Plant Soil (2015) 386:273–283
DOI 10.1007/s11104-014-2252-0

REGULAR ARTICLE

Sugarcane performance under phosphorus deficiency: physiological responses and genotypic variation

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Received: 28 May 2014 / Accepted: 25 August 2014 / Published online: 17 September 2014

Abstract

Background and aim Developing genotypes with enhanced performance under phosphorus (P) deficiency can be described as an approach to improving production sustainability. This study investigated the physiological responses of sugarcane varieties to varying P availability and the plant traits contributing to P efficiency (shoot dry matter production under low P availability).

Methods Sugarcane varieties IACSP94-2101, IACSP95-5000, RB86-7515, IAC91-1099, IACSP94-2094 and IAC87-3396 were grown under low (25 mg P kg^{-1} soil) and high (400 mg P kg^{-1} soil) P supply, and the leaf gas exchange, photochemical activity, plant growth and P uptake were evaluated.

Results The sugarcane varieties responded distinctly to a low P supply, as indicated by differences in root and shoot growth, leaf area, net CO_2 assimilation, photosynthetic P utilization efficiency, leaf P concentration and P uptake. The following ranking was obtained for P efficiency: IACSP94-2094 = IACSP95-5000 > IAC87-3396 = RB86-7515 = IACSP94-2101 = IAC91-1099.

Conclusion Greater leaf area, net CO_2 assimilation and P acquisition efficiency were combined in the more P-efficient varieties but not in the less efficient ones. Although it was not possible to separate cause and effect, such finding might be explained by the positive effect of improved leaf P concentration on leaf area and net CO_2 assimilation, which in turn contributed to sustaining improved plant performance under a low P supply.

Keywords Nutrient efficiency · Root growth · Nutritional stress · Leaf area · Photosynthesis · *Saccharum* spp.

Abbreviations

APA	acid phosphatase activity (μmol *p*-nitrophenol g^{-1} of fresh tissue h^{-1})
ETR	apparent electron transport rate (μmol m^{-2} s^{-1})
$\Delta F/F_m$'	effective quantum efficiency of photosystem II
k	instantaneous carboxylation efficiency (μmol m^{-2} s^{-1} Pa^{-1})
C_i	intercellular CO_2 concentration (μmol mol^{-1})

Responsible Editor: Hans Lambers.

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LA	leaf area (cm^2 pot^{-1})
R	leaf respiration (μmol m^{-2} s^{-1})
F_v/F_m	maximum quantum efficiency of photosystem II
A_N	net CO$_2$ assimilation (μmol m^{-2} s^{-1})
PRUpE	phosphorus root uptake efficiency (μg cm^{-1})
q_P	photochemical quenching
PPUE	photosynthetic P use efficiency (μmol mg^{-1} s^{-1})
WUE	photosynthetic water use efficiency (μmol mmol^{-1})
PUtE	physiological P utilization efficiency (g mg^{-1})
RDM	root dry matter (g pot^{-1})
RL	root length (m pot^{-1})
SDM	shoot dry matter (g pot^{-1})
SRL	specific root length (m g^{-1})
g_s	stomatal conductance (mol m^{-2} s^{-1})
E	transpiration (mmol m^{-2} s^{-1})

Introduction

Sugarcane cultivation is an important option for biofuel production due to its high potential of biomass production per area and adaptability to several edaphoclimatic conditions. However, stalk yield of sugarcane is frequently restricted in tropical soils due to poor phosphorus (P) availability (Landell et al. 2003; Zambrosi 2012). Therefore, the use of high rates of P fertilizers is a widespread practice for sugarcane production in such soils, as this nutrient enhances yields by increasing tiller production, the weight per cane and the final stalk population (Gopalasundaram et al. 2012).

Taking into account that the efficiency of P fertilization is normally very low due to the complex dynamics of P in the soil (Takahashi and Anwar 2007), the use of plant genotypes with improved P efficiency has become an important approach for increasing the sustainability of cultivation in low P soils (Vance et al. 2003). In this scenario, P efficiency is defined as the ability of plants to maintain growth and yield at low P availability. For instance, it was demonstrated that P-efficient sugarcane varieties have higher and more stable yields under a low P supply than less efficient varieties (Sundara 1994). Despite the benefits obtained by using varieties with enhanced P efficiency, the plant traits contributing to the differential tolerance to P deficiency in sugarcane have not been elucidated. Furthermore, the initial screening of sugarcane genotypes able to grow under low soil P availability might be enhanced by knowledge of the plant traits associated with tolerance to P deficiency.

Overall, the differential performance of crop genotypes under low P availability depends on both the ability of plants to acquire P from the soil and the conversion of the absorbed P into biomass (Lambers et al. 2006; Shen et al. 2011). Additionally, genotypic differences in response to P deficiency might be related to the maintenance of leaf area and photosynthesis, i.e., photosynthate production capacity, under low P supply (Fujita et al. 2004). Indeed, this assumption is supported by the fact that the impaired plant growth under this nutritional stress is also associated with the negative effects of P deficiency on leaf area development and on the photosynthetic rate of individual leaves, resulting in an insufficient supply of photosynthate for adequate biomass production (Pieters et al. 2001; Sato et al. 2010; Zambrosi et al. 2011). Within this context, the present study was based on the hypothesis that sugarcane varieties cultivated under conditions of low P availability exhibit differential abilities in maintaining photosynthate production capacity and that such characteristic would be present in the more P efficient varieties. Accordingly, we aimed to investigate plant growth, P uptake and photosynthetic parameters of six sugarcane varieties grown under P deficiency and to characterize the plant traits contributing to the distinct performance of sugarcane under this nutritional stress.

