

Atributos morfológicos y fisiológicos de genotipos de *Brachiaria* en suelo con bajo fósforo disponible y alta saturación de aluminio

Morphological and physiological attributes of genotypes of *Brachiaria* in soil with low available phosphorus and high aluminium saturation.

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REC.: 02-11-07

ACEPT.:14-12-07

FORMA DEFINITIVA: 13-02-09

RESUMEN

Se evaluaron en invernadero con suelo de Matazul, Meta, Colombia durante 6 semanas, 2 genotipos de *Brachiaria* (uno mejor adaptado a bajo P, *B. decumbens* y otro menos adaptado, *B. ruziziensis*) y 8 progenies del cruzamiento entre ellos (mejores adaptados: H-7, H-40, H-28, H-58; menos adaptados: H-190, H-94, H-82, H-179). Los genotipos mejor adaptados presentaron mayor biomasa aérea, explicada por mayor área foliar, mayor absorción de P en el tallo y mayor volumen de raíz. Las características morfológicas de las raíces, principalmente longitud, peso seco y contenido de P, permitieron mejor adaptación a bajo P disponible en el suelo. El periodo de evaluación fue suficiente para que se presentaran diferencias entre progenies.

Palabras clave: *B. decumbens*; *B. ruziziensis*; híbridos; absorción de fósforo.

ABSTRACT

In the present work 2 *Brachiaria* genotypes (*Brachiaria decumbens*, considered as more adapted and *Brachiaria ruziziensis*, considered as less adapted) and 8 progenies from the cross of these two (H-7, H-40, H-28 and H-58 more adapted; H-190, H-94, H-82 y H-179 less adapted) were evaluated for differences in shoot and root attributes when grown for 6 weeks in an acid soil from Matazul, Meta, Colombia. Results indicated that the more adapted genotypes to low P had more shoot biomass, this could be explained by a greater leaf area, more P uptake in the stem and greater root volume. The root attributes: root length; root dry weight; and

root P content contributed to greater adaptation to low P availability in acid soil. Six weeks of plant growth was not adequate to distinguish differences in adaptation to low P between the two parents, but it was adequate time to identify superior progenies from the crosses.

Keywords: *B. decumbens*; *B. ruziziensis*; hybrids; phosphorus uptake.

INTRODUCTION

Although the forage potential of the genus *Brachiaria* was first recognized 40 years ago in Australia, only since its cultivation in tropical America in the last 20 - 25 years, has its great importance as a forage crop been perceived (Miles *et al.*, 2004). In Brazil the productivity of over 70 million hectares has been increased 5 - 10 times with respect to the native grasslands.

However, the pastures degrade in only a few years as a result of problems with acidity and deficiencies of N and P (Rao *et al.*, 1998a, b; 1999). Ecotypes of *Brachiaria* have been identified as adapted to acid soils (Rao *et al.*, 1993; 1998a), and *B. decumbens* is characterized as highly resistant to Al (Wenzl *et al.*, 2001). The efficiency of P adsorption refers to the ability of the gramineae to absorb a large quantity of this element through a unit length of the root (Rao, 2001a), or to the ability of tolerant genotypes to absorb P when planted under conditions of low P availability (Schaffert *et al.*, 2001; Alves *et al.*, 2002).

Absorption of P by *Brachiaria* can be improved through increased contact of the root system with the P in the soil, increased absorption by unit length of root, a capacity to utilize insoluble forms of P, and the effect of the association with vesicular-arbuscular mycorrhizae (Rao *et al.*, 1998b).

The objective of this study was to investigate morphological and physiological characteristics that may be involved in the adaptation to low P availability in the soil in the presence of aluminium in two *Brachiaria* genotypes (*B. decumbens*,

considered better adapted and *B. ruziziensis*, less adapted) and eight progeny of the crosses between them.

