

## Foliar phosphorus application enhances nutrient balance and growth of phosphorus deficient sugarcane

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### Abstract

Although it is well known that nutrient imbalance in shoot tissues may impair plant performance, the interactive effect between foliar phosphorus (P) application and varying P availability in the rooting medium on the nutritional status of sugarcane has not been well studied. To fill this research gap, four sugarcane varieties (IAC91-1099, IACSP94-2101, IACSP94-2094 and IACSP95-5000) were evaluated using a combination of two concentrations of P in nutrient solution (P-deficient, PD = 0.02 mmol L<sup>-1</sup> and P-sufficient, PS = 0.5 mmol L<sup>-1</sup>) and foliar P application (none and 0.16 mol L<sup>-1</sup>). The spray was applied until drip point three times during the experiment with 15 days intervals, after which the plants were harvested to quantify growth and shoot concentration of nitrogen (N), P, magnesium (Mg), sulphur (S) and manganese (Mn). The responses of sugarcane plants to foliar P spray at different levels of P supply in the rooting medium was not genotype-dependent. It was demonstrated for the averaged values across varieties, that foliar P application enhanced sugarcane performance under low P, as revealed by improvements of leaf area and dry matter production of shoot and root of PD plants. Under P limitation we also observed diminished shoot concentration of N, P, Mg, S and increased concentration of Mn. However, foliar P spray increased the concentrations of N, P, S and reduced shoot Mn. Furthermore, shoot P:N, P:Mg, P:S, P:Mn and Mg:Mn concentration ratios exhibited a positive relationship with shoot dry matter production. In conclusion, low P supply in the rooting medium impairs nutrient balance in shoot tissues of sugarcane at early growth; however, this effect was ameliorated by foliar P application which merits further study under field conditions.

**Keywords:** fertilisation management, manganese, nitrogen, nutritional stress, *Saccharum* spp.

### 1 Introduction

Since sugarcane productivity in the tropics is frequently limited by low phosphorus (P) availability in soils and a large fraction of the P added through fertilisers is rendered unavailable in high-P fixing soils, more efficient management practices of P fertilisation should be adopted in farming systems to enhance nutrient-use efficiency (Devi *et al.*, 2012; Zambrosi, 2012). Accordingly, foliar P application has been advocated as a complementary management strategy to supply this nutrient

to crops grown in P-deficient soils and/or when roots are unable to acquire the necessary amounts of P to meet plant's growth requirements (Noack *et al.*, 2010; Fernández & Brown, 2013). For sugarcane, the benefits of foliar P application under low soil P have already been shown by an enhanced commercial cane sugar content (Pawar *et al.*, 2003), but the mechanisms behind improved crop yield under such practice are not completely understood. Therefore, we wanted to investigate how foliar P application influences absorption and transport of nutrients to the shoot in P-deficient plants, since plant's performance is directly related to balance of essential elements in shoot tissues (Clemens, 2001; Salt *et al.*, 2008) and the effects of P spray on the nutritional status of sugarcane under P deficiency remains to be revealed.

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The influence of foliar P spray on shoot nutrient concentration of sugarcane plants under P stress might be particularly important for nitrogen (N) and sulphur (S), since the assimilatory pathways of these nutrients are closely connected (Nikiforova *et al.*, 2005) and N is often a limiting nutrient for sugarcane yield (Ishikawa *et al.*, 2009; Robinson *et al.*, 2011). The effect of low P on nutrient metabolism may also extend to magnesium (Mg), because P deficiency reduces the concentration of Mg in leaf tissue most likely due to an impaired translocation to the shoot (Skinner & Matthews, 1990). In addition to such direct effect on Mg nutrition, another study has revealed that P deficiency also increases shoot manganese (Mn) concentration and contributes to imbalance between Mg and Mn in plant tissues (Zambrosi, 2016), with subsequent risks of impairment in Mg functions (Marschner, 1995).

In view of the above, we conducted a greenhouse study under controlled hydroponic conditions to test the hypothesis that foliar P application benefits sugarcane performance under low P condition at early growth by its ability to improve the nutrient balance in the affected shoot tissue. Our specific objectives were (i) to investigate the interactive effects of foliar P application with P availability in the rooting medium on plant growth and shoot concentration of selected nutrients (N, P, Mg, S and Mn), and (ii) to gain insights into the mechanisms by which foliar P application influences early sugarcane growth under P limitation.

## 2 Materials and methods

The experiment was carried out in an unshaded greenhouse from March to June 2012 with a mean air temperature of 35/25 °C (day/night). Stalks of four contrasting sugarcane varieties (IAC91-1099, IACSP94-2101, IACSP94-2094 and IACSP95-5000) were cut into stem-node segments and propagated in plastic pots containing moistened vermiculite. At 30 days after planting, when the sugarcane plants had 4–5 leaves, a total of 24 plants of each variety were transferred to plastic boxes containing 12 L of  $\frac{1}{2}$  ionic strength nutrient solution and no P for establishment. Each plastic box contained two plants of the same variety.