Materials and methods

Plant material and growth conditions

The experiment was carried out under greenhouse conditions, with an average air temperature of 35/25 °C (day/night). The maximum photosynthetically active radiation (PAR) was approximately 1,100 μmol m^{-2} s^{-1}, and the minimum air relative humidity was 40 %. The sugarcane varieties (*Saccharum* spp.) IACSP95-5000, IACSP94-2101, RB86-7515, IAC91-1099, IACSP94-2094 and IAC87-3396 were selected because of their distinct adaptation to several soil types, as observed in field trials (Landell and Bressiani 2008).

Homogenous stalks of the selected varieties were collected from 10 month-old plants in the same field.

The stalks were cut into stem-node segments and then further propagated in plastic pots containing moistened vermiculite. Beginning at 1 week after planting, plants were fertigated with 1 mM $CaNO_3$ every other day for 2 weeks. At 21 days after planting, when the sugarcane plants had 4 to 5 leaves and were 9 to 12 cm height, three plants of each variety were transplanted into plastic pots filled with 16.5 kg of a typical Oxisol with very low P availability (P-resin = 3 mg dm^{-3}). Such soil had been collected from the surface layer (0–0.25 m) of a pasture land. The soil was air-dried, sieved and analyzed for physical (clay content = 617 g kg^{-1} and sand content = 333 g kg^{-1}) and chemical (pH in 0.01 M $CaCl_2$ = 4.5; K = 0.9 $mmol_c$ dm^{-3}, Ca = 7.0 $mmol_c$ dm^{-3} and Mg = 3.0 $mmol_c$ dm^{-3}) attributes (Camargo et al. 1986; van Raij et al. 2001). Previously to the beginning of the experiment, dolomite was applied to increase the soil base saturation to 60 %, and the soil was incubated for 45 days.

The selected varieties were grown for 90 days either under low (25 mg P kg^{-1} soil, P_{25}) or high (400 mg P kg^{-1} soil, P_{400}) P supply. According to these two P treatments, portions of limed soil were mixed with soluble P sources (KH_2PO_4, K_2HPO_4 and $NH_4H_2PO_4$) and used to fill plastic pots. The following basal fertilization was also applied for both P treatments at planting (mg kg^{-1} of soil): 60.0 N; 80.0 K; 60.0 S; 5.0 Zn; 1.2 Cu; 4.0 Mn; 0.5 B; 0.25 Mo and 5.0 Fe. The different amounts of N and K added by P sources between P_{25} and P_{400} were balanced with NH_4NO_3 and KCl. Top-dress fertilizations with N and K occurred every week until 60 days after planting to achieve 180 mg N kg^{-1} of soil and 140 mg K kg^{-1} of soil. Plants were watered daily to maintain the soil moisture between 75 and 80 % of the maximum water holding capacity.

Physiological evaluations

After 83 days of P treatments, eight leaf discs (5 mm in diameter, excluding the main vein) were taken from mature leaves, placed in plastic bags and stored in an insulated box with ice for determining the in vivo acid phosphatase activity (APA) (Besford 1980; Elliott and Läuchli 1986). Then, the leaf discs were placed into glass tubes with 2.1 mL of the substrate *p*-nitrophenyl-1-phosphate (12.5 mM), which was prepared in 50 mM Na-acetate buffer at pH 5.0. The glass tubes were kept warm in a water bath at 30 °C for 15 min. The reaction was stopped by transferring 1 mL of the incubation extract to a glass tube containing 2 mL of 2 M NaOH. The formation of *p*-nitrophenol was quantified at 410 nm using a spectrophotometer with a standard calibration curve for *p*-nitrophenol.

Leaf gas exchange was measured from 09:00 to 12:00 h using a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, NE, USA) equipped with a modulated fluorometer (LCF-40 LI-COR Inc., Lincoln, NE, USA) under 2,000 μmol m^{-2} s^{-1} PAR and air CO_2 concentration of 380 μmol mol^{-1}. The measurements of net CO_2 assimilation (A_N), transpiration (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were performed in fully expanded leaves after 85 days of P treatments, and the photosynthetic water use efficiency (WUE = A_N/E) and instantaneous carboxylation efficiency ($k = A_N/C_i$) were calculated. Chlorophyll fluorescence was evaluated simultaneously with gas exchange in light-exposed leaves. The effective quantum efficiency of photosystem II ($\Delta F/F_m$'), apparent electron transport rate (ETR) and photochemical quenching (q_P) were estimated according to Roháček (2002) by applying a saturation pulse (λ=630 nm, PAR ~8,000 μmol m^{-2} s^{-1}, 0.8 s). Minimum fluorescence (F_o') was measured after photosystem I excitation with far-red light (λ=740 nm, PAR ~5 μmol m^{-2} s^{-1}, 2.0 s). Leaf respiration (R) and the maximum quantum efficiency of photosystem II (F_v/F_m) were evaluated in dark-adapted leaves (30 min). Measurements were obtained under temporal stability and when the total coefficient of variation was lower than 5 %.

