment. Among the traits associated with root system growth, only RL at standardP level environment presented statistically zero genetic variance. In the other cases, h^2 varied between 0.48 and 0.69 for these traits. Among the traits related to P content, SPC and RPC presented higher h^2 at standardP environment, with values of 0.32 and 0.22, respectively; at low P environment, these values were 0.13 for SPC and approximately

zero for RPC, indicating a strong environmental influence when measuring this trait. The phosphorus use and efficiency in shoot (PUE_SDM) and root (PUE_RDM) development showed higher h^2 in standardP environment, these indices yielded higher h^2 than P uptake and P utilization efficiency in both environments. Exception of PUpE_SDM in standardP environment and PUtE_RDM in low P environment all indices related with PUE provide h^2 statistically higher than zero ($p<0.05$).

Table 4. Estimates of genetic variance (V_g), environmental variance (V_e), heritability (h^2), and accuracy (Acc) for traits related to phosphorus efficiency in 29 popcorn lines under two P levels.

Trait class	Traits	High P					Low P				
		Vg	Ve	h^2	p	acc	Vg	Ve	h^2	p	acc
Shoot development	LL	63.51	32.84	0.66	0.00	0.92	57.29	27.88	0.67	0.00	0.93
	PH	14.10	6.96	0.67	0.00	0.93	8.96	5.69	0.61	0.00	0.91
	SD	1.57	0.91	0.63	0.00	0.92	0.83	0.75	0.53	0.00	0.89
Root development	RL	0.12	423772.80	0.00	1.00	0.00	367611.83	402900.97	0.48	0.00	0.87
	RS	29827.85	13111.05	0.69	0.00	0.93	36673.80	22197.00	0.62	0.00	0.92
	RV	88.44	89.71	0.50	0.00	0.88	20.21	15.46	0.57	0.00	0.90
P content	RPC	$5.3x10^{-3}$	$1.9x10^{-2}$	0.22	0.01	0.71	$5.20x10^{-10}$	$5.14x10^{-3}$	0.00	1.00	0.00
	SPC	0.10	0.22	0.32	0.00	0.79	$6.5x10^{-3}$	$4.3x10^{-2}$	0.13	0.11	0.60
P Use and Efficiency in Shoot	PUE_SDM	154.36	53.84	0.74	0.00	0.94	268.43	592.04	0.31	0.00	0.79
	PUpE_SDM	405.97	5597.40	0.07	0.40	0.47	4607.12	19418.35	0.19	0.02	0.69
	PUtE_SDM	$7.3x10^{-4}$	$1.5x10^{-3}$	0.32	0.00	0.80	$3.7x10^{-3}$	$1.7x10^{-2}$	0.18	0.04	0.67
P Use and Efficiency in Root	PUE_RDM	22.75	11.63	0.66	0.00	0.93	135.03	148.17	0.48	0.00	0.87
	PUpE_RDM	16.54	10.89	0.60	0.00	0.91	10.64	27.53	0.28	0.00	0.77
	PUtE_RDM	$7.6x10^{-3}$	$3.5x10^{-2}$	0.18	0.03	0.67	$5.0x10^{-7}$	0.89	0.00	1.00	0.00

[1] The assessed traits were length of the last leaf (LL) , plant height (PH), stem diameter (SD), root length (RL), root surface area (RS), root volume (RV), shoot P content (SPC) and root P content (RPC). In addition the indices P use efficiency (PUE), P uptake efficiency (PUpE), P utilization efficiency of shoot dry matter (PUtE), were applied in shoot and (SDM) root dry matter (RDM). p: *p-value of the hypothesis test – H0: $h^2=0$; H1: $h^2>0$, by the likelihood ratio test.

It is expected that individuals with high vegetative growth at one environment also show high grow that another environment

In most practical aspects of breeding, assessment of genotypes at contrasting environments, especially for P availability, is impracticable. In this sense, to infer the consequence that a selection applied to an environment can promote in another, from bi-environment assessments, components of genetic, environmental, and phenotypic correlation of traits between environments were estimated (Fig. 1).

The traits RL, RPC and PUtE_RDM were not included due to the absence of convergence to the bi-environment model, which is supposedly due to the high environmental influence, reflected in an h^2 approximately zero in standardP (RL) or low P environment (RPC and PUtE_RDM). Genetic correlation values between −0.87 and 0.99, most of the genetic correlations presented high estimates ($r_g \geq 0.85$), with a large part of them statistically different from zero ($p<0.1$). All traits related to shoot vegetative growth presented high genetic correlations between environments (0.93 to 0.99).

For traits related to root system growth, high-magnitude genetic correlations (0.89 and 0.99; $p<0.01$) were observed. The results of high genetic correlations for traits related to shoot and root system growth indicate that the selection of individuals with high vegetative growth at an environment will provide a positively correlated response to another environment. For SPC, the genetic correlation was −0.87 ($p<0.05$), indicating that individuals with a high SPC at high P environment are expected to present a low SPC at low P environment. The PUE_SDM showed high genetic correlation between the environments (0.98), whereas for PUtE_SDM and PUpE_SDM these genetic correlations were low (-0.79 and -0,87 respectively), however for PUpE_SDM this correlation was non-significant ($p>0.1$), due probably the non-significant h^2 in standardP environment. In the case of RDM the genetic correlation between environments were estimated for PUE_RDM and PUpE_RDM, for both cases these estimations were high (0.93 and 0.99 respectively; $p<0.01$).

Regarding environmental correlations, estimates ranging from −0.02 to 0.25 indicated that the environment did not present a great influence on phenotypic correlations. In addition, because no trait showed a high h^2 together with a high r_g estimation for both environments, no high phenotypic correlation values (r_p from −0.19 to 0.65) were observed.

