



## Phosphorus uptake and use efficiency of different cotton cultivars in savannah soil (Acrisol)

Nericlenes Chaves Marcante<sup>1\*</sup>, Takashi Muroaka<sup>1</sup>, Isabeli Pereira Bruno<sup>2</sup> and Marcos Antonio Camacho<sup>3</sup>

<sup>1</sup>Universidade de São Paulo, Avenida Centenário, 303, 13400-970, Piracicaba, São Paulo, Brazil. <sup>2</sup>Instituto de Agronomia do Paraná, Londrina, Paraná, Brazil. <sup>3</sup>Universidade Estadual do Mato Grosso do Sul, Aquidauana, Mato Grosso do Sul, Brazil. \*Author for correspondence. E-mail: marcante1987@gmail.com

**ABSTRACT.** Low soil phosphorus (P) is a limiting factor for plant growth in the Brazilian savannah, where P diffuses slowly and has a high fixation rate in soil (as Al-P and Fe-P). In this study, we investigated the variation in P uptake by different cotton cultivars grown in savannah soil. We conducted a greenhouse experiment using a fully factorial 2 x 17 randomized design with two P treatments (low P = 20 mg kg<sup>-1</sup> and high P = 120 mg kg<sup>-1</sup>) and 17 cotton cultivars. The plants were potted in Acrisol soil labeled with radioisotope <sup>32</sup>P. There was genetic variation in the P use efficiency among the cotton cultivars. The P treatments significantly influenced the dry weight of shoots (DWS), P accumulation, the specific activity of <sup>32</sup>P, and the L-value (both the standardized and L-values discounted the P present in the cottonseed). Hierarchical clustering analysis classified the cotton cultivars into distinct, internally homogeneous clusters. Our results suggest that cotton cultivars could be selected to maximize P absorption efficiency in conditions of low plant-available P. The cultivars FMT 523, FM 910 and CNPA GO 2043 were the most responsive to P fertilization, while Barbadense 01, FM 966LL, IPR Jataí, BRS Aroeira and BRS Buriti were most efficient at absorbing plant-available P.

**Keywords:** <sup>32</sup>P activity, *G. barbadense*, *G. hirsutum*, Brazilian Cerrado.

## Eficiência de absorção e utilização de fósforo por cultivares de algodão em um solo de Cerrado (Latosolo Vermelho Amarelo)

**RESUMO.** A baixa disponibilidade de fósforo (P) é um fator limitante para o crescimento de plantas no Cerrado Brasileiro, onde a difusão do P é baixa e o solo tem grande capacidade de fixação do P por óxidos de ferro e alumínio. Nesse estudo foi investigado a variação da absorção de P por diferentes cultivares de algodão cultivados em solo proveniente da região do Cerrado. Foi conduzido um experimento de casa de vegetação em delineamento inteiramente casualizado em esquema fatorial 2 x 17, dois tratamentos com P (baixo P = 20 mg kg<sup>-1</sup> e alto P = 120 mg kg<sup>-1</sup>) e 17 cultivares de algodão. As plantas foram cultivadas em vasos com um solo marcado com o radioisótopo <sup>32</sup>P. Houve variação genética para eficiência uso de P entre cultivares de algodão. O tratamentos com P influenciaram significativamente a matéria seca da parte aérea, acúmulo de P, atividade específica do <sup>32</sup>P, valor L e valor L descontando o P proveniente das sementes. A análise multivariada classificou os cultivares de algodão em grupos distintos e homogêneos internamente. Os resultados desse trabalho sugerem que pode-se selecionar cultivares de algodão mais eficientes em absorver e utilizar P em solos com baixa disponibilidade de P para as plantas. Os cultivares FMT 523, FM 910 e CNPA GO 2043 foram os mais responsivos a adubação fosfatada, enquanto que os cultivares Barbadense 01, FM 966LL, IPR Jataí, BRS Aroeira e BRS Buriti foram os mais eficientes na utilização de P.

**Palavras-chave:** atividade do radioisótopo <sup>32</sup>P, *G. barbadense*, *G. hirsutum*, Cerrado.

### Introduction

Phosphorus (P) availability is a major constraint on plant growth in many terrestrial ecosystems, especially in the tropics and subtropics (Vance, Uhde-Stone, & Allan, 2003). Limited P availability inhibits the growth of many crops, such as soybean (*Glycine max* L.), corn (*Zea mays* L.), wheat (*Triticum* spp. L.), bean (*Phaseolus vulgaris* L.), rice (*Oryza saliva*

L.) and cotton (*Gossypium hirsutum* L.). Low P availability can trigger various physiological adaptive responses, such as increased root surface area and root hair density, as well as biochemical responses, such as the exudation of protons and organic acids that alter the chemistry of the rhizosphere, allowing the uptake of previously unavailable P (George, Turner, Gregory, Cade-Menun, & Richardson, 2006). The variability in these responses indicates

that developing cultivars that have a greater capacity to grow in soils with low P availability could offer an alternative to P fertilizers (Zhang et al., 2012).

Research on phosphorus efficiency has been conducted in a variety of crop species, including soybean (Wang, Guppy, Watson, Sale, & Tang, 2011), corn (Fageria & Baligar, 1997), common bean (Liao et al., 2004), rice (Fageria, Baeta, & Alexandre, 2011), and wheat (Ma et al., 2011). However, very little is known about cotton P use efficiency. P efficiency studies require two levels of P availability to identify efficient and inefficient cultivars (Fageria & Baligar, 1997; Ahmad, Hans-peter, Johann, Josef, & Walter, 2014). Although previous research has found a strong correlation between cotton P uptake and the proportion of P bonded to iron and aluminum oxides (Dohary, Rochester, & Blair, 2004; Wang, Tang, Guppy, & Sale, 2008) as well as a weak correlation between cotton P uptake and P fertilization in alkaline soils (Bronson et al., 2001), further work is needed to better understand and evaluate the P use efficiency of cotton plants.