Growth parameters

The sugarcane varieties were harvested after 90 days of P treatments by cutting plants at soil level. Tillers were counted, and leaves were separated from stalks to evaluate the total leaf area (LA) using a digital planimeter (LI-3000, LI-COR Inc., Lincoln, NE, USA). Roots were collected by sieving the entire amount of soil contained in each pot. A representative portion of roots was sampled and spread on a 4-cm grid pattern for measuring the total root length (RL), according to the line-intercept method (Tennant 1975). The specific root length (SRL) was also estimated. Leaves, stalks and roots were washed with tap water, rinsed with deionized water, dried for 72 h at 60 °C and weighed to quantify the shoot (SDM) and root (RDM) dry matter production.

Phosphorus concentration in plant organs and P use efficiency measurements

Dried leaves, stalks and roots were ground and digested in nitric-perchloric acid before the P concentration was determined using an inductively coupled argon plasma emission spectrophotometer (Bataglia et al. 1983). The total P accumulated in shoots (leaves plus stalks) and roots was calculated by multiplying P concentration and dry matter of each plant portion. The P root uptake efficiency (PRUpE) was calculated as the ratio between the entire P accumulation in the plants and RL. The physiological P utilization efficiency (PUtE) for shoot and root was calculated as the ratio between SDM or RDM and the respective P content (Rose and Wissuwa 2012). The photosynthetic P use efficiency (PPUE) was also calculated as A_N per leaf P concentration expressed on an area basis.

Statistical analysis

The experiment was carried out in a complete factorial design (six sugarcane varieties x two P rates) and was arranged in randomized blocks with four replicates (four plastic pots with three plants in each pot). The data were analyzed using a two-way analysis of variance. When the interaction between the causes of variation was significant, the effects of the varieties within each P rate and the effects of the P rates within each variety were compared ($p<0.05$) using Duncan's multiple range and F test, respectively. When an interaction was non-significant ($p>0.05$), Duncan's multiple range and F test were applied to differentiate the means of the main factors.

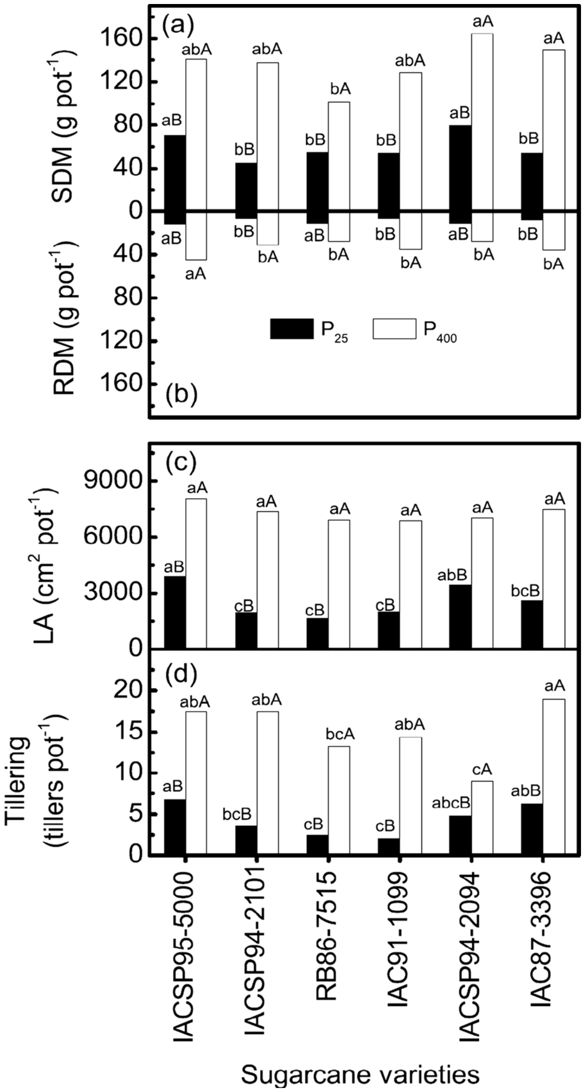

Fig. 1 Shoot (SDM in a) and root (RDM) dry matter (in b), leaf area (LA in c) and tillering (in d) of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

Results

Sugarcane growth under varying P availability

The low P supply reduced SDM by 46 to 71 % (Fig. 1a), RDM by 59 to 80 % (Fig. 1b), LA by 52 to 72 % (Fig. 1c) and tillering by 47 to 81 % (Fig. 1d) across the varieties. Furthermore, IACSP95-5000 and IACSP94-2094 exhibited higher SDM than the other varieties under P_{25}. Based on this parameter, the following ranking was obtained for P efficiency: IACSP94-2094 = IACSP95-5000 > IAC87-3396 = RB86-7515 = IACSP94-2101 = IAC91-1099. IACSP95-5000, IACSP94-2094 and RB86-7515 exhibited a higher RDM than IACSP94-2101, IAC91-1099 and IAC87-3396 under P_{25}. In this same P treatment, IACSP95-5000 and IACSP94-2094 also showed the highest LA. With regard to tillering, IACSP95-5000 had greater values than IACSP94-2101, RB86-7515 and IAC91-1099. Under P_{400}, IACSP94-2094 and IAC87-3396 showed a higher SDM than RB86-7515, whereas no difference was found among the other varieties (Fig. 1a). In this same P treatment, IACSP95-5000 had the highest RDM (Fig. 1b), but there was no variation with respect to LA (Fig. 1c). Concerning tillering under a high P supply, IACSP94-2094 showed the lowest amount of tillers (Fig. 1d).