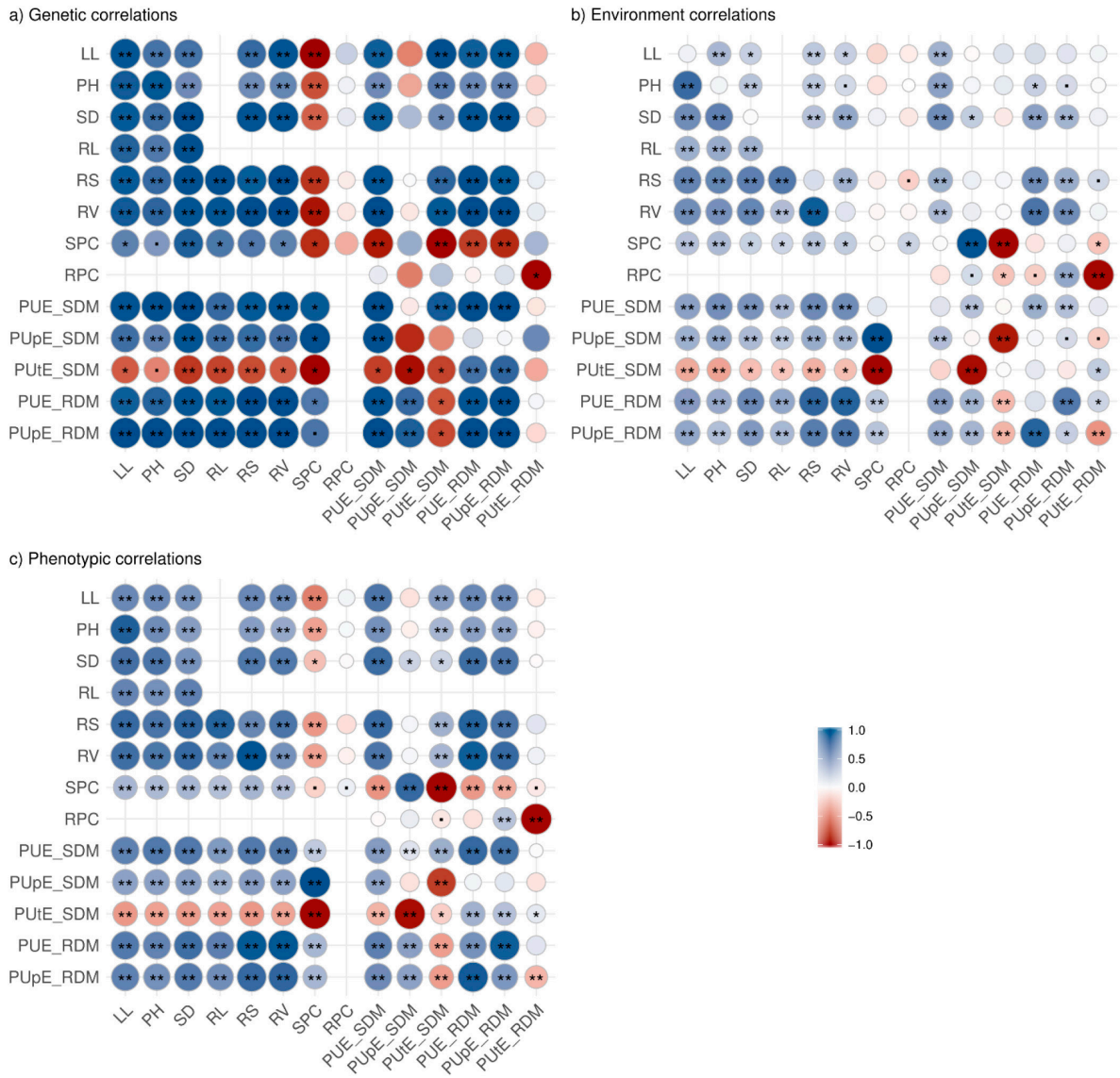

Figure 1. Estimates of genetic (a), environmental (b), and phenotypic (c) correlations among traits assessed at high (upper diagonal) and low (lower diagonal) P environments in 29 popcorn lines. The diagonal are the correlations of the same trait between the environments. The assessed traits were length of the last leaf (LL) , plant height (PH), stem diameter (SD), root length (RL), root surface area (RS), root volume (RV), shoot P content (SPC) and root P content (RPC). In addition the indices P use efficiency (PUE), P uptake efficiency (PUpE), P utilization efficiency of shoot dry matter (PUtE), were applied in shoot and (SDM) root dry matter (RDM). The traits RL, RPC and PUtE_RDM did not provide a conversion to the bi-trait model at high and low P environments, respectively. **, * and · (dot): $p<0.01$, $p<0.05$ and $p<0.1$, respectively.

Indirect selection of traits related to shoot vegetative growth

Several traits related to plant vegetative growth at low nutrient environments are difficult to measure, making unfeasible their assessment at certain phases of breeding programs. For instance, traits related to root system development, in which plants need to be removed from soil (or substrate), and traits related to nutrient uptake, in which plants need to be destroyed even before the reproductive stage. Thus, the influence of genetic and environmental factors on phenotypic correlation was partitioned to better understand the impact of selecting traits related to vegetative growth on these hard-to-measure traits (Fig. 1).

Genetic correlations between traits varied from −0.99 to 0.99. At both environments, most of the traits related to shoot vegetative growth presented positive and significant genetic, environmental, and phenotypic correlations ($p<0.05$) with traits related to root system growth, indicating that plants with a greater shoot vegetative growth naturally present a well-developed root system, regardless the assessed environment.

SPC presented negative and significant genetic and phenotypic correlations with almost all traits related to growth and P use efficiency both in shoot and

root system when assessed at standardP conditions. However, at the P-restricted environment, correlations between SPC and traits related to shoot and root system development were positive ($p<0.05$). On the contrary, RPC did not present genetic correlation statistically different from zero with any trait at standardP environment and the correlation study with this trait was not possible in low P environment because RPC presented $h^2=0$.