Cotton is primarily cultivated in China, India, USA, Pakistan and Brazil, but it is a crucial global resource for textile manufacturing, accounting for 50% of all fiber used in this industry. In Brazil, cotton fiber production is concentrated in large savannah habitats (Cerrado), which commonly have acidic soils with low P availability. P deficiency in cotton crops causes slow shoot development, dark green leaves, flower bud necrosis, and the yellowing of older leaves. Low P availability may also directly affect flower bud development, and decrease the absorption of N and Mg, as a result of inhibited cell growth (Dohary et al., 2004). The development of P-efficient cotton cultivars coupled with good management practices could result in a decrease in the use of phosphate fertilizer, thereby improving the sustainability of cotton production.

Several legume species are able to access sparingly available P. Among these, white lupin (*Lupinus albus* L.) is considered to be the standard P-efficient plant. White lupin has some traits that have made it ideal for growth in soils where P availability is low, such as the formation of cluster roots, and the exudation of citrate and malate (phosphate mobilizing substances) (Dissanayaka, Maruyama, Masuda, & Wasaki, 2015). These traits may partially account for the use of white lupin as a control, when evaluating the P efficiency of other crops in soil with low available phosphorus (Hocking, Keerthisinghe, Smith, & Randall, 1991; Lambers, Clements, & Nelson, 2013).

In the present study, a set of 15 herbaceous and 2 wild cotton cultivars was used to evaluate the extent

of genetic variability in P use efficiency and provide insight into the selection and breeding of cultivars with enhanced P use efficiency.

## Material and methods

### Plant materials

Fifteen herbaceous cotton cultivars (*Gossypium hirsutum* L.), two wild cotton cultivars (*Gossypium barbadense* L.) and one white lupin cultivar (*Lupinus albus* L.) were grown in pots in a greenhouse experiment. We used white lupin as a control to evaluate the P efficiency of the cotton cultivars. The 15 agricultural cotton were: BRS – Aroeira; BRS – Buriti; CNPA – GO 2043; IPR – Jataí; IPR – 05-513; IPR – 140; FMT – 523; FMT – 701; FM – 910; FM – 966<sub>LL</sub>; FB – 0403; IAC – 25; NuOpal®; Sicala – 40; and the 2 wild cottons were: Barbadosense – 01 and Barbadosense – 02.

### Soil properties

The topsoil (0 to 20 cm) was collected from a typical savannah biome in the city of Aquidauana, Mato Grosso do Sul State, Brazil - (20° 28' S and 55° 40' W). The soil meets the classification requirements of Acrisol, according to the World Reference Base for Soil Resources, or Argissolo Vermelho-Amarelo Litólico, according the Brazilian Soil Classification System (Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2013). This soil type was selected because it best represents the weathered soils of the savannah biome (Cerrado), where there are subsurface soil layers that contain clay particles and abundant hydrous oxides of aluminum and iron.

The collected soil was air-dried, sieved with a 2 mm mesh, and homogenized. The physical (Camargo, Moniz, Jorge, & Valadares, 1986) and chemical (Raj, Andrade, Cantarella, & Quaggio, 2001) characteristics of the soil were as follows: clay, silt, and sand content was 225, 62, and 713 g kg<sup>-1</sup>, respectively; the pH (CaCl<sub>2</sub>) was 4.2; resin extractable P was 3.0 mg dm<sup>-3</sup>; organic matter was 11.0 g kg<sup>-1</sup>; S was 5.0 mg kg<sup>-1</sup>; and K, Ca, Mg and H+Al were 2.7, 15.0, 10.0 and 34.0 mmol<sub>c</sub> kg<sup>-1</sup>, respectively. The soil base saturation was raised to 70% by applying lime with 95% relative total power of neutralization (27.50% CaO and 18.5% MgO).

### Labelling soil with <sup>32</sup>P

The P availability was assessed with an isotopic-dilution technique based on the specific activity (Sa) of <sup>32</sup>P (DMP μg P<sup>-1</sup>) and the L-value method (Larsen, 1952). The air-dried soil (3 kg per pot) was uniformly labeled with <sup>32</sup>P by adding 250 ml of

radioisotope  $^{32}\text{P}$  solution (6.9 KBq pot<sup>-1</sup>), free of P-charger, to each pot. After the solution was applied, the soil was incubated for 15 days until isotopic equilibrium between the  $^{32}\text{P}$  and  $^{31}\text{P}$  was reached.

### Experimental design

This experiment had a fully factorial, randomized design (17 cultivars x 2 P treatments X 4 replications). The two P treatments were the following: (1) low P (20 mg kg<sup>-1</sup>) and (2) high P (120 mg kg<sup>-1</sup>). The phosphorus source was triple superphosphate, which was readily available to plants.

### Growth conditions

The experiment was conducted in a greenhouse, with maximum and minimum temperatures of 35 and 22°C, respectively. Plants were grown in plastic pots containing 3 kg of sieved soil; five seeds were sown per pot. At the seedling stage, N and K were applied (100 mg kg<sup>-1</sup> of N as ammonium sulfate, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and 200 mg kg<sup>-1</sup> K as potassium sulfate, K<sub>2</sub>SO<sub>4</sub>). Micronutrients were also applied in nutrient solutions (0.5 mg kg<sup>-1</sup> B as boric acid, 1.5 mg kg<sup>-1</sup> Cu as copper sulfate, 1.5 mg kg<sup>-1</sup> Zn as zinc sulfate, and 0.1 mg kg<sup>-1</sup> Mo as ammonium molybdate). At 7 days post-emergence, plants were thinned to two plants per pot. Then, at 15 days post-emergence, 100 mg kg<sup>-1</sup> of N as ammonium sulfate was applied. The plants were irrigated and the soil moisture was monitored and maintained at approximately 70% of the maximum water retention.

### Harvest and analysis of plant material

At 40 days post-emergence, shoots were harvested, washed, dried at 70°C for 72h, and weighed to determine the dry weight of shoots (DWS). Dried shoots were subsequently milled and sieved through a 0.30 mm screen. Sub-samples of the ground tissue (500 mg) were used to determine P concentration in shoots ([P]) via nitric-perchloric acid digestion. The nutrient content of the tissue was determined with the metavanadate colorimetric method, as described by Malavolta, Vitti and Oliveira (1996). The Activity of  $^{32}\text{P}$  in the extract of nitric-perchloric acid digestion was determined using a liquid scintillation counter (Vose, 1980).