RB86-7515 and IACSP95-5000 presented the highest RL under P_{25} (Fig. 2a), whereas IACSP94-2101, RB86-7515 and IAC91-1099 had higher SRL values than IACSP95-5000 and IACSP94-2094 (Fig. 2b). RB86-7515 also showed higher root:shoot DM ratio than IACSP94-2101, IACSP94-2094 and IAC87-3396 under P_{25} (Fig. 2c). Although RL had reduced and SRL had increased under low P availability (Fig. 2a–b), IACSP94-2101 was the unique variety in which SRL was not affected by P availability. No variation in the root:shoot DM ratio was found for RB86-7515 and IACSP94-2094 due to P treatments, whereas the other varieties presented higher values under P_{400} (Fig. 2c).

Phosphorus concentration in plant organs and acid phosphatase activity in leaves

The leaf P concentration was 36 to 56 % lower under P_{25} compared to P_{400} (Fig. 3a), whereas the root P

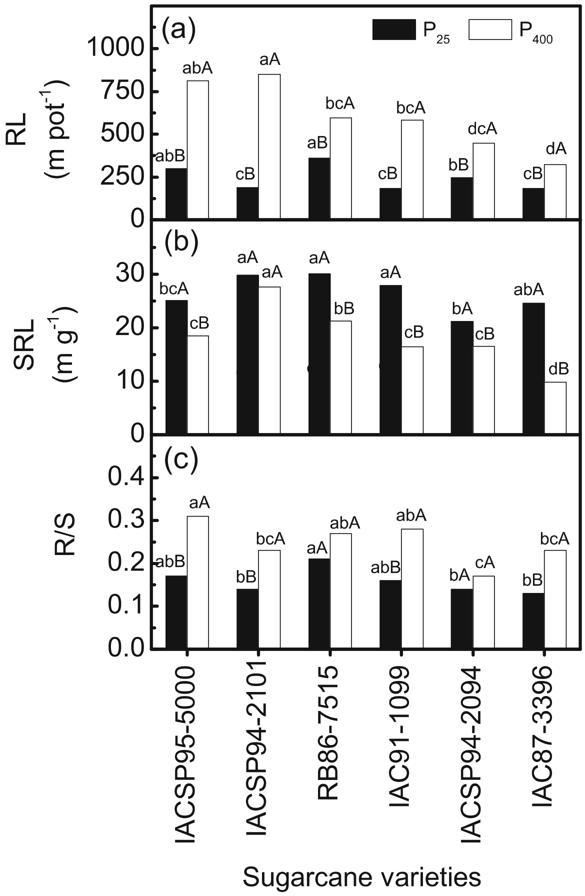

Fig. 2 Root length (RL in a), specific root length (SRL in b) and root:shoot DM ratio (R/S in c) of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

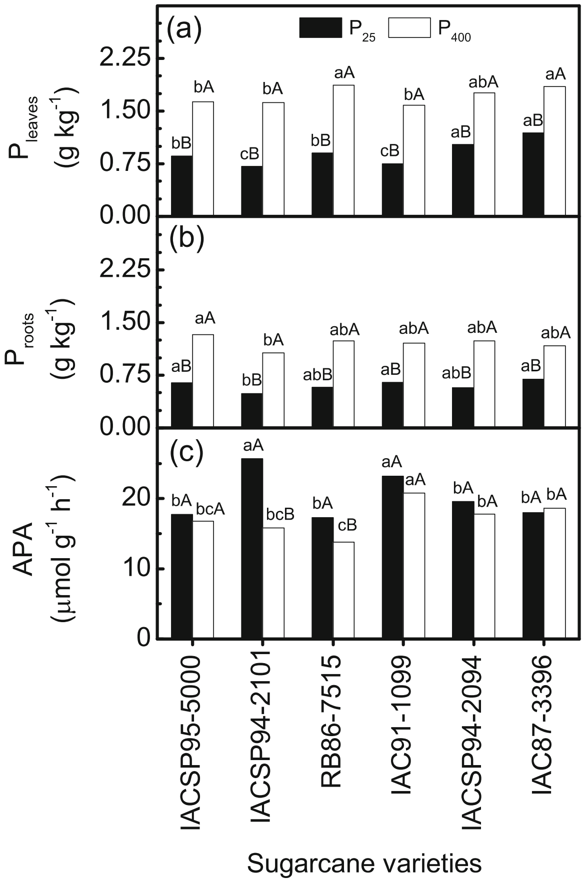

Fig. 3 Concentration of P in leaves (P_{leaves} in a) and roots (P_{roots} in b), and leaf acid phosphatase activity (APA in c) of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

concentration was 41 to 54 % lower under the limiting P condition (Fig. 3b). IACSP94-2094 and IAC87-3396 had the highest leaf P concentration under P_{25}, whereas the lowest values were found for IACSP94-2101 and IAC91-1099. Accordingly, IAC87-3396 had the highest stalk P concentration under P_{25} (data not shown). IACSP94-2101 exhibited lower root P concentration than IACSP95-5000, IAC91-1099 and IAC87-3396, whereas no difference was observed among the other varieties under P_{25}. In general, the differences in leaf and root P concentrations among the varieties were lower under P_{400} compared to P_{25} (Fig. 3a–b).