The correlation within and among traits related with shoot and root development, as well PUE_SDM and PUE_RDM were positive ($p<0.01$) in both environment, reflected the plant with more vigor in shoot are also the plants with more vigor in root. When analyzing only the use and efficiency of P with P uptake, the PUE_RDM showed high correlation with PUpE_RDM in both environment and PUE_SDM showed high correlation with PUpE_SDM only in low P environment. When analyzed the correlation of P use and efficiency and P internal utilization in shoot the correlation between PUE_SDM and PUtE_SDM were high in standardP environment and low in low P environment, in the root this correlation could be estimated only standardP environment only the estimation were statically equal zero ($p>0.1$).

Potential lines for breeding

With trait measurements and PUE indices, the assessed lines were discriminated regarding the predicted genotypic values (Fig. 2–4). Shoot vegetative growth superiority can be determined mainly by PUE_SDM (Fig. 2ab). When considering a selection of superior 30 %, lines L66, P2, P3, P4, P7, P8, and P9 stood out by being among the best selected at both environments for SDM. In addition, these best lines for PUE_SDM, also provided great performance for PUE_RDM (Fig. 3ab), that is the most important characteristic for root development.

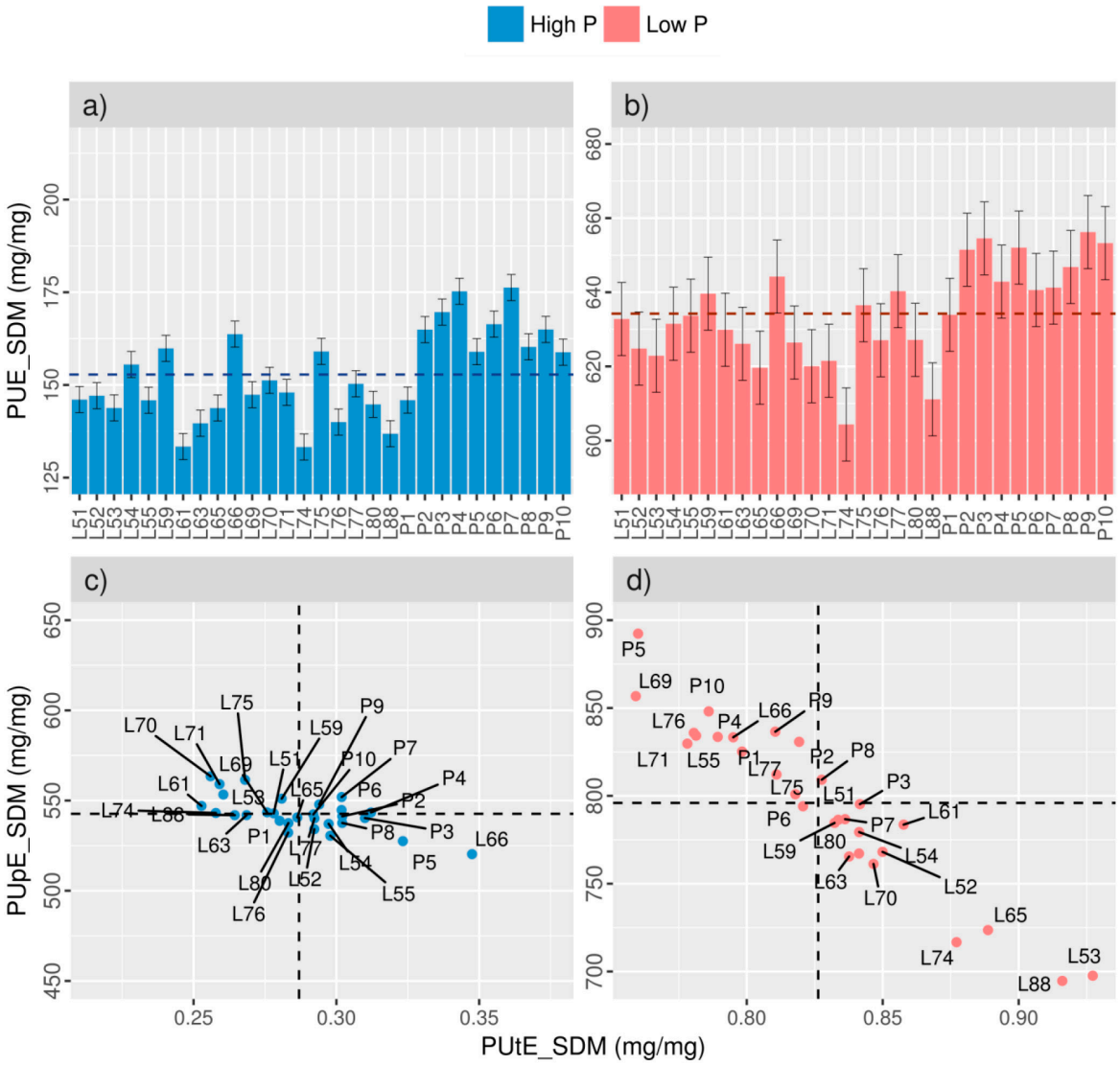

Figure 2. P use efficiency of shoot (PUE_SDM) at high (a) and low P (b) environment and theirs related indices PUpE_SDM and PUtE_SDM at high P (c) and low P (d) environment in 29 popcorn lines.