After five days in the diluted nutrient solution, the plants were shifted to a full-strength nutrient solution containing the following P treatments in the rooting medium: P-deficient (PD, 0.02 mmol L<sup>-1</sup> of P) and P-sufficient (PS, 0.5 mmol L<sup>-1</sup> of P). The full-strength nutrient solution had the following composition

in mmol L<sup>-1</sup>: 13.0 N (8.0% as NH<sub>4</sub><sup>+</sup>), 5.0 Ca, 3.0 K, 1.3 Mg, 1.3 S; and, in  $\mu$ mol L<sup>-1</sup>, 41.6 B, 46.7 Fe, 8.2 Mn, 3.5 Zn, 1.0 Cu and 1.3 Mo (Zambrosi *et al.*, 2011). The pH of the solution was adjusted to approximately 5.5 and the hydroponic solutions were replaced every 15 days.

The PD and PS treatments were combined with two foliar P treatments: no foliar spray or a foliar spray of a solution containing 0.16 mol L<sup>-1</sup> of P. The spray solution contained 0.5% mineral oil as a surfactant and was applied during the morning until the drip point. Ammonium phosphate ((NH<sub>4</sub>)<sub>3</sub>PO<sub>4</sub>) was used for foliar P application, and the total amount of N present in this solution was balanced with ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) in the treatment without foliar P application. A randomized complete-block design was used with a split-split-plot arrangement and three replications. The main plots were the P concentrations in the nutrient solution (two concentrations), the sub-plots corresponded to foliar P treatments (no foliar spray and foliar spray) and the sub-sub-plots contained the sugarcane varieties (four varieties).

Three foliar P sprays were applied during the growth period, the first spray being 15 days after the onset of P treatments in the nutrient solution and the two remaining applications occurring at 15-day intervals thereafter. To avoid contamination of the nutrient solution with the shoot-applied solution, the lids of the plastic boxes were covered with toilet paper. Nutrient solutions were replaced the same day when foliar P application was made.

All plants were harvested 60 days after the onset of the P treatments in the nutrient solution and leaves were separated from the stalks to determine total leaf area using a digital planimeter (LI-3000, LI-COR Inc., Lincoln, NE, USA). Leaves, stalks and roots were washed with tap water, rinsed with deionized water, dried for 72 hours at 60 °C and weighed to quantify shoot (leaves plus stalks), dry matter (DM) and root DM. The dried shoots were ground and subjected to an acid digestion before the mineral composition was determined using an inductively coupled argon plasma emission spectrophotometer (Bataglia *et al.*, 1983).

We analysed the data using a factorial analysis of variance (ANOVA) considering three-way interactions for P supply in the nutrient *versus* foliar P application *versus* sugarcane varieties. Linear and quadratic regressions were also used to describe the relationship between variables, and the model with higher regression coefficient ( $P < 0.05$ ) was selected.

### 3 Results

We did not notice significant ( $P > 0.05$ ) three-way interaction for P supply in the nutrient *versus* foliar P application *versus* sugarcane varieties on the evaluated parameters of plant growth and shoot nutrient concentrations. There was also no influence ( $P > 0.05$ ) of the varieties on such responses of sugarcane plants to foliar P application (data not shown). Therefore, and in concordance with the main objectives of the present study, the averaged values of the four sugarcane varieties were used to investigate the effects of foliar P spray on growth and nutritional status of sugarcane plants at sufficient and deficient P availability in the rooting medium.