The highest leaf APA was found in IACSP94-2101 and IAC91-1099 under P_{25}. Variation in the leaf APA also occurred under P_{400}, and the highest activity was found in IAC91-1099 (Fig. 3c).

Leaf gas exchange and photochemistry

Low P availability reduced A_N by 25 to 51 %, with RB86-7515 and IAC91-1099 being the most sensitive varieties (Fig. 4a). IAC87-3396 had the highest A_N under P_{25}, followed by IACSP95-5000 and IACSP94-2094, which exhibited similar A_N. With the exception of IACSP94-2094, k was 23 to 59 % lower under P_{25} compared to P_{400} (Fig. 4b). IACSP94-2101, RB86-7515 and IAC91-1099 had similar and lower k values than the other varieties under P deficiency. Under this same stressful condition, IACSP95-5000 and IACSP94-2094 exhibited higher PPUE than the other varieties, with exception of IAC87-3396 (Fig. 4c). Moreover, IACSP95-5000 and IACSP94-2094 showed increases in PPUE under P_{25}. Under P_{400}, IACSP95-5000 and IAC87-3396 had the highest A_N, and IACSP94-2101 exhibited the lowest values (Fig. 4a). No variation in k and PPUE was found among the varieties under P_{400}, with exception of IACSP94-2101 that exhibited the lowest k and PPUE values (Fig. 4b–c).

Non-significant interactions ($p > 0.05$) between varieties and P rates were found for g_s, E, $\Delta F/F_m$' and q_P; however, there were differences among the varieties and the low P availability caused reductions in all these variables (Fig. 5a–d). Regarding WUE and ETR, non-significant differences were found among the varieties ($p > 0.05$), and the averaged values were significantly ($p < 0.05$) reduced due to low P availability: WUE from 9.8 to 7.5 µmol mmol^{-1} (−24 %) and ETR from 163 to 115 µmol m^{-2} s^{-1} (−30 %). C_i, R and F_v/F_m were not affected ($p > 0.05$) by either variety or P rate, and the averaged values across P rates and varieties were 91.6 µmol mol^{-1}, 1.48 µmol m^{-2} s^{-1} and 0.791, respectively.

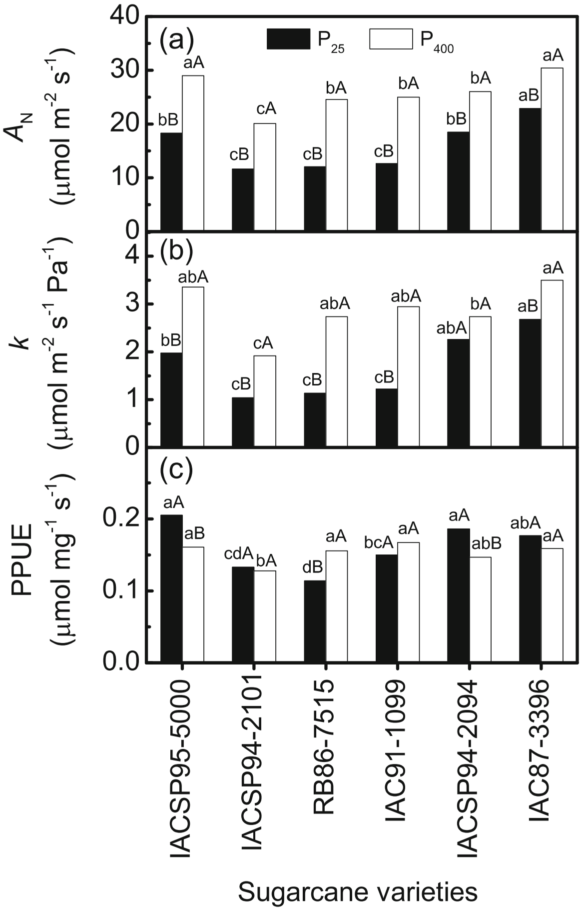

Fig. 4 Net CO_2 assimilation (A_N in a), instantaneous carboxylation efficiency (k in b) and photosynthetic P use efficiency (PPUE in c) of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p < 0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p < 0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

Phosphorus use efficiency measurements

Both shoot and root P uptakes were reduced in all varieties under P_{25} compared to P_{400} (Fig. 6a–b). IACSP94-2101 and IAC91-1099 exhibited lower shoot P uptake than IACSP95-5000, IACSP94-2094 and IAC87-3396