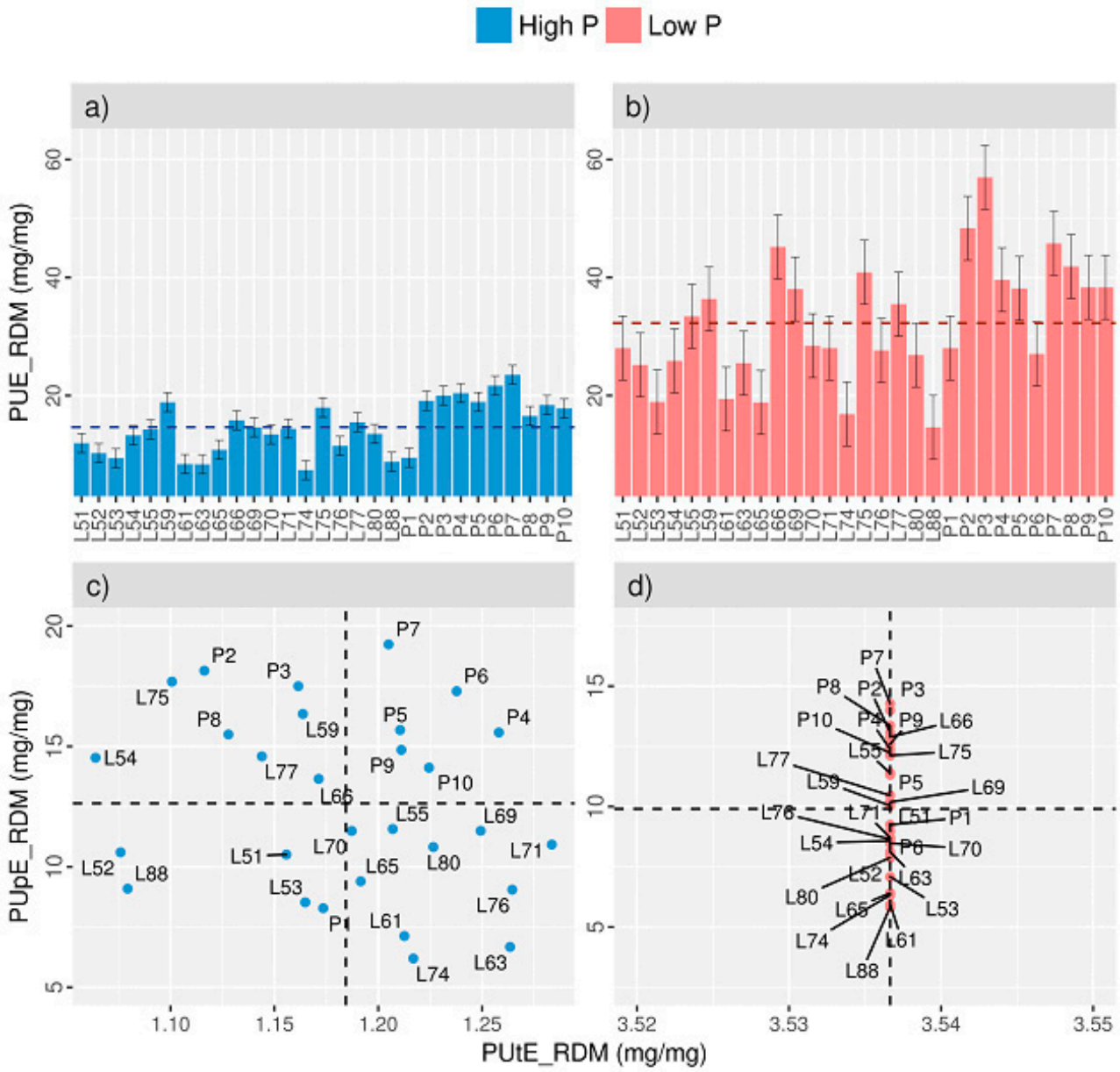

Figure 3. P use efficiency of root (PUE_RDM) at high (a) and low P (b) environment and theirs related indices PUpE_RDM and PUtE_RDM at high P (c) and low P (d) environment in 29 popcorn lines.