MATERIALS AND METHODS

The study was performed in a greenhouse (20-42°C, 30-90% relative humidity, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photons) in CIAT (Palmira, Cauca Valley, Colombia, 3° 30' N, 76° 21' E y 965 MASL). Ten genotypes of *Brachiaria* were evaluated; two species considered more (*B. decumbens*) and less adapted (*B. ruziziensis*) to soils with low availability of P; and eight progeny F1 chosen from 190 crosses between the two parent types (better adapted: H-7, H-40, H-28, H-58; less adapted: H-190, H-94, H-82, H-179).

Planting was performed in an Oxisol from Matazul, Meta, Colombia (4° 9' N, 72° 38' E, 160 MASL y 2649 mm annual precipitation), washed with deionised water to extract the roots (Table 1). Two levels of phosphorus were used (0 and 50 kg ha⁻¹) (Table 2). The remaining nutrients were applied at high levels (80N, 100K, 66Ca, 29Mg, 20S and micronutrients). One stolon per genotype was planted per plastic pot (2.6 kg). The pots were watered regularly with deionised water to maintain them near 100% of capacity in the field.

Table 1. Soil analysis for Matazul (0-20cm), Meta, before and after washing, without applying nutrients. CIAT, Palmira, 2006.

TIPO MUESTRA	M.O. %	pH	Al meq/100g	Ca meq/100g	Mg meq/100g	K meq/100g	NO ₃	NH ₄	P	B	Cu	Fe	Zn	Mn	D.R g cm ³
S. lavado	2.8	4.7	1.2	0.14	0.05	0.03	0.15	0.57	2.8	0.4	0.3	27.3	0.4	0.5	2.6
S. sin lavar	3.0	4.4	1.4	0.07	0.03	0.03	0.32	0.56	1.6	0.4	0.1	21.0	0.2	0.1	2.6

O.M.: Organic Material, D: Density

The plants were harvested six weeks after planting. The roots were scanned and analyzed with the programme WinRhizo V- 3.1 for Windows (Root- image-analysis software) to calculate the total length of the root (the sum of the principal and lateral roots), the diameter, and the volume of the root. The foliar area was measured from the leaves (LI300, LI-COR, Inc., Lincoln, NE). Additionally, the dry

weights (60 °C for 48 h) of the roots, stems and leaves were calculated. The concentration (%), the absorption and the efficiency of use of P were estimated in each part of the plant (Salinas y Garcia, 1985).

Table 2. Analysis of soil from Matazol, Meta after applying nutrients at the point of planting and harvesting. CIAT, Palmira, 2006.

TIPO MUESTRA	pH	Sat. Al %	Al	Ca	Mg	K	P	B	Cu	Fe	Zn	Mn
			meq/100g						ppm			
0P siembra	4.55	75.3	1.10	0.16	0.08	0.12	2.7	0.67	43.07	1.35	0.65	0.25
50P siembra	4.49	68.2	0.95	0.19	0.07	0.18	18.2	0.43	13.01	0.75	0.46	0.22
0P cosecha	4.92	70.3	1.15	0.25	0.09	0.14	3.4	1.10	41.15	1.32	0.59	0.42
50P cosecha	4.84	75.9	1.20	0.31	0.05	0.02	14.1	0.94	46.42	1.36	0.46	0.24

Al. Sat.: Aluminium saturation

A design of completely randomized blocks was utilized with three repetitions. The following analyses were conducted using the programme SAS (SAS/STAT, 2002-2003): Analysis of variance and MSD (minimum significant difference) for each variable; multiple regression analysis using the aerial biomass as dependent variable; simple Pearson correlations between the aerial biomass and the independent variables – foliage area, leaf dry weight, stem dry weight, root dry weight, root length, root diameter, root volume, phosphorus absorption in the leaves, stems, and roots, aerial biomass, total biomass, and use efficiency of P.

RESULTS AND DISCUSSION

Significant differences were observed in the majority of the variables evaluated in both 0P and 50P. Few differences were seen between the parental types. In six weeks *B. decumbens* did not present morphological or physiological characteristics that explain its better adaptation, but one of the authors observed that after 82 days this species has a larger total biomass than *B. ruziziensis*. In the F1 progeny at 0 P, the majority of the variables were significantly different; at 50P there were few differences. This indicates that with an adequate provision of P, total biomass production is increased (Correa, 1993; Rao *et al*, 1998a). The majority of the variables of H-94 presented similar values to the better adapted genotypes.