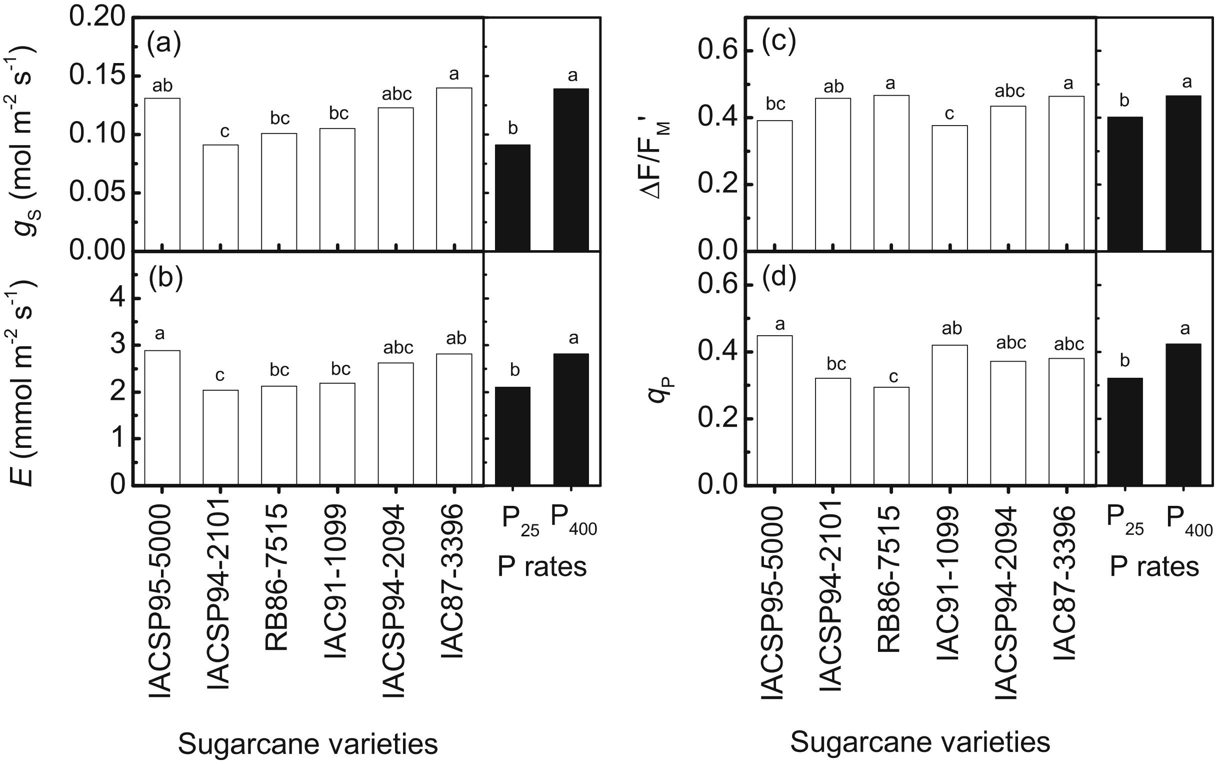

Fig. 5 Stomatal conductance (g_s in a), transpiration (E in b), effective quantum efficiency of photosystem II ($\Delta F/F_m$' in c) and photochemical quenching (q_P in d) as affected by sugarcane varieties and P rates applied to the soil at planting. Variety comparison: columns followed by different letters for the average of P rates are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different letters for the average of varieties are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

under P_{25}. Root P uptake of IACSP95-5000 was higher than that of IACSP94-2101 and IAC91-1099 but similar to the other varieties. The variation in both shoot and root P uptake among the varieties was less pronounced in P_{400} than in P_{25} (Fig. 6a–b).

In general, IACSP94-2094 and IAC87-3396 presented higher PRUpE than the other varieties, regardless P availability (Fig. 7a). All varieties exhibited reductions in PRUpE due to low P availability. While IACSP94-2101 showed the highest PUtE$_{shoot}$ under P_{25}, IAC87-3396 exhibited the lowest value (Fig. 7b). IACSP94-2101 exhibited higher PUtE$_{root}$ than IACSP95-5000, IAC91-1099 and IAC87-3396 under P_{25} (Fig. 7c). PUtE$_{shoot}$ and PUtE$_{root}$ of all the varieties were lower under P_{400} compared to P_{25} (Fig. 7b–c).

Discussion

Our hypothesis that sugarcane varieties would exhibit differential abilities to maintain photosynthate production capacity when grown under low P availability was confirmed. Although there were reductions in both LA and A_N of all varieties under P_{25} compared to P_{400}, a significant genotypic variation for these traits was found under the P-deficient condition (Figs. 1c and 4a). Such variation in LA and A_N under P_{25} was a response to P nutritional stress because no difference among the varieties was observed for LA under P_{400} and the ranking for A_N was not the same under P_{400} and P_{25}. Based on the combined values of LA and A_N across the varieties, our results suggested that IACSP95-5000, IACSP94-2094 and IAC87-3396 exhibited a higher photosynthate production capacity than IACSP94-2101, RB86-7515 and IAC91-1099 under low P supply. Additionally, differences for P efficiency (Fig. 1a), leaf P concentration (Fig. 3a) and shoot P uptake (Fig. 6a) were observed when comparing IACSP95-5000 and IACSP94-2094 to IACSP94-2101 and IAC91-1099. Taken together, such results revealed that these two groups of varieties exhibited the most contrasting responses to low P supply and that enhanced P