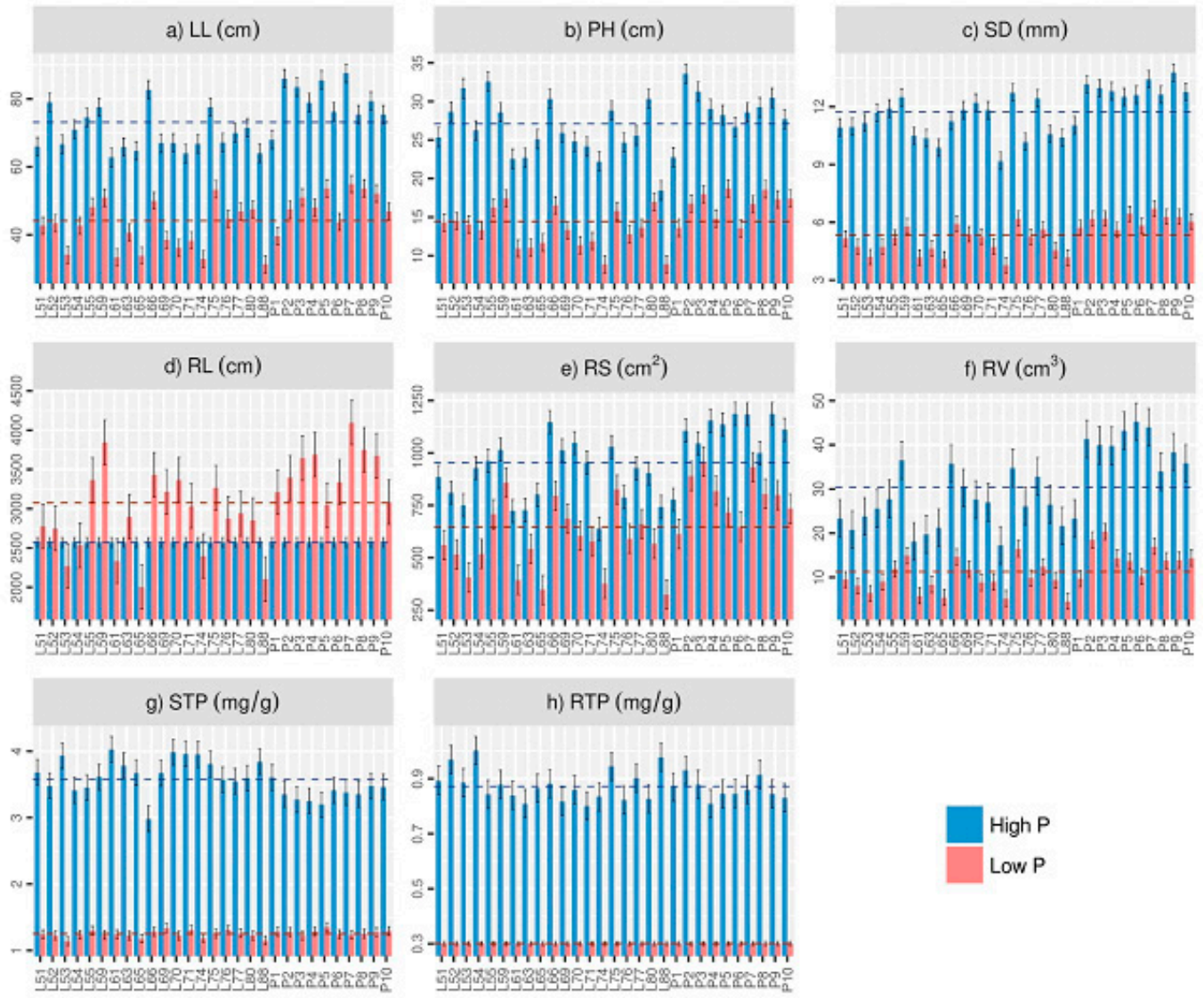

Figure 4. Predicted genotypic values in 29 popcorn lines assessed at high and low P environments for the following traits: a) length of the last leaf (LL); b) plant height (PH); c) stem diameter (SD); d) root length (RL); e) root surface area (RS); f) root volume (RV); g) shoot P content (SPC); and h) root P content (RPC). Dashed blue and red lines represent the mean at high and low P environment, respectively.

The PUE depend of PUtE and PUpE, for PUE_SDM in high P environment this index were most influenced by PUtE_SDM (Fig. 2c), whereas in low P environment PUpE_SDM and PUtE_SDM showed strong negative correlation thus no line yield high PUpE and PUtE for SDM in low P environment, however in this case the best lines for PUE_SDM showed higher PUpE_SDM and lower PUtE_SDM (Fig. 2d). The evaluation of PUE_RDM (Fig. 3ab) in their related indices, showed that in low P environment only PUpE_RDM contributed with PUE variation (Fig. 3d), whereas in standardP environment the best lines for PUE also provided best results for PUpE and PUtE (Fig. 3d).

Among these superior lines for PUE, the group P2, P3, P8, and P9 were also among the 30 % selected at both environments for PH and SD (Fig. 4ab). In addition, these lines presented high values for LL (Fig. 4c). Regarding the traits RS, RL, and RV, which are related to root system development (Fig. 4d–f), lines P2, P4, P7, and P9 stood out belonged the 30 % superior genotypes at both environments. For RL, all genotypes presented similar results at standardP environment, being the only trait in which lines presented higher averages at low P environment when compared with high P environment, showing that P restriction promoted root growth (Fig. 4d). Considering SPC (Fig. 4g), the selection of 30 % of lines with high P content at high P level environment (L51, L53, L61, L63, L70, L71, L74, L75, and L88) did not present a high vegetative performance. However, at low P environment, the most lines with the satisfactory shoot and/or root system growth presented SPC above the average. For RPC, the discrimination of genotypes at low P environment was not possible ($h^2 \approx 0$) (Fig. 4f). In contrast, at standardP environment, in the group of

30 % superior genotypes, the lines L51, L53, and L74 also presented a high SPC; and the lines P2 and P8 which showed RPC over the average also presented a high vegetative growth. When assessing the dispersion between dry matter production with P content, line L75 presented a genotypic value above the average, except for RPC at low P, which presented a null h^2. In other words, L75 produced above-average dry matter in all cases and stood out by the high P concentration in the tissues.

Genetic diversity measured by SSR–EST markers distinguish groups for potential crosses and inheritance studies

By means of SSR–EST markers, the investigation of genetic diversity was carried out aiming at guiding the allocation of crosses between lines (Fig. 5). Based on the results of PUE indices (Fig. 3,4) and traits (Fig. 4), a group with four lines (P2, P4, P7, and P9) that stood out was detached as a superior group, in addition to a second group with lines that presented undesirable results for the assessed traits, which come from an open pollinated population denominated BRS Angela (represented by the red color). Thus, crosses between these two groups can be used to inheritance study.