The data were used to calculate the specific  $^{32}\text{P}$  activity (Sa) of each plant (DPM μg P<sup>-1</sup>), according equation 1:

$$\text{Sa} = \frac{{}^{32}\text{P Shoot}}{{}^{31}\text{P Shoot}} \quad (1)$$

Isotopically exchangeable P values (L-values) were calculated from the following equation 2:

$$L - \text{value} = \frac{\text{Sa applied}}{\text{Sa shoots}} - 1 \quad (2)$$

Phosphorus uptake (PU) mg pot<sup>-1</sup> was calculated as equation 3:

$$\text{PU} = [\text{P}] \times \text{DWS} \quad (3)$$

Before beginning the greenhouse experiment, 10 seeds of each cotton cultivar and white lupin were analyzed for P content. The P content in the seeds was subtracted from the total content of P in plants, to standardize the measurement of P uptake from the soil (L<sub>seed</sub> value mg kg<sup>-1</sup>), according equation 4.

$$\text{Lseed} = \frac{Y[\text{XT} - \text{Z}]}{\text{YT} - \text{X}} \quad (4)$$

where:

Lseed is the L-value, discounting the P from the seed (P mg kg<sup>-1</sup>); Y is the  $^{32}\text{P}$  activity in the solution applied per pot (DPM); XT is the total P activity in the plant (mg); YT is the  $^{32}\text{P}$  activity in the plant (DPM); Z is the total P from the seed (mg); and X is the dose of  $^{31}\text{P}$  charger applied per pot (mg).

### Data analysis

The data were analyzed using analysis of variance (ANOVA), Pearson correlation, and hierarchical clustering in SAS®, 'Statistical Analysis System'. Comparisons were made between the means (T-student) of the yield, DWS, [P], PU, Sa, L-value, Lseed, and the value for both levels of P.

The hierarchical cluster analysis was used to verify similarities among the cotton cultivars in DWS, [P], PU, Sa, L-value and L<sub>seed</sub>; all variables were weighted equally. Prior to the hierarchical cluster analysis, the data were standardized before calculating the Euclidean distances because the variables had different scales. Thus, the averages for each variable for each cultivar were divided by the same standard deviations, so that all variables were given the same weight in the cluster analysis. After normalization, all means were equal to zero, and variances were equal to one (Manly, 2008). The results were presented as dendrograms; the cluster classification of cotton cultivars in dendrograms was based on achieving greater homogeneity within each cluster and greater heterogeneity between different clusters, based on the average values of the variables in each cluster and the Euclidean distances between cotton cultivars.

## Results and discussion

The white lupin data are presented separately from those of the cotton cultivars, as the white lupin data would affect the analysis of variance and hierarchical cluster analysis, and would thus prevent the classification of cotton cultivars into distinct, internally homogeneous clusters. The mean values for DWS, [P] and PU, Sa, L-value and  $L_{seed}$  in the high and low P levels for white lupin are presented in Table 1. The mean values for DWS, [P] in the high and low P levels for cotton cultivars are presented in Table 2.

**Table 1.** Mean values of DWS, [P], PU, Sa, L,  $L_{seed}$  of white lupin cultivated in high P (120 mg kg<sup>-1</sup>) and low P (20 mg kg<sup>-1</sup>) conditions.

	120 mg kg <sup>-1</sup>					
	DWS <sup>a</sup>	[P] <sup>b</sup>	PU <sup>c</sup>	Sa <sup>d</sup>	L <sup>e</sup>	$L_{seed}$ <sup>f</sup>
White Lupine	g	g kg <sup>-1</sup>	mg pot <sup>-1</sup>	DPM μg P <sup>-1</sup>	mg kg <sup>-1</sup> soil	
	1.1	5.8	3.8	3.7	1236.2	967.1
	20 mg kg <sup>-1</sup>					
White Lupine	1.3	3.8	3.4	1.1	3298.0	1627.0

<sup>a</sup>DWS represent dry weight of shoots; <sup>b</sup>[P] represent concentration of phosphorus; <sup>c</sup>PU represent phosphorus uptake; <sup>d</sup>Sa represent specific activity; <sup>e</sup>L represent L-value; <sup>f</sup> $L_{seed}$  represent L-value discounting the P in the plant from the seed.

**Table 2.** Mean values of DWS, [P] and PU of cotton cultivated in high P (120 mg kg<sup>-1</sup>) and low P (20 mg kg<sup>-1</sup>) conditions.