Aerial Biomass Production

The production of aerial biomass (dependent variable) was best explained by a linear model. The variable that best explained the variability in absence of P was foliage area (R^2 : 0.82), and, to a lesser extent, root volume, and absorption of phosphorus by the stem (R^2 : 0.89). These are expected results given that under low P conditions the plant directs the assimilated energy to root growth and not to the foliage (Rao, 1996; Rao *et al.*, 1998b; Yun y Keappler, 2001). As root growth was least affected, variation in this variable coincided with variation in aerial biomass. In 50P, the root dry weight explained the greatest part of the variation (R^2 : 0.7), followed by P absorption in the stem, root diameter, and foliage area (R^2 : 0.91) (Table 3).

In both 0P and 50P positive high correlations were observed between aerial biomass and foliage area, dry weight (of leaves, stems and roots), root length, root volume, and phosphorus absorption (in stems, roots, aerial biomass and total biomass). Rao *et al.* (1997) found correlations between aerial biomass and P absorption in the aerial portion, foliage area and root biomass. Root diameter did not show significant correlations at either P level. P absorption in the leaves showed a positive correlation at 0P, and in the efficiency of use of P at 50P (Table 3).

Table 3. Coefficients of determination (R^2) and simple correlation (r) between aerial biomass (g planta^{-1}) and other variables in genotypes evaluated in a greenhouse with soil from Matazul, Meta, Colombia. CIAT, 2006.

Variables Independientes Tratamientos (kg P ha^{-1})	BIOMASA AEREA (g planta^{-1})				
	0P		50P		
Coef. determinación y correlación	R^2	r	R^2	r	
Area foliar	0.82	0.91 **	0.03	0.83	**
Peso seco de hojas		0.88 **		0.86	**
Peso seco de tallos		0.94 **		0.96	**
Peso seco de raíz		0.88 **	0.70	0.84	**
Longitud de raíz		0.85 **		0.80	**
Diámetro de raíz		0.01	0.04	-0.21	
Volumen de raíz	0.05	0.89 **		0.76	**
Absorción de P en hojas		0.53 **		0.24	
Absorción de P en tallos	0.02	0.69 **	0.14	0.65	**
Absorción de P en raíz		0.66 **		0.37	*
Absorción de P en biomasa aérea		0.76 **		0.56	**
Absorción total de P		0.74 **		0.57	**
Eficiencia de uso de P		0.08		0.60	**
R^2 Total	0.89		0.91		

*, ** Significant at 0.05 y 0.01% probability, respectively.

Application of 50P affected the production of aerial biomass (Figure 1), similarly to that reported by Rao (2001a). At 0P the better adapted progeny presented better values. The marked differences (Table 4) between the best adapted genotype H-7 ($1.35 \text{ g planta}^{-1}$) and H-82 ($0.5 \text{ g planta}^{-1}$) can be explained by the morphology of the radicular system, the activity of the enzyme acid phosphatase in the leaves and roots, and the concentration of Pi in leaves and roots (Rao *et al.*, 1997).