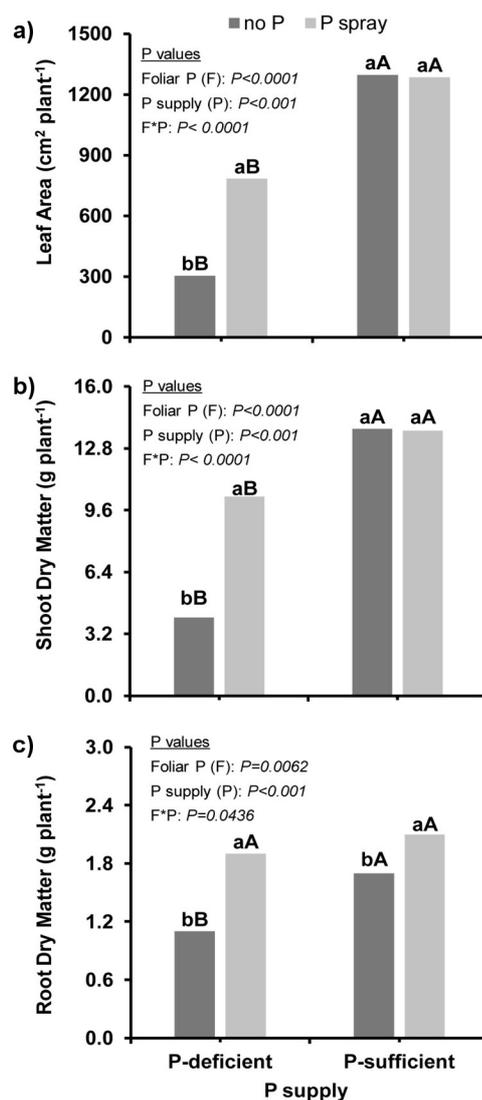
Shoot growth of PS plants was larger than of PD plants regardless of foliar P treatment (Fig. 1a-b). However, increases in leaf area (158%) and shoot DM (156%) following foliar P spray were only significant for PD plants. Positive effect of foliar P application on root growth was observed for plants under both PD and PS, even if it was more pronounced in the former (73% *versus* 24%; Fig. 1c). Furthermore, root DM of plants receiving foliar spray presented similar values in both regimes of P supply in the nutrient solution.

Foliar P application increased shoot P concentration in both PD (100%) and PS plants (29%) (Fig. 2). Shoot P concentration of plants with sufficient P in the nutrient solution were higher than those grown in a PD substrate regardless of foliar P treatment. Additionally, shoot P concentration was positively related to plant DM production, and the nutrient concentration associated with maximum shoot growth was  $3.4 \text{ g kg}^{-1}$  (Fig. 3).

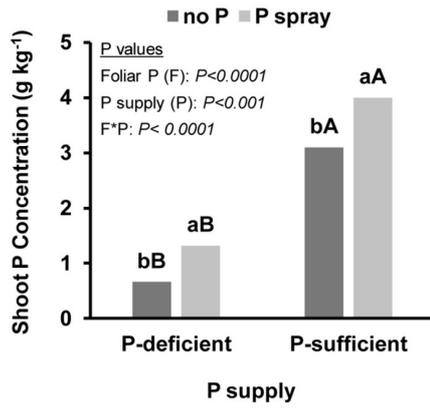
Shoot N concentration was reduced by 18–29% in the PD treatment compared to PS (Table 1); however, foliar P application increased the concentration of this nutrient by 20% in PD plants. Magnesium concentration in shoot was not influenced by foliar P spray and the averaged value across foliar P treatments was 15% higher in PS compared to PD plants (Table 1). Foliar P application increased shoot concentration of S by 72% in PD plants as compared with those that did not receive a P spray (Table 1). PS plants without a P spray showed higher shoot S concentration (50%) than PD plants, but there was no difference under foliar treatment. Foliar P application reduced shoot Mn by 26% in PD plants compared with the absence of P spray (Table 1). It was also observed a significant reduction of shoot Mn concentration under PS compared with PD nutrient solution in both foliar P treatments.

There also was a positive relationship of P:nutrient ratios (concentration basis,  $\text{g kg}^{-1}$ ) with shoot DM production of sugarcane (Fig. 4). Based on these data, maximum plant growth may be achieved with the following nutrient ratios: P:N = 0.17, P:Mg = 1.24, P:S = 0.62, and P:Mn = 38.0. Moreover, there was a positive association between shoot DM production and Mg:Mn ratio, with maximum plant growth attained at a ratio value of 34.0 (Fig. 5).