Cultivar	120 mg kg <sup>-1</sup>			20 mg kg <sup>-1</sup>		
	DWS <sup>a</sup>	[P] <sup>b</sup>	PU <sup>c</sup>	DWS <sup>a</sup>	[P] <sup>b</sup>	PU <sup>c</sup>
	g	g kg <sup>-1</sup>	mg pot <sup>-1</sup>	g	g kg <sup>-1</sup>	mg pot <sup>-1</sup>
CNPA GO 2043	14.2 a	5.0 ab	72.7 a	9.8 a	3.7 cb	36.3 a
BRS Aroceira	13.0 a	4.7 a-c	59.0 ab	10.4 a	3.3 bc	36.0 a
IAPAR 40	12.9 a	4.3 b-d	54.7 bc	9.5 ab	3.7 cb	35.0 ab
FM 993	10.6 b	3.6 d	38.3 de	6.6 c-e	4.0 ab	26.0 c-f
FM 910	10.5 b	4.7 a-d	47.0 b-d	6.8 c-e	4.8 a	32.3 a-e
IAC 25	10.5 b	4.2 b-d	43.7 c-e	8.0 bc	4.3 ab	34.0 a-c
FM 966 LL	10.0 b	4.0 cd	36.7 de	6.1 e	4.3 ab	25.3 d-f
BRS Buriti	10.0 b	4.3 b-d	41.0 c-e	6.9 c-e	3.7 bc	24.3 ef
IPR 05-513	10.0 bc	3.7 cd	39.0 de	6.9 c-e	3.7 bc	24.7 ef
IPR Jataí	9.9 bc	4.3 b-d	41.7 c-e	6.0 e	5.0 a	28.3 a-f
Barbadense 1	9.6 bc	4.7 a-d	41.3 c-e	8.9 b-d	3.0 bc	28.7 d-f
FMT 523	9.5 bc	4.7 a	48.7 bc	6.3 d-e	4.0 bc	23.7 f
FMT 701	8.9 bc	4.0 b-d	39.3 de	7.0 c-e	3.7 bc	26.0 c-f
Barbadense 2	8.8 bc	4.7 ab	42.3 c-e	7.3 c-e	3.3 c	23.0 f
Sicala 40	8.5 bc	5.0 ab	42.7 c-e	7.0 c-e	5.0 ab	33.0 a-d
FB 0403	8.3 cd	5.0 ab	41.7 c-e	6.4 c-e	4.3 ab	27.3 b-f
NuOpal	7.1 d	4.7 a-d	32.0 e	6.9 c-e	4.3 ab	29.0 a-f
Mean	10.1	4.5	45.0	7.4	4.0	30.0
F	6.4**	2.4*	3.6**	4.8***	2.0*	2.6**
LSD	2.1	0.9	14.6	1.7	0.9	8.2
CV (%)	12	15	19	12	15	17

<sup>a</sup>DWS represent dry weight of shoots; <sup>b</sup>[P] represent concentration of phosphorus; <sup>c</sup>PU represent phosphorus uptake; <sup>d</sup>\*, \*\*, \*\*\* Significant levels of probability of 5, 1 and 0.1%, respectively, from T-test.

Significant variation was observed for [P] and PU within the cotton cultivars and white lupin (Tables 1 and 2) both two P levels. The DWS of all cotton cultivars in the low P treatment was significantly reduced (> 50%) because of the low plant-available P in the Acrisol (Table 2). However, there was no influence of low P availability on the DWS of white lupin (Table 1). Low P conditions resulted in a small reduction in the [P] of cotton

cultivars (Table 2), but for white lupin the reduction was significant (35%) (Table 1). The cotton cultivars differed significantly in PU between high and low P treatments (Table 2), while no difference was observed between treatments in white lupin (Table 1). In white lupin, the L-value and  $L_{seed}$  varied considerably between high and low P treatments (Table 1).

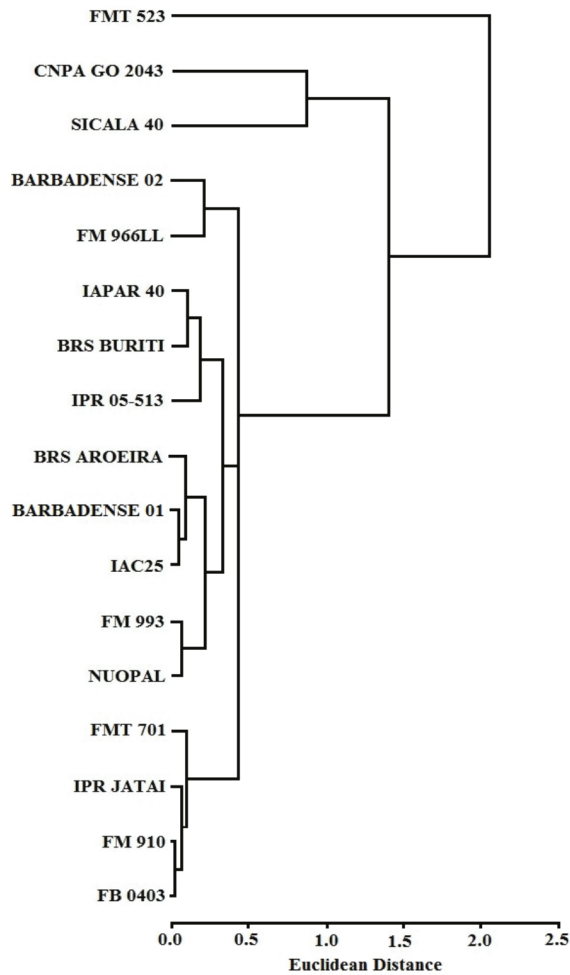
The correlation coefficients (correlation matrix) between DWS, [P], PU, Sa, L-value, and  $L_{seed}$  in the two P treatments are shown in Table 3. In addition to the significance of the correlations (Table 3), we also considered their practical importance when drawing the dendrograms. Therefore, the results were presented in two dendrograms: the first based on the DWS, [P], and PU; and the second based on the Sa, L-value and  $L_{seed}$  for both P treatments. The correlation between the DWS and the PU was 0.86\*\*\* and 0.73\*\*\* in the high P and low P treatments, respectively, and the correlation between the Sa and the L-value was -0.73\*\*\* and 0.90\*\*\* in the high P and low P treatments, respectively (Table 3).

**Table 3.** Correlation coefficients between the DWS, [P], PU, Sa, L and  $L_{seed}$  of cotton cultivars grown in high P (120 mg kg<sup>-1</sup>) and low P (20 mg kg<sup>-1</sup>) conditions.