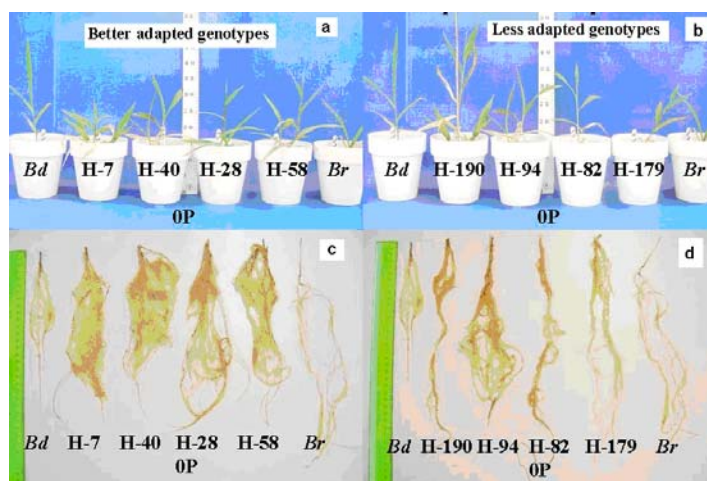


Figure 1. Effect of low P in *B. decumbens*, *B. ruziziensis* and 8 F1 progenies in soil from Matazul, Meta six weeks after planting. a y b) aerial part of plants c y d) plant roots.

Foliage Area

Significant differences in foliage area were observed among genotypes at both levels of phosphorus. In 0P the better adapted progenies showed a greater foliage area (87.2 Vs 46.9 cm² plant⁻¹). This result confirms that under a low application of P the expansion of the leaf and foliage area is reduced (Rao *et al.*, 1996). The genotype H-40 was six times better than H-82 (100 Vs 15.9cm² plant⁻¹). In 50P no differences were observed between the better and less adapted progenies. (Table 4, Figure 1). As foliage expansion is strongly related with the extension of epidermal cells, the low P content may limit this process (Rao, 2001b).

Production of Root Biomass

In 0P, the better adapted progenies showed double the root dry weight (0.36 Vs 0.18 g plant⁻¹). Watanabe *et al.* (2006) found that the absence of phosphorus in the presence of aluminium reduced to a lesser extent the weight of roots in a hybrid of *Brachiaria* compared with *Andropogon gayanus*. In 50P no differences were seen between progenies. Between the parental types, there were no differences at either level of P (Table 4).

The plants respond to a deficiency in P by increasing the formation and lengthening of the lateral roots, and by reducing the lengthening of the primary root. The changes in root morphology and growth are proportional to the concentration of growth regulators, particularly auxins, ethylene and cytokines. The augmented production of ethylene in plant roots with P deficiencies may be responsible for root hair formation. Levels of cytokines are reduced in plants with P deficiency. Genes which influence the expression of auxins, AIR1, 3, 9,12, HRGP and LRP1, which control root lateral development have been identified (Hammond *et al.*, 2004).

Root length

The parent types did not show any significant differences in the two levels of phosphorus. In 0P root length in the better adapted progenies was almost double

(51.9 Vs 27.1 m plant⁻¹) (Figure 1). In 50 P no differences were observed between the progenies (Table 4). Root length in *B. dictyoneura* in 0 and 50P indicated good performance in low P and a good response to P application (Rao *et al.*, 1996). The most prominent root characteristic in forage grasses is the high length resulting from the large surface area and the high correlation of root surface with aerial dry weight. Both parameters improve the absorption and availability of P as a result of improved access to less mobile nutrients due to the fine division and the rapid development of the root system. (Rao, 2001a,b).

Root diameter

At both levels of phosphorus, *B. ruziziensis* presented the greatest root diameter, and *B. decumbens* presented diameters similar to the progenies. This is concordant with results obtained in nutrient solutions by A Louw-Gaume¹. No differences were seen between better and less adapted progenies in either 0 or 50P (Table 4). Root diameter is an important attribute in P absorption in deficient soils, and defines the maximum soil volume that can be exploited for photosynthate production, and can vary among species and depending on the plant age (Alves *et al.*, 2002). Generally, root diameter is less in plants tolerant to low P (Rao *et al.*, 1999).

Root volume

At 0P the better adapted progenies presented almost double the root volume compared to the less adapted (3.36 Vs 1.75cm³ plant⁻¹). No differences were observed between progenies at 50P (Figure 2), nor between the parentals at either P level (Table 4).