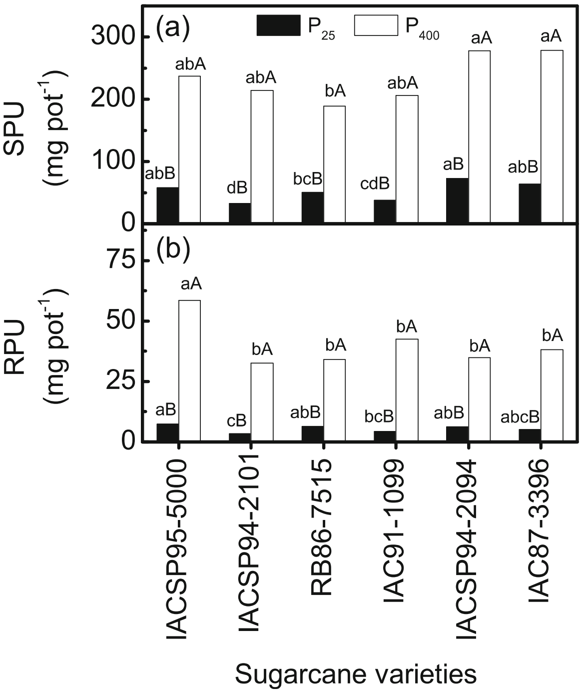

Fig. 6 Shoot (SPU in a) and root (RPU in b) P uptake of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

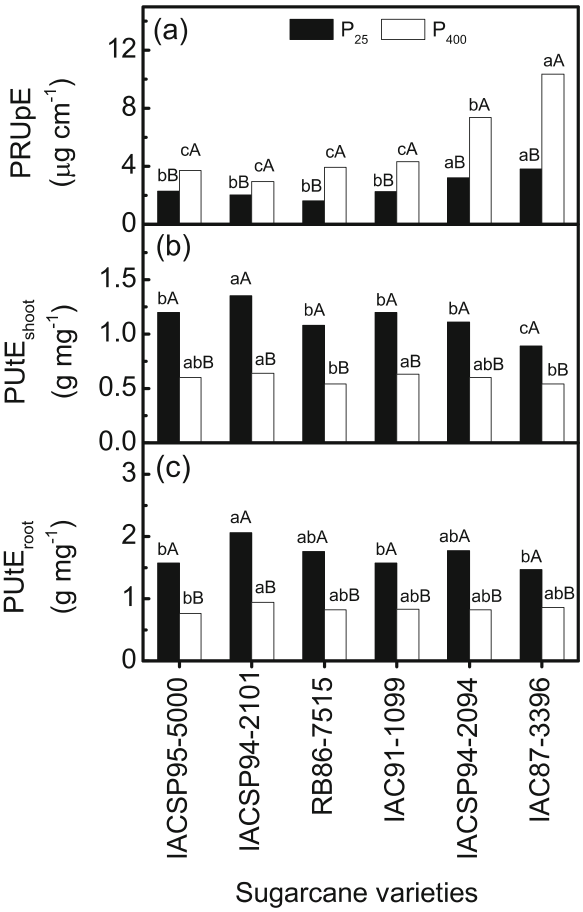

Fig. 7 Phosphorus root uptake efficiency (PRUpE in a) and physiological P utilization efficiency in shoots ($PUtE_{shoot}$ in b) and roots ($PUtE_{root}$ in c) of sugarcane varieties as affected by P rates applied to the soil at planting. Variety comparison: columns followed by different lowercase letters within the same P rate are significantly different by Duncan's multiple range test ($p<0.05$). P rate comparison: columns followed by different uppercase letters for the same variety are significantly different by the F test ($p<0.05$). P_{25} = application of 25 mg P kg^{-1} of soil; P_{400} = application of 400 mg P kg^{-1} of soil

acquisition efficiency and photosynthate production capacity are present in the more P efficient genotypes (IACSP95-5000 and IACSP94-2094).

The effect of P deficiency on the photosynthate production capacity of the sugarcane varieties appeared to be more pronounced on LA than on A_N, as the reduction in LA reached 72 % and A_N varied from 25 to 51 % due to low P availability. Accordingly, the differences in plant growth between P-deficient and -sufficient treatments were mainly attributed to the amount of absorbed radiation than on radiation use efficiency in maize (Mollier and Pellerin 1999). Furthermore, the averaged values of LA and A_N of IACSP95-5000 and IACSP94-2094 were 84 and 52 %, respectively, higher than those found in IACSP94-2101 and IAC91-1099, suggesting that LA rather than A_N imposed a greater influence on sugarcane P efficiency. Therefore, the maintenance of a greater LA might be a desirable trait in sugarcane genotypes to improve biomass production under low P conditions, as already observed in other nutritional stresses (Zhao et al. 2014). Herein, the potential of radiation interception by sugarcane depended more on the capacity of each variety to maintain growth of individual leaves than on tillering ability and leaf appearance. This assumption was supported by the fact that varieties with similar tillering (Fig. 1d) and leaf number (data not shown) exhibited distinct LA (Fig. 1c) under P_{25}, probably because the varieties maintained differential rates of leaf cell production and elongation (Assuero et al. 2004; Kavanová et al. 2006). Despite the fact that improvements in A_N might have a more limited scope than in LA in order to increase sugarcane photosynthate production, the varieties exhibiting a lower sensitivity of LA to low P supply also had higher tolerance to decreasing A_N. In addition, there was a positive association between these traits across the varieties under P_{25}, which might contribute to maximize photosynthate production under limiting P condition.