		120 mg kg <sup>-1</sup>				
		[P] <sup>b</sup>	PU <sup>c</sup>	Sa <sup>d</sup>	L-value <sup>e</sup>	$L_{seed}$ <sup>f</sup>
		g kg <sup>-1</sup>	mg pot <sup>-1</sup>	DMP μg P <sup>-1</sup>	mg kg <sup>-1</sup> soil	mg kg <sup>-1</sup> soil
DWS <sup>a</sup>	g pot <sup>-1</sup>	-0.009 <sup>ns</sup>	0.86***	0.08 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.28 <sup>ns</sup>
[P] <sup>b</sup>	g kg <sup>-1</sup>		0.47 <sup>ns</sup>	0.054 <sup>ns</sup>	0.29 <sup>ns</sup>	0.34 <sup>ns</sup>
PU <sup>c</sup>	mg pot <sup>-1</sup>			0.18 <sup>ns</sup>	0.10 <sup>ns</sup>	-0.06 <sup>ns</sup>
Sa <sup>d</sup>	DMP μg P <sup>-1</sup>				-0.73***	-0.79***
L-value <sup>e</sup>	mg kg <sup>-1</sup> solo					0.76***
		20 mg kg <sup>-1</sup>				
DWS <sup>a</sup>	g pot <sup>-1</sup>	-0.49*	0.73***	0.67***	-0.50*	-0.46 <sup>ns</sup>
[P] <sup>b</sup>	g kg <sup>-1</sup>		0.24 <sup>ns</sup>	-0.56 <sup>ns</sup>	0.35 <sup>ns</sup>	0.25 <sup>ns</sup>
PU <sup>c</sup>	mg pot <sup>-1</sup>			0.28 <sup>ns</sup>	-0.24 <sup>ns</sup>	-0.28 <sup>ns</sup>
Sa <sup>d</sup>	DMP μg P <sup>-1</sup>				-0.90***	-0.85***
L-value <sup>e</sup>	mg kg <sup>-1</sup> soil					0.95***

<sup>a</sup>DWS represent dry weight of shoots; <sup>b</sup>[P] represent concentration of phosphorus; <sup>c</sup>PU represent phosphorus uptake; <sup>d</sup>Sa represent specific activity; <sup>e</sup>L-value represent L value; <sup>f</sup> $L_{seed}$  represent L value discounting the P in the plant from the seed; <sup>ns</sup>, \*, \*\*, \*\*\* not significant, significant at probability 0.1 and 5%, respectively.

Under high P conditions, the hierarchical cluster analysis divided the cotton cultivars into four clusters, based on their responsiveness to P fertilization, as measured by their DWS, [P], and PU (the cutoff was 1 Euclidean distance): (1) highly responsive (high DWS, [P] and PU), (2) responsive (higher DWS and moderate PU), (3) poorly responsive (low DWS), and (4) low responsiveness (low DWS, [P] and PU). The clusters were comprised by the following cotton cultivars (Figure 1): (1) FMT 523; (2) CNPA GO 2043; (3) Sicala 40; and (4) Barbadense 02, FM 966LL, IAPAR 40, BRS Buriti, IPR 05-513, BRS Aroceira, Barbadense 01, IAC 25, FM 993, NUOPAL, FMT 701, IPR Jataí, FM 910, and FB 0403 (Figure 1).

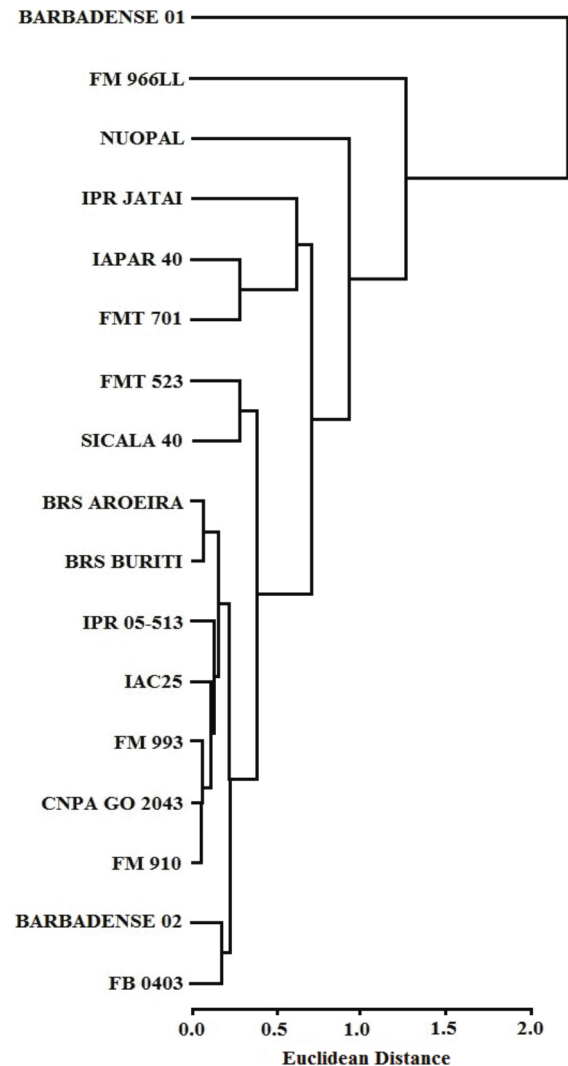


**Figure 1.** Dendrogram resulting from the hierarchical cluster analysis of cotton cultivars based on the dry weight of shoots (DWS), concentration of phosphorus ([P]) and the phosphorus uptake (PU) in high P (120 mg kg<sup>-1</sup>) conditions.

Under low P conditions, the DWS was correlated with [P] (-0.49<sup>\*</sup>) and PU (0.73<sup>\*\*\*</sup>) (Table 3). Hierarchical cluster analysis divided the cotton cultivars into three clusters, according to their efficient absorption of low plant-available P, as measured by their DWS, [P], and PU (the cutoff was 1.5 Euclidean distance): (1) highly efficient (high DWS, [P] and PU); (2) efficient (high DWS, moderate [P] and PU), and (3) low efficiency (low DWS, [P] and PU). These clusters comprised the following cotton cultivars (Figure 2): (1) Barbadense 01; (2) FM 966LL and (3) NuOpal, IPR Jataí, IAPAR 40, FMT 701, FMT 523, Sicala 40, BRS Aroeira, BRS Buriti, IPR 05-513, IAC 25, FM 993, CNPA GO 2043, FM 910, Barbadense 02, and FB 0403.

Table 4 shows the mean values of the Sa, L-value and L<sub>seed</sub> of cotton cultivars in both P treatments. There were no significant differences in Sa values

among cotton cultivars grown in the high P treatment. Conversely, cotton cultivars grown in the low P treatment had a significant decrease in Sa and increase in the L-value and L<sub>seed</sub> (p > 0.01%) (Table 4).