The principal strategy of *Brachiaria* species to absorb P from applied fertilizer is to produce an extensive root system to explore a greater volume of soil, as well as establishing associations with MVA fungi (Rao *et al.*, 1998a). Root characteristics such as length, diameter, number, and length and duration of the root hairs are

¹ Annabé Louw-Gaume. Personal Communication. Swiss Federal Institute of Technology, ETH. 2007.

important in determining the P absorption capacity in deficient soils (Rao *et al.*, 1999; Alves *et al.*, 2002)

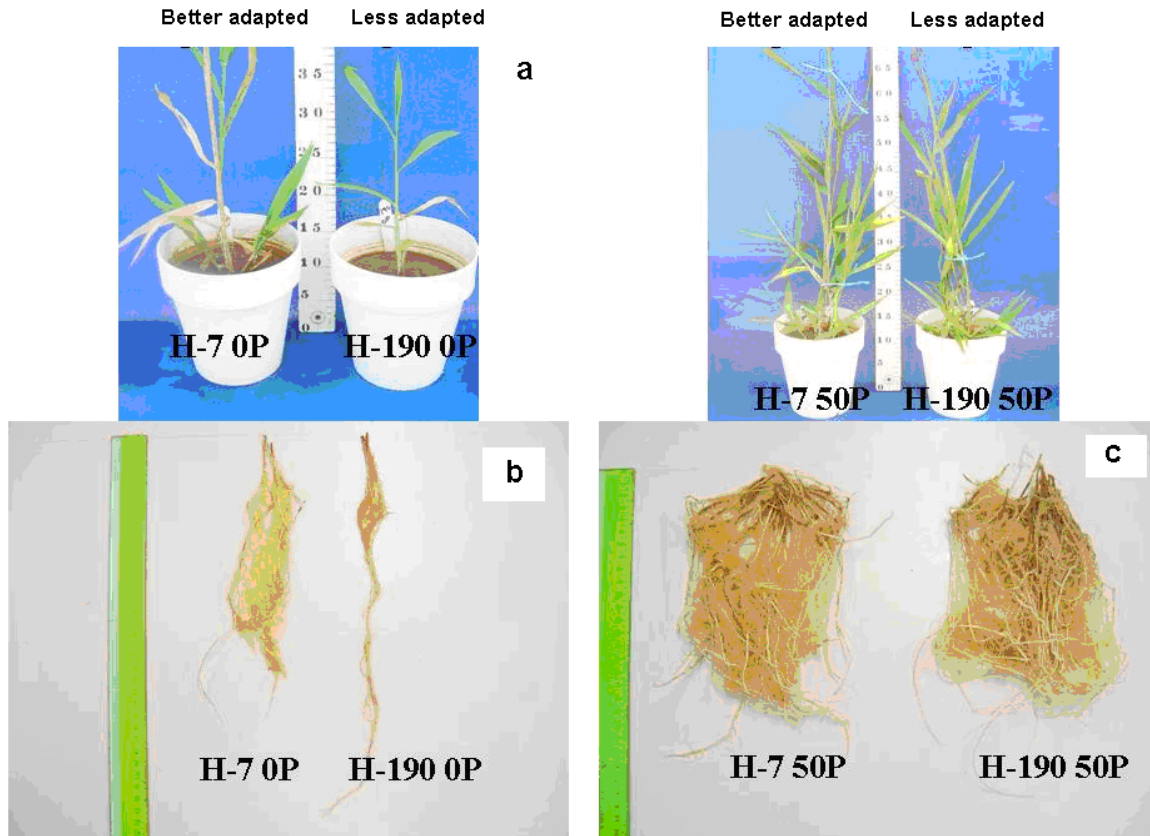


Figure 2. Effect of low and high P in two genotypes with contrasting adaptation to low P six weeks after planting. a) Aerial part of the better adapted genotype H-7, and the less adapted H-190 in low and high P b) roots of the better adapted genotype H-7 and the less adapted H-190 in low P c) roots of the better adapted H-7 and the less adapted H-190 in high P.