Impaired A_N in P-deficient plants compared to P-sufficient ones is attributed to increased mesophyll resistance, diminished levels of RuBP and its regeneration, the accumulation of starch in chloroplasts, impaired stomatal conductance and low sink demand (Foyer and Spencer 1986; Fredeen et al. 1989; Rao and Terry 1995; Pieters et al. 2001). Overall, our results suggested that contrasting A_N in varieties grown under P_{25} (Fig. 4a) was caused by differential sensitivity of the biochemical reactions of photosynthesis rather than stomatal sensitivity to P deficiency. Such argument was based on C_i, which was not affected by P rates and did not vary among varieties, and on *k*, which was higher in IACSP95-5000, IACSP94-2094 and IAC87-3396 than in IACSP94-2101, RB86-7515 and IAC91-1099 under P_{25} (Fig. 4b). Regarding photochemistry, our data also revealed that ATP and NADPH production by electron flow through PSII did not cause the differential response of A_N under P_{25}. This was supported by the lack of consistent differences in $\Delta F/F_m$' and q_P between IACSP95-5000, IACSP94-2094 and IAC87-3396 vs. IACSP94-2101, RB86-7515 and IAC91-1099 (Fig. 5c–d) and the absence of significant variation ($p>0.05$) for F_v/F_m and ETR among the varieties. However, an evaluation of PPUE revealed that A_N per unit of P was increased in IACSP95-5000 and IACSP94-2094 under low P availability but not in the other varieties (Fig. 4c). These results suggest that an improved PPUE is an important physiological adjustment to reduce the impact of P deficiency on sugarcane A_N and support the idea that high PPUE is a valuable functional trait for plant adaptation to low P soils (Hidaka and Kitayama 2009).

Taking into account that the effects of P deficiency on plant growth might precede those on leaf photosynthesis (Plénet et al. 2000) and that impaired root growth under P deficiency was not due to source limitation (Wissuwa et al. 2005), even without recognizing the primary effect, we may argue that the greatest P efficiency of IACSP95-5000 and IACSP94-2094 was driven by their greater P acquisition efficiency. Further, the performance of these varieties under P_{25} was favored because a higher leaf P concentration alleviated the negative impact of low P availability on LA and A_N and thus on photosynthate supply for biomass production. In addition, a greater photosynthate production capacity might have also contributed to the improved P efficiency of IACSP95-5000 and IACSP94-2094 because carbon costs of P acquisition are more elevated under limiting P condition (Lynch and Ho 2005). An evaluation of the root traits across the varieties indicated that the greater P acquisition of IACSP95-5000 compared to IACSP94-2101 and IAC91-1099 was solely associated with its enhanced RL, whereas in IACSP94-2094 it was due to both increased RL and PRUpE. Although it is difficult to ascertain whether a higher amounts of absorbed P are the result or the cause of a larger root size found in IACSP95-5000 and IACSP94-2094 (Gahoonia and Nielsen 2004), P acquisition efficiency was enhanced because a larger root size increases soil exploration and reduces the effects of low P mobility to the rhizosphere (Schachtman et al. 1998; Raghothama and Karthikeyan 2005). Indeed, root growth would be the most efficient manner to improve tolerance to P deficiency, even though, it might also be favored by a greater PRUpE because the positive effects of absorbed P on root growth and thus in P acquisition (Wissuwa 2003).

Our results also revealed that the root mechanisms underlying P acquisition from the soil influence the performance of sugarcane under low P supply. For instance, the combination of similar shoot P uptake and lower $PUtE_{shoot}$ of IAC87-3396 compared to IACSP95-5000 and IACSP94-2094 under P_{25} (Figs. 6a and 7b) suggested that the former variety had an increased carbon cost for root P acquisition under limiting P condition. This phenomenon might have caused a decrease in shoot carbon availability to be used in biomass production (Nielsen et al. 2001), which in turn resulted in the lower P efficiency of IAC87-3396 (Fig. 1a). The idea that the impaired shoot growth of IAC87-3396 was due to increased carbon cost in roots and not to source limitation was also supported by the fact that this variety had LA similar to IACSP94-2094 and even higher A_N than both IACSP95-5000 and IACSP94-2094. Moreover, no variation was found with regard to leaf respiration among these varieties. In another study, contrasting P efficiency of potato genotypes showing similar A_N and leaf respiration was attributed to variation in root respiratory demand due to higher root biomass and/or higher carbon loss through root exudation (Balemi and Schenk 2009). Therefore, such responses should be further investigated in sugarcane to improve our understanding about the relationship between P efficiency and the carbon costs of P acquisition.

Conclusions

From a practical point of view, our results are important for the management of sugarcane areas because they reveal the variation in P efficiency and thus the opportunity of obtaining gains in plant biomass production by growing more efficient varieties. Herein, we showed that the sugarcane varieties with superior performance under low P availability (IACSP95-5000 and IACSP94-2094) exhibited a combination of greater leaf P concentration, shoot P uptake and photosynthate production capacity (leaf area and net CO_2 assimilation), alleviating thus, the limiting effect of low P supply on biomass production. Accordingly, the sugarcane varieties more tolerant to low P availability presented as strategies more efficient P acquisition from soil and less sensitivity of leaf area and net CO_2 assimilation to limiting P condition. Therefore, our data also suggest the possibility of exploiting these plant traits in combination to obtain sugarcane varieties more adapted to P-deficient soils.

Acknowledgments We thank the São Paulo State Research Foundation (FAPESP, Brazil) for financial support (Grant # 2011/18446-0) and Guilherme Z. Garcia for help during the experiment. The authors also acknowledge the scholarship (PERM) and fellowships (RVR; HC) granted by the National Council for Scientific and Technological Development (CNPq, Brazil).

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