**Figure 2.** Dendrogram resulting from the hierarchical cluster analysis of cotton cultivars based on the dry weight of shoots (DWS), concentration of phosphorus ([P]) and phosphorus uptake (PU) in low P (20 mg kg<sup>-1</sup>) conditions.

In high P conditions, the cotton cultivar Sa values were strongly correlated with the L-values and L<sub>seed</sub>, - 0.73<sup>\*\*\*</sup> and - 0.79<sup>\*\*\*</sup>, respectively, and the L-values were strongly correlated with L<sub>seed</sub> value (0.76<sup>\*\*\*</sup>) (Table 3). Hierarchical analysis divided the cotton cultivars into four clusters (Figure 3), according to their P absorption efficiency: (1) highly efficient (low Sa, high L-value and L<sub>seed</sub>); (2) efficient (low Sa, moderate to high L-value and L<sub>seed</sub>); (3) moderately efficient (low Sa, moderate L-value and L<sub>seed</sub>); and (4) low efficiency (high Sa, low

L and  $L_{\text{seed}}$  values). These groups were comprised by the following cotton cultivars (the cutoff was 1 Euclidean distance): (1) FM 910; (2) CNPA GO 2043; (3) IPR Jataí; and (4) FM 966LL, BRS Buriti, IAC 25, Nuopal, FM 993, FMT 701, Barbadense 02, FMT 523, Sicala 40, IPR 05-513, BRS Aroeira, IAPAR 40, Barbadense 01, and FB 0403.

**Table 4.** Mean values of Sa, L and  $L_{\text{seed}}$  of cotton cultivars grown in the high P (120 mg kg<sup>-1</sup>) and low P (20 mg kg<sup>-1</sup>) conditions.

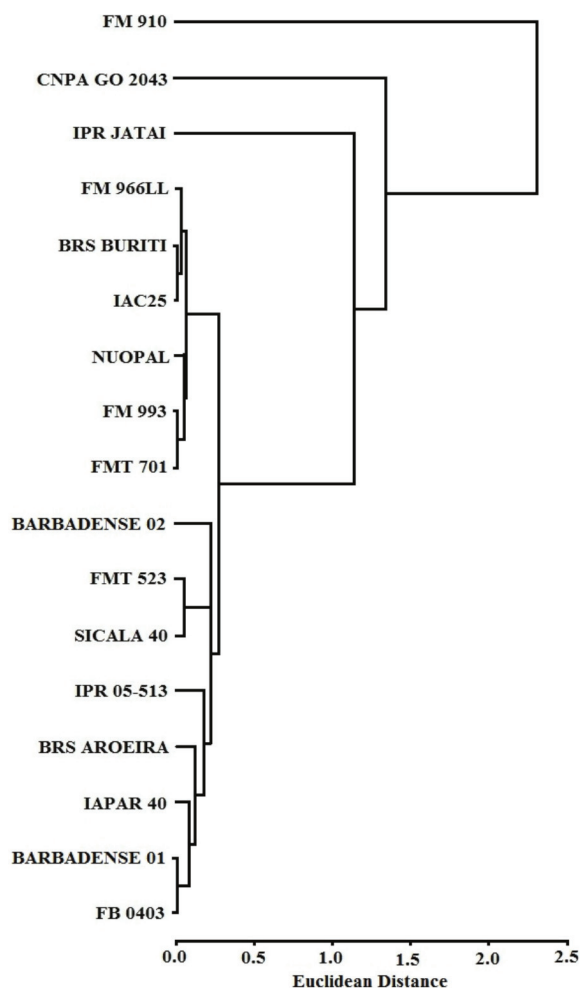
Cultivar	120 mg kg <sup>-1</sup>			20 mg kg <sup>-1</sup>		
	Sa <sup>a</sup> DPM μg P <sup>-1</sup>	L <sup>b</sup> mg kg <sup>-1</sup> soil	$L_{\text{seed}}$ <sup>c</sup> mg kg <sup>-1</sup> soil	Sa <sup>a</sup> DPM μg P <sup>-1</sup>	L-value <sup>b</sup> mg kg <sup>-1</sup> soil	$L_{\text{seed}}$ <sup>c</sup> mg kg <sup>-1</sup> soil
FM 966LL	8.9	342.2	170.6	4.0 hi	414.3 c-e	257.1 bc
BRS Buriti	8.5	340.1	169.7	7.5 c-f	347.4 cf	173.5 b-e
FMT 701	8.3	327.8	163.6	2.8 i	776.8 a	495.5 a
BRS Aroeira	7.7	340.4	170.0	10.9 ab	236.4 fg	118.0 e
Barbadense 01	7.6	344.7	164.2	10.7 a-d	240.4 fg	97.4 e
IAPAR 40	7.4	350.0	174.7	10.0 a-c	265.1 fg	132.2 c-e
IPR 05-513	7.3	353.3	176.4	9.0 b-e	291.0 e-g	144.9 b-e
IAC 25	7.1	401.3	200.3	6.7 e-g	408 c-e	203.5 b-e
NuOpal	6.8	401.4	267.2	8.7 b-e	301.9 e-g	150.5 b-e
Barbadense 02	6.8	275.5	134.1	12.0 a	219.5 g	109.4 e
FMT 523	6.6	414.9	221.9	5.3 f-i	506.5 b-d	252.3 b-d
FM 993	6.4	423.7	211.6	8.3 b-e	322.9 e-g	160.9 b-e
FB 0403	6.0	438.7	303.3	4.2 hi	532.9 bc	265.9 b
CNPA GO	5.9	433.6	216.5	11.0 ab	243.3 fg	121.5 ed
FM 910	5.4	367.9	319.0	6.9 d-g	331.9 e-g	197.0 b-e
IPR Jataí	5.2	417.9	268.2	6.4 e-g	402.2 de	200.5 b-e
Sicala 40	4.4	516.3	306.7	4.8 g-i	547.0 b	222.7 b-e
Mean	6.8	381.7	235.5	8.5	375.7	194.3
F	0.7 <sup>ns</sup>	1.3 <sup>ns</sup>	1.2 <sup>ns</sup>	8.5 <sup>***</sup>	11.1 <sup>***</sup>	4.2 <sup>**</sup>
LSD	4.1	145.0	140.1	2.7	126.0	131.1
CV (%)	17.8	14.9	36.9	33.8	39.1	48.4

<sup>a</sup>Sa represent specific activity; <sup>b</sup>L represent L value; <sup>c</sup> $L_{\text{seed}}$  represent L value discounting the P in the plant from the seed; <sup>ns</sup>, <sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup> not significant, significant at probability 0.1, 1.0 and 5%, respectively, from T-test.