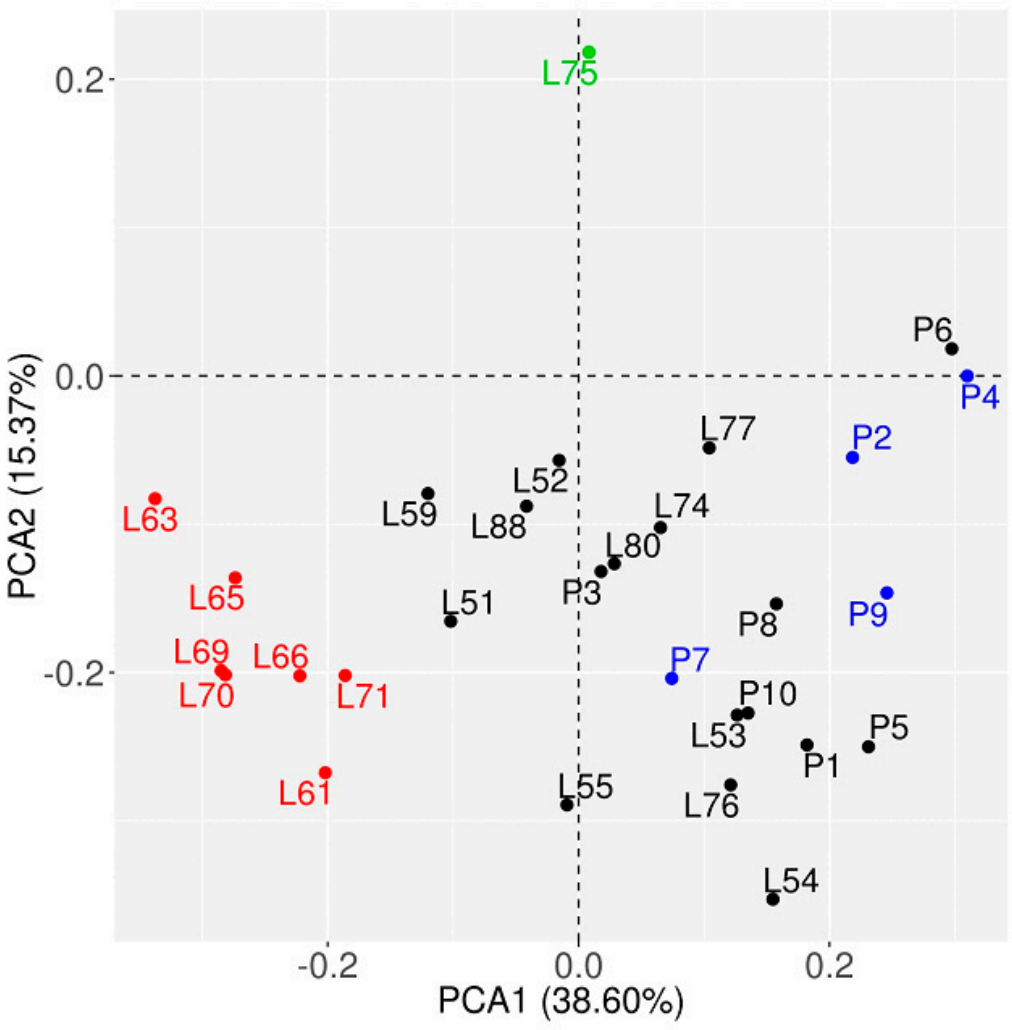

Figure 5. Principal Component Analysis (PCA) based on distance by the Gower algorithm among 29 popcorn lines using 15 SSR–EST primers.

Finally, the line L75 was detached, which was genetically divergent from the others, presenting predicted genotypic values above the average for all traits related to shoot and root system vegetative development. In addition, only this line presented P content in the tissues (SPC and RPC) above the average at both environments. Because L75 presented genetic merit for the assessed traits and was quite divergent from the highly merited lines (P2, P4, P7, and P9), crosses involving L75 and lines P2, P4, P7, and P9 could be an opportunity to explore a genetic complementarity.

Discussion

Due to the socio-environmental needs for food production aiming the lowest possible impact on nature, breeding programs that consider the selection of genotypes adapted to environments with both lower and higher fertilizer supply are essential. In the second case, from an agronomic point of view, individuals that respond adequately to potential environmental improvements are desirable. In this study, a panel of popcorn lines from the Germplasm Bank of UENF, from different regions, was assessed for traits related to plant adaptation at environments with restricted and unrestricted P supply.

In order to initiate a breeding program to select individuals under certain environmental conditions, it is interesting to estimate the genetic and environmental influence magnitude on the phenotypic variation (Falconer and Mackay, 1996), as well as the accuracy of genetic predictions (Resende and Duarte, 2007). Most of traits and PUE presented greater heritability and accuracy at high P level environment. Traits related to tissue P content (root and shoot) presented the lowest heritability at both P levels, as in the study developed by Van de Wiel et al. (2016) under conditions of P stress, in which heritability was generally low. Furthermore, Fritsche-Neto et al. (2010) and Meirelles et al. (2016) reported that the experimental accuracy at the P-restricted environment was lower for most traits assessed in maize. The analytical measurement of P content in SPC and RPC was carried out by chemical protocols, being more complex the measurement in RPC since it is more susceptible to contamination with P present in soil remnants in the root. Thus, the results confirm that the assessment of popcorn genotypes under stress requires meticulous environmental attention. In the case of P, soil standardization is required and more replications are desirable, especially for complex-to-measure traits such as RPC. In addition, an improvement in the measurement accuracy of PUpE and PUtE, which are indices that combines two traits (dry matter and P content), is essential to reach a high precision measurement.

One of the major breeding problems is the genotype-environment interaction because, with a pronounced interaction effect, the best genotypes at an environment will not be good in another. According to Falconer and Mackay (1996), this interaction occurs whether the genetic correlation is not positive; when negative, the selection at an environment will promote negatively correlated response to another environment. Except for traits that presented $h^2 \approx 0$ (RL, RPC and PUtE_RDM), genotypic, environmental, and phenotypic correlations were estimated among environments and most of traits and PUE presented a high-magnitude genetic correlation. These results are in accordance with the study conducted by Mundim et al. (2013), who observed positive genetic correlations between high and low P environments for traits of shoot and root system development and PUE. Because the assessments at environments are quite complicated when a restriction exclusively in P occurs, the selection may be carried out only at the traditional cultivation environment and thus indirectly selecting the individuals that will stand out at the P-restricted environments.

SPC and PUtE_SDM presented a significant negative genetic correlation between environments ($p<0.05$) and PUpE_SDM yielded statistic non-significant correlation. In contrast with these finds, Mundin et al. (2013) reported strong positive correlation of P uptake in SDM and non-significative genetic correlation between environment for PUtE_SDM. The PUpE_SDM non-significant correlation reported in this study, this is probably due low heritability in high P environment, whereas in the report of Mundim et al. (2013) PUpE showed high h^2 in environment with low and high P available. At high P environment, genotypes with greater vegetative development presented a low P concentration in the shoot. This was probably because P was not restricted, and plants absorbed it according to their needs and in this case plant with more vegetative vigor were the plants with more PUtE. In counterpart, at low P environment, the lines of greater vegetative development were more efficient in acquiring the scarce P and not necessary in the internal utilization of this nutrient.