Biomass fractionation

The relationship between dry aerial biomass production and root dry weight is greater at 0P than at 50P (3.8 Vs 3.3 g g^{-1}) (Table 5). This behaviour is characteristic of Pi deficiency, where it is assumed that the majority of the assimilated energy is used for root growth (Rao, 1996; Yun y Keappler, 2001). Rao *et al.*(1998b) found that in low fertility root growth in various *Brachiaria* genotypes was less affected, indicating the change in carbon fractionation as an adaptive trait.

Table 4. Dry Aerial biomass, foliage area, root dry weight, root length, root diameter, and root volume of *B. decumbens*, *B. ruziziensis* and 8 F1 progenies in soil from Matazul, Meta six weeks after planting. CIAT, Palmira, 2006.

Genotype	Aerial biomass		Foliage area		Dry weight root		Length root		Diameter root		Volume root	
	g plant ⁻¹		cm ² plant ⁻¹		g plant ⁻¹		m plant ⁻¹		mm plant ⁻¹		cm ³ plant ⁻¹	
	0P	50P	0P	50P	0P	50P	0P	50P	0P	50P	0P	50P
<i>Bd</i>	0.45	6.2	35	432	0.08	1.65	11.9	118	0.28	0.38	0.71	12.3
<i>Br</i>	0.75	5.8	54	368	0.21	1.90	10.9	94	0.39	0.46	1.35	15.0
H-7	1.35	10.9	93	650	0.42	3.29	67.8	218	0.30	0.38	4.63	22.8
H-40	1.16	10.5	100	592	0.38	3.45	62.7	225	0.28	0.36	3.95	22.0
H-28	0.98	6.2	72	358	0.38	2.32	42.5	167	0.27	0.33	2.70	13.9
H-58	1.09	13.1	83	650	0.27	3.98	34.5	249	0.28	0.33	2.18	21.7
H-190	0.59	10.4	49	491	0.12	3.33	21.6	241	0.26	0.34	1.21	21.8
H-94	1.15	10.5	81	476	0.29	2.74	43.5	224	0.28	0.32	2.85	18.1
H-82	0.50	9.9	16	427	0.13	3.09	18.9	183	0.31	0.35	1.42	17.9
H-179	0.90	10.1	42	502	0.19	2.36	24.3	160	0.28	0.33	1.51	14.2
Better adapted (H)	1.14	10.2	87	562	0.36	3.26	51.9	215	0.28	0.35	3.36	20.1
Less adapted (H)	0.79	10.2	47	474	0.18	2.88	27.1	202	0.28	0.33	1.75	18.0
Average	0.89	9.4	63	494	0.27	3.07	39.5	208	0.28	0.34	2.56	19.0
MSD_{0.05}	0.45	2.4	34	144	0.14	0.99	19.7	43.8	0.03	0.07	1.32	6.01
Significance	**	**	**	**	**	**	**	**	**	**	**	**

*, ** Significant at 0.05 y 0.01% probability, respectively.

Phosphorus absorption in different plant parts

No differences were observed in P absorption in the leaves and stems at either P level. Root P absorption presented significant differences at 0P, with the better adapted progenies being more than double that of the less adapted genotypes (0.14 Vs 0.06 mg plant⁻¹). Between the parentals at 0P, differences in absorption in the leaves and roots were seen, with *B. ruziziensis* having the highest levels of absorption (Table 5), coinciding with that seen in nutritive solutions by Louw-Gaume.

Table 5. Aerial / root biomass relationship, leaf, stem, and root P absorption, and P usage efficiency of *B. decumbens*, *B. ruziziensis* y 8 F1 progenies in soil from Matazul, Meta six weeks post-planting. CIAT, Palmira, 2006.