For cotton cultivars in the low P treatment, Sa was strongly correlated with the L and  $L_{\text{seed}}$  values, - 0.90<sup>\*\*\*</sup> and - 0.85<sup>\*\*\*</sup>, respectively, and the L-value was correlated with  $L_{\text{seed}}$  value (0.95<sup>\*\*\*</sup>) (Table 3). Hierarchical cluster analysis divided the cotton cultivars into five clusters (Figure 4), according to their P absorption efficiency in low P conditions: (1) highly efficient (low Sa, high L-value and  $L_{\text{seed}}$ ), (2) very efficient (low Sa, high L-value and moderate  $L_{\text{seed}}$ ), (3) moderately efficient (low to moderate Sa, high to moderate L-value and  $L_{\text{seed}}$ ), (4) poor efficiency (moderate Sa, L-value and  $L_{\text{seed}}$ ), and (5) low efficiency (high Sa, low L and  $L_{\text{seed}}$  values). These clusters were comprised by the following cotton cultivars (the cutoff was 1 Euclidean distance): (1) IPR Jataí; (2) BRS Aroeira and BRS Buriti; (3) FMT 701; and (4) IAPAR 40, CNPA GO 2043, FMT 523, FM 993, IAC 25, Barbadense 02, IPR 05-513, Nuopal, FM 966LL, Barbadense 01 and FM 910; (5) Sicala 40, and FB 0403.

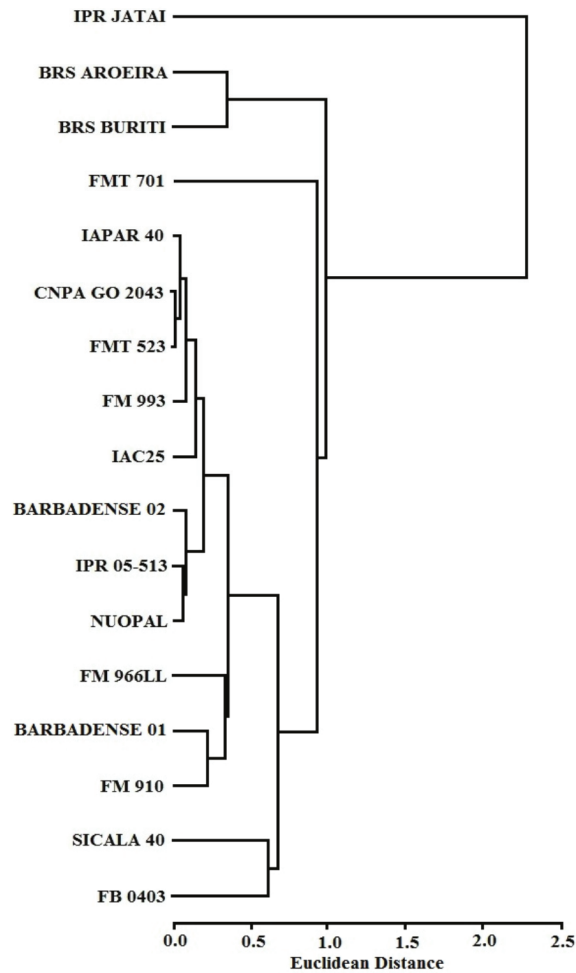
The P uptake efficiency can be determined from the Sa of <sup>32</sup>P present in the plant, i.e. the lower the Sa, the higher the P absorption efficiency of cultivar,

and the higher the Sa is, the lower the P absorption efficiency. If all cotton cultivars and white lupin had identical P uptake efficiencies, they would have the same Sa, and an equal DPM μg<sup>-1</sup> of P (Larsen, 1952; Hocking et al., 1991). However, white lupin had a lower Sa, indicating it was able to access P in the soil that was less available to the other plants. White lupin most likely increased its access to otherwise unavailable soil P by excreting organic acid (citrate) or extruding protons, thereby acidifying the rhizosphere, and allowing it to solubilize P-Al and P-Fe.



**Figure 3.** Dendrogram resulting from hierarchical cluster analysis of cotton cultivars based on specific activity (Sa), L-value (L) and L-value discounting the P present in the cottonseed ( $L_{\text{seed}}$ ) in high P (120 mg kg<sup>-1</sup>) conditions.

Despite differences in the concentration and accumulation of P between white lupin and cotton cultivars, their Sa of <sup>32</sup>P in DWS can nonetheless be compared because the two species were grown under the same conditions and had access to the same P source (Larsen, 1952).



**Figure 4.** Dendrogram resulting from hierarchical cluster analysis of cotton cultivars based on specific activity ( $S_a$ ), L-value (L) and L-value discounting the P present in the cottonseed ( $L_{seed}$ ) in low P ( $20 \text{ mg kg}^{-1}$ ) conditions.

In this study, white lupin had a lower  $S_a$  in both high and low P treatments, indicating the greater ability of this species to take up P compared to the cotton cultivars (Shane, Lambers, Cawthray, Kuhn & Schurr, 2008). Hocking, Keerthisinghe, Smith and Randall (1991) showed that compared to a variety of crop species, white lupin is able to access up to six times more P from low P soils. White lupin has been reported to emit organic compounds into the rhizosphere (such as citrate), which could increase P solubility and availability, and result in increased P absorption (Richardson, Hocking, Simpson & George, 2009; Fageria & Moreira, 2011). Additionally, this species has been shown to develop cluster roots as an adaptation for nutrient acquisition in nutrient-poor soils. Under low P conditions, cluster roots increase the root surface area, thereby releasing larger amounts of P-solubilizing compounds, as well as augmenting the efficiency of solubilized P recovery (Shane et al., 2008). The

anion citrate most likely solubilizes inorganic and organic soil P by several mechanisms, including anion exchange, chelation, and suppressing the reabsorption and precipitation of inorganic P (Hocking et al., 1991).