When considering the correlations between traits, the main traits that measure shoot vegetative growth (including PUE_SDM) presented positive genetic and phenotypic correlations at both environments with traits related to root system development and PUE_RDM. P acquisition is closely related to root system growth (Wang et al., 2010), which is in agreement with other studies in which, at contrasting P environments, genotypes with a better-growth root system show higher shoot growth (Mundim et al., 2013; Neto et al., 2016). Based on these results, when selecting individuals with the best-developed shoot, regardless the environment, the breeder will indirectly select the individuals with a more developed root system. Thus, these results are very important for the applied breeding since the measurement of root traits is difficult because the genotypes need necessarily to be removed from the soil.

At standardP environment, RPC presented statistically null genetic correlations with traits related to development, whereas, at low P environments, RPC presented statistically null genetic variance. This indicates that at high P environment, there are plants with high and low vegetative growth that presented high relative P levels in the roots. In the case of low P environment, the measurement of RPC requires

a greater precision to verify the reliable genetic difference between individuals.

When assessing the genetic correlations between P content in the shoot with traits related to vegetative development, negative correlations were observed at standardP environment and positive correlations at low P environment. These results are closely related to genetic correlations between SPC at low and standardP environments, indicating that the same genes might not act increasing SPC at both environments.

When assessing the components related with PUE, for SDM in high P environment only the PUtE_ SDM contributed for PUE_SDM variation, whereas for RDM in the same environment were found plants superior for both PUtE_RDM and PUpE_RDM, consequently these lines showed the highest PUE_ RDM. Mundim et al. (2013) could find lines that overcame for PUtE_SDM and PUpE_SDM regardless the environment with low or standardP available, however in this study the authors computed these component considering the P extracted in the whole plant, and here since we computed the PUE for SDM and RDM, the PUtE and PUpE considered only the P extracted in the specific tissues (shoot matter or root mater). The finds reported here, suggested that in environment with high P available, the lines uptake the P enough for shoot, then the plants with higher SDM were more efficient in the utilization of P acquired, and in the case of RDM the best lines were more efficient in the utilization and in uptake P.

Moreover, in the standardP environment the genetic correlation of PUE_RDM with PUpE_RDM was positive and strong and the correlation between PUE_RDM and PUtE_RDM was close zero. In assessment of PUE_SDM Mundin et al. (2013) reported that the genetic correlation between PUE and PUpE were much higher than PUE with PUtE. In agreement of these results, in the low P environment, the plants with higher PUE_SDM and PUE_RDM were the plants with more P uptake ability and these plants showed low P utilization ability for SDM, in case of PUtE_RDM the lines provided similar results. Probably these results are reflecting that these lines came from selection of traits related with plant vigor, what indirectly result in plants with more ability of develop root system, and then uptake P more efficiency. These lines never were selected based on physiological traits what would improve the biochemistry mechanism of PUtE. Thus, to improve PUE_SDM, the breeder must understand the biochemistry complex of plants and find the capital physiologic traits for selection plants more efficient in the internal use of nutrients.

For the predicted genetic values, RL was clearly higher at low P environment. Similar results were found by Mundim et al. (2013) indicating that the P-restricted environment promotes a stimulus in root development so that the plants can absorb more this scarce nutrient. However, root system at standardP environments is more voluminous and dense, which is closely related to plant growth in general, in contrast to the results observed by Mundin et al. (2013) who found a higher average of root dry mass at the P-restricted environment. According to Van de Wiel et al (2016), low P environments promote plant roots with diverse architecture, which may be more elongated than dense. It may be seen as a balance of carbon use efficiency so that when combining an increase in density and length of roots, it would lead to a high physiological cost. Therefore, root elongation would be more efficient for plants to explore different soil regions in search of the missing nutrient.

This study also aimed to identify potential individuals for integrating a popcorn breeding line to obtain cultivars that develop well under low P conditions and be responsive to environmental improvement. For this, lines P2, P4, P7, and P9, in addition to presenting a better shoot and root system development at both environments, also showed an above-average P content in the shoot, which reinforces the indication of these materials to be included in intercross programs. In order to implement inheritance studies, a group of lines from BRS Angela population was identified, being phenotypically contrasting to the highest merit lines, in addition to being genetically divergent. Thus, crossing between these materials is indicated for future genetic studies. Finally, the line L75 presented merit for all assessed traits, being relatively divergent in relation to the superior lines and with a high potential for the exploitation of heterosis via allelic complementarity through crosses with the elite lines.

Conclusions

The phosphorus use and efficiency or PUE in low P environments were determinate majority per P uptake. The selection of plant with more shoot vigor in one environment, would provide positive response selection for shoot development traits in the other environment and for root vigor in both environment. This study highlighted a group of superior lines (P2, P4, P7, and P9), also suggested crosses to heritance study and to explore complementarity.

References

AMARAL JÚNIOR, A.T.; GONÇALVES, L.S.A.; FREITAS JÚNIOR, S.P.; CANDIDO, L.S.; VITTORAZZI, C.; PENA, G.F.; RIBEIRO, R.M.; SILVA, T.R.C.; PEREIRA, M.G.; SCAPIM, C.A.; VIANA, A.P.; CARVALHO, G.F. 2013. CULTIVAR RELEASE UENF 14 : a new popcorn cultivar. **Crop Breeding and Applied Biotechnology**, 13(3):218–220.

BUTLER, D. 2009. **asreml: asreml fits the linear mixed model**. Available from URL. www.vsni.co.uk.

BRIEN, C. 2016. **asremlPlus: Augments the Use of "ASReml-R" in Fitting Mixed Models**. Available from URL. https://cran.r-project.org/package=asremlPlus

DAHER, R.F.; PEREIRA, M.G.; TUPINAMBÁ, E.A.; AMARAL JÚNIOR, A.T.; ARAGÃO, W.M.; RIBEIRO, F.E.; OLIVEIRA, L.O.; SAKIYAMA, N.S. 2002. Assessment of coconut tree genetic divergence by compound sample RAPD marker analysis. **Crop Breeding and Applied Biotechnology**, 2(3):431-438.