Genotipo	Relación B.A/raíz g g ⁻¹		Absorción P en hojas mg planta ⁻¹		Absorción P en tallo mg planta ⁻¹		Absorción P en raíz mg planta ⁻¹		Eficiencia uso de P g g ⁻¹	
	0P	50P	0P	50 P	0P	50 P	0P	50 P	0P	50 P
	<i>Bd</i>	5.88	3.85	0.14	3.88	0.27	4.87	0.09	1.64	1077
<i>Br</i>	4.23	3.28	0.41	4.64	0.21	4.27	0.32	2.15	873	590
H-7	3.16	3.39	0.31	3.50	0.37	5.20	0.16	2.21	1595	1018
H-40	3.02	3.08	0.31	3.07	0.28	3.89	0.14	1.67	1563	1242
H-28	2.50	2.68	0.36	3.11	0.27	3.17	0.12	1.29	1328	832
H-58	4.05	3.29	0.18	4.20	0.44	6.99	0.16	2.08	1460	989
H-190	5.30	3.18	0.22	4.83	0.16	4.65	0.04	1.76	1761	927
H-94	4.11	3.86	0.27	3.14	0.48	6.34	0.09	1.36	1460	983
H-82	3.54	3.26	0.07	3.36	0.13	5.32	0.04	1.57	1999	967
H-179	4.97	4.29	0.10	4.53	0.26	5.08	0.06	1.46	2135	905
Mejor adaptados(H)	3.18	3.11	0.29	3.47	0.34	4.81	0.14	1.81	1486	1020
Menos adaptados(H)	4.48	3.65	0.17	3.96	0.26	5.35	0.06	1.53	1839	945
Media	3.83	3.38	0.24	3.82	0.29	4.98	0.12	1.72	1525	905
DMS_{0.05}	2.36	0.87	0.21	2.04	0.24	2.46	0.06	0.92	495	226
Significancia	**	*	NS	NS	NS	NS	**	NS	*	**

*, ** Significant at 0.05 y 0.01% probability, respectively.

NS: not significant

When the source of Pi is limited, generally the plants produce more roots, increase the absorption in the roots, transferring Pi from older leaves and using up the vacuole stores of Pi; additionally, mycorrhizal infection increases. Conversely, when plants possess an adequate source of Pi and are absorbing this element in rates higher than that required, various processes act, such as conversion of Pi to stored organic compounds, and a reduction in the absorption rate in order to prevent the accumulation of toxic concentrations (Lee *et al.*, 1990).

Efficiency in phosphorus use

Differences were seen among genotypes at both levels of P, at 0P use was much more efficient. No differences were seen between the parentals, however, *B. decumbens* tended to show a greater efficiency. Rao *et al.* (1995) reported greater efficiency in P use in *B. decumbens* compared with other cultivars of *Brachiaria*, and Correa (1993) found greater efficiency in *B. decumbens* than in *B. brizantha* or *P. maximun*. Greater efficiency of use permits *B. decumbens* to survive in soils with low phosphorus.

The efficiency of absorption is attributed to morphological, physiological and biochemical adaptations in the roots (Rao *et al.*, 1999; Lynch y Brown, 2001; Abel *et al.*, 2002). Morphologically, the structure of the root changes, or the quantity, density, and length of the lateral, adventitious roots, and root hairs is modified (Bates y Lynch, 2000). Additionally, the roots respond with the expression and secretion of acid phosphatase and RNAases (Trull y Deikman, 1998), with the synthesis and exudation of organic acids (Jones, 1998) and with an increase in the synthesis of Pi transporters (Raghothama, 2000; Jain *et al.*, 2007).

CONCLUSIONS

- The *Brachiaria* genotypes best adapted to low P presented greater aerial biomass, explained by the greater foliage area, greater P absorption in the stem and greater root volume.
- Root morphological characteristics, principally length, dry weight and P content permitted better adaptation to low P conditions in the soil.
- The F1 progenies showed little difference in root diameter, with a tendency to have fine roots like the parent type most tolerant to low P availability (*B.decumbens*) but in a lower proportion in those less adapted.
- The evaluation time of six weeks was sufficient to see differences between the progenies, but not between the parental types.

ACKNOWLEDGEMENTS

To ZIL-ETH (Zürich, Switzerland), CIAT y CORPOICA (Colombia) for logistical, technical and financial support for the doctoral thesis of S. Mejía K. from which was derived the present article.

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