In a comparison of the P efficiency of corn hybrids using white lupin as a control crop, Fernandes and Muraoka (2002) reported that white lupin had a higher P efficiency than the corn hybrids tested. White lupin had a  $S_a$  that was 2.3 times lower than the most efficient hybrid. This supports the findings of the present study, where white lupin was more P efficient in both P treatments, with lower  $S_a$  and higher L-values and  $L_{seed}$  than the cotton cultivars (Table 1). The higher L-value and  $L_{seed}$  in white lupin under low P conditions compared to high P conditions further supports its greater P efficiency, and indicates that this species was accessing a larger pool of available P than the cotton cultivars (Hocking et al., 1991). The L-value is an estimate of the total quantity of plant-available P in the soil and soil solution (Larsen, 1967). The resin extractable P soil test indicated that the soil would be classified as P deficient ( $3.0 \text{ mg dm}^{-3}$ ) for cropping, but it appeared adequate for white lupin.

The results of this study indicate that there is substantial genetic variation for P efficiency in the 17 tested cotton cultivars. The cotton cultivars had significant differences in both growth under low P conditions and their response to P fertilization (high P conditions) (Table 2 and 4). Referring to Figure 1 and the average values shown in Table 2, the cultivars CNPA GO 2043 and FMT 523 were the most responsive to P fertilization, when cultivated in high P conditions. The DWS is considered a good indicator of maximum economic yield and, therefore, can be used as selection criteria in the evaluation of cultivars for nutritional efficiency (Fageria, Baligar, Moreira, & Portes, 2010). According to Wang, Tang, Guppy and Sale (2008), P treatment ( $0 - 20 \text{ mg kg}^{-1}$ ) significantly influenced cotton DWS. The same authors found that cotton grown in high P soil had a 38% greater DWS compared to plants grown in the low P treatment. The higher DWS in low P conditions indicates that DWS is a reliable P efficiency screening metric for plant cultivars (Ahmad, Gill, Qureshi, & Hamud-Ur-Rehman, 2001). The variation in DWS among cotton cultivars (Table 2) indicates a genetic difference in the accumulation of biomass when grown in soil with different amounts of available P. A small decrease in DWS in P-deficient soil can be used to categorize specific cultivars as more efficient (Baligar, Fageria, & He, 2001); high-yielding cultivars that display little change in DWS between high and low P conditions would be considered



suitable for cultivation in areas where the concentration of P is limiting (Ahmad et al., 2001). A study comparing the P efficiency of two genotypes of rapeseed (*Brassica napus*) in low P conditions (5  $\mu$ M), found that the more efficient genotype had an 80.5% greater DWS than the less efficient genotype (Hu, Ye, Shi, Duan, & Xu, 2010). Furthermore, the authors observed that there was greater variation in DWS between the high and low P treatments in the less efficient genotype, confirming that efficient genotypes can be more tolerant to the stress caused by P deficiency.

The variation in PU between cotton cultivars is related to the morphological characteristics of the plants, which have a significant impact on their ability to cope with the stress caused by P deficiency. The exploitation of the soil through the proliferation and extension of a metabolically efficient root system can determine plant fitness in low P conditions (Baligar et al., 2001; Lynch & Ho, 2005). According to Wang et al. (2008) cotton plants grown in field conditions are not able to release organic acids through their roots, and so have not changed the rhizosphere chemistry to increase the immobilization of P. P uptake by cotton plants primarily depends on the extent of the roots in soil layers (Wang et al., 2011). The cotton cultivar CNPA GO 2043 had the greatest PU, but had a 51% reduction in accumulated P under low P conditions. The same trend was observed for most cultivars. It is possible that this reduction is linked to the stress caused by P deficiency, which reduces the rate of cell division in meristematic apex shoots (Akhtar, Oki, & Adachi, 2001), and limits plant growth (Table 2).

An efficient cultivar is one that has the ability to absorb relatively high amounts of soil nutrients, has high biomass production per unit of nutrient absorbed, and has low storage of nutrients in the straw (Fageria & Baeta, 2008). Plant P uptake efficiency may be affected by several factors, such as the formation of proteoid roots observed in P-efficient species (white lupin), the release of organic acids of low molecular weight from the root system, and the acidification of the rhizosphere (Wang et al., 2008). In addition to biochemical adaptations, physiological changes in root architecture also increase the P acquisition efficiency of a root system (Raghothama & Karthikeyan, 2005; Clair & Lynch, 2010). Identifying cotton cultivars with a high nutrient absorption and utilization efficiencies could reduce the amount of P applied in fertilizer inputs, as well as allow cotton to be cultivated in regions with nutrient poor soils (Fageria & Baeta, 2008). In a study using the

radioisotopes  $^{32}\text{P}$  and  $^{33}\text{P}$ , Dohary, Rochester and Blair (2004) found that over 95% of P present in cotton plants came from the soil and only 5% from the fertilizer applied. Thus, it is evident that P uptake by cotton plants in field conditions is based primarily on the extent of the roots in soil.

## Conclusion

Cotton cultivars differ in their P absorption capacity. There is substantial genetic variation in P efficiency between 17 cotton cultivars and this could be exploited in breeding to produce new P-efficient, high-yielding cultivars for P-deficient acid soils. The cultivars FMT 523, FM 910, and CNPA GO 2043 were the most responsive to phosphate fertilizer in areas with a sufficient level of P. The cultivars Barbadense 01, FM 966LL, IPR Jataí, BRS Aroeira, and BRS Buriti were the most efficient in accumulating P when grown in low available P conditions.

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