DOVALE, J.C. AND FRITSCHE-NETO, R. 2013. Genetic control of traits associated with phosphorus use efficiency in maize by REML/BLUP. **Revista Ciência Agronômica**, 44:554–563.

DOYLE, J.J. AND DOYLE, J.L. 1990. Isolation of plant DNA from fresh tissue. **Focus**, 12:13-15.

FALCONER, D.S. AND MACKAY, T.F.C. 1996. **Introduction to quantitative genetics**. 4th ed. Longman, Harlow, 123p.

FRITSCHE-NETO, R.; DOVALE, J.C.; LANES, É.C.M.; RESENDE, M.D.V.; MIRANDA, G.V. 2012. Genome-Wide Selection for tropical maize root traits under conditions of nitrogen and phosphorus stress. **Acta Scientiarum.Agronomy**, 34(4):389–395.

FRITSCHE-NETO, R.; MIRANDA, G.V.; DE LIMA, R.O.; SOUZA, L.V.; SILVA, J. 2010. Herança de caracteres associados à eficiência de utilização do fóssforo em milho. **Pesquisa Agropecuária Brasileira**, 45(5):465–471.

GAXIOLA, R.A.; EDWARDS, M.; ELSER, J.J. 2011. A transgenic approach to enhance phosphorus use efficiency in crops as part of a comprehensive strategy for sustainable agriculture. **Chemosphere**, 84(6):840–845

GOOD, A.G.; SHRAWAT, A.K.; MUENCH, D.G. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? **Trends Plant Science**, 9(12):597–605.

GOWER, J.C. 1971. A general coefficient of similarity and some of its properties. **Biometrics**, 27:857-874

GUNES, A.; IANL, A.; ALPASLAN, M.; CAKMAK, I. 2006. Genotypic variation in phosphorus efficiency between wheat cultivars grown under greenhouse and field conditions. **Soil Science and Plant Nutrition**, 52(4):470–478.

GOWER, J.C. 1971. A general coefficient of similarity and some of its properties. **Biometrics**, 27(4):857-874.

MEIRELLES, W.F.; PARENTONI, S.N.; GUIMARÃES, L.J.M.; GUIMARÃES, P.E. DE O.; PACHECO, C.A.P.; DE OLIVEIRA, A.C.; MENDES, F.F.; SCAPIM, C.A. 2016. Análise dialélica de linhagens de milho quanto à responsividade ao fósforo e à sua eficiência de uso. **Pesquisa Agropecuária Brasileira**, 51(1):224–232.

MOLL, R.H.; KAMPRATH, E.J.; JACKSON, W.A. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. **Agronomy Journal**, 74:562–564.

MUNDIM, G.B.; VIANA, J.M.S.; DE LIMA, R.O.; ALMEIDA, C. 2014. Early evaluation of popcorn hybrids for vegetative use efficiency of nitrogen and phosphorus and secondary traits. **Maydica**, 59(3):321-328.

MUNDIM, G.B.; VIANA, J.M.S.; MAIA, C. 2013. Early evaluation of popcorn inbred lines for phosphorus use efficiency. **Plant Breeding**, 132(3):613–619.

NETO, A.P.; FAVARIN, J.L.; HAMMOND, J.P.; TEZOTTO, T.; COUTO, H.T.Z.; BELL, R.W. 2016. Analysis of phosphorus use efficiency traits in coffea genotypes reveals *Coffea arabica* and *Coffea canephora* have contrasting phosphorus uptake and utilization efficiencies. **Frontiers in Plant Science**, 7:408.

OCHOA, I.E.; BLAIR, M.W.; LYNCH, J.P. 2006. QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. **Crop Science**, 46:1609–1621.

PETERS, J.B. 2005. **Wisconsin Procedures for Soil Testing, Plant Analysis and Feed & Forage Analysis: Plant Analysis**. Soil Science Department, College of Agriculture and Life Sciences, University of Wisconsin-Extension, Madison, WI. Available from URL. http://uwlab.soils.wisc.edu/files/procedures/plant_icp.pdf

R CORE TEAM. 2016. **R: A Language and Environment for Statistical Computing**. Available from URL. http://www.r-project.org/.

RAMAEKERS, L.; REMANS, R.; RAO, I.M.; BLAIR, M.W.; VANDERLEYDEN, J. 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. **Field Crops Research**, 117(2–3):169–176.

RESENDE, M.D.V. AND DUARTE, J.B. 2007. Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical**, 37(3):182–194.

SAWAZAKI, E. 2001. A cultura do milho pipoca no Brasil. **O Agronômico**. 53(2):11-13.

SHAROPOVA, N.; MCMULLEN, M.D.; SCHULTZ, L.; SCHROEDER, S.; SANCHEZ-VILLEDA, H.; GARDINER, J.; BERGSTROM, D.; HOUCHINS, K.; MELIA-HANCOCK, S.; MUSKET, T.; DURU, N.; POLACCO, M.; EDWARDS, K.; RUFF, T.; REGISTER, J.C.; BROUWER, C.; THOMPSON, R.; VELASCO, R.; CHIN, E.; LEE, M.; WOODMAN-CLIKEMAN, W.; LONG M.J.; LISCUM, E.; CONE, K.; DAVIS, G.; COE JR, E.H. 2002. Development and mapping of SSR markers for maize. **Plant Molecular Biology**, 48(5–6):463-81.

VAN DE WIEL, C.M.; VAN DER LINDEN, C.G.; SCHOLTEN, O.E. 2016. Improving phosphorus use efficiency in agriculture: opportunities for breeding. **Euphytica**, 207(1):1–22.

WANG, X.; SHEN, J.; LIAO, H. 2010. Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? **Plant Science**, 179(4):302–306.

WISSUWA, M. AND AE, N. 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. **Plant Breeding**, 120(1):43–48.