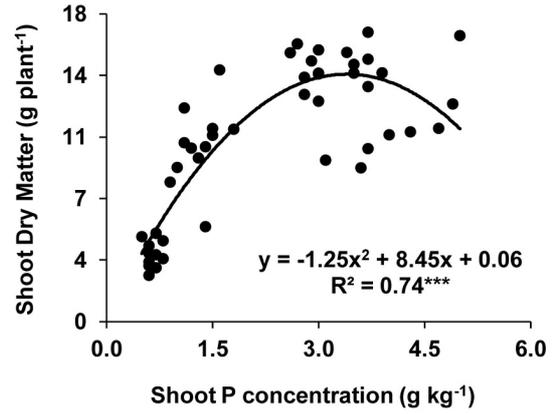
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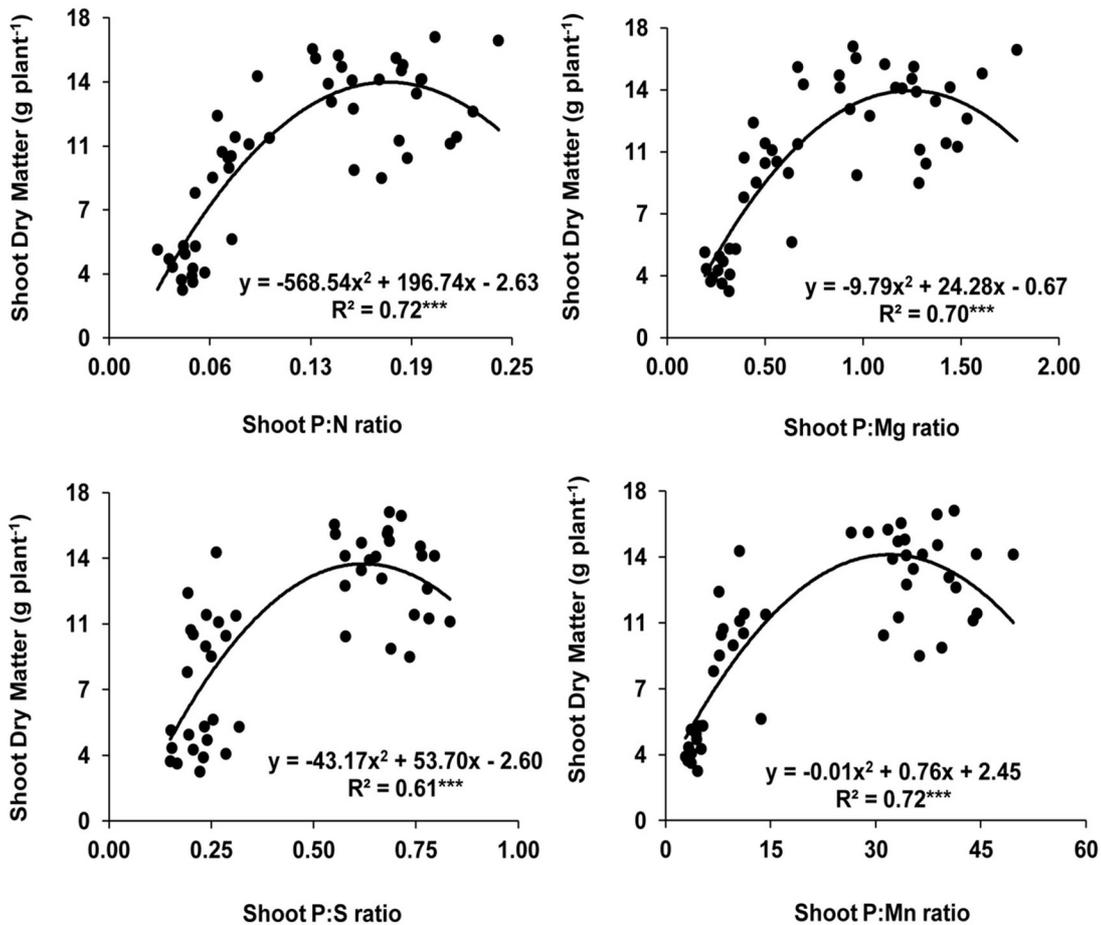
**Fig. 1:** Effect of foliar P application and P supply in the nutrient solution on the averaged growth of four sugarcane varieties. Foliar P comparison: columns followed by different lowercase letters with the same P supply in the nutrient solution are significantly different at  $P < 0.05$ . P supply comparison: columns followed by different uppercase letters for the same foliar P treatment are significantly different at  $P < 0.05$ . Foliar P supply consisted of three applications of a solution containing  $0.16 \text{ mol L}^{-1}$  of P as ammonium phosphate –  $(\text{NH}_4)_3\text{PO}_4$  – applied in 15-day intervals. P-deficient nutrient solution:  $0.02 \text{ mmol L}^{-1}$  of P; P-sufficient nutrient solution:  $0.5 \text{ mmol L}^{-1}$  of P.



**Fig. 2:** Averaged shoot P concentration of four sugarcane varieties as affected by foliar P application and P supply in the nutrient solution. Foliar P comparison: columns followed by different lowercase letters within the same P supply are significantly different at  $P < 0.05$ . P supply comparison: columns followed by different uppercase letters for the same foliar P treatment are significantly different at  $P < 0.05$ . P spray: three applications of a solution containing  $0.16 \text{ mol L}^{-1}$  of P as ammonium phosphate –  $((\text{NH}_4)_3\text{PO}_4)$  – in 15-day intervals. P-deficient nutrient solution:  $0.02 \text{ mmol L}^{-1}$  of P; P-sufficient nutrient solution:  $0.5 \text{ mmol L}^{-1}$  of P.



**Fig. 3:** Relationship between shoot P concentration and dry matter production across four sugarcane varieties, foliar P application and two levels of P supply in the nutrient solution ( $n = 48$ ). \*\*\*  $P < 0.05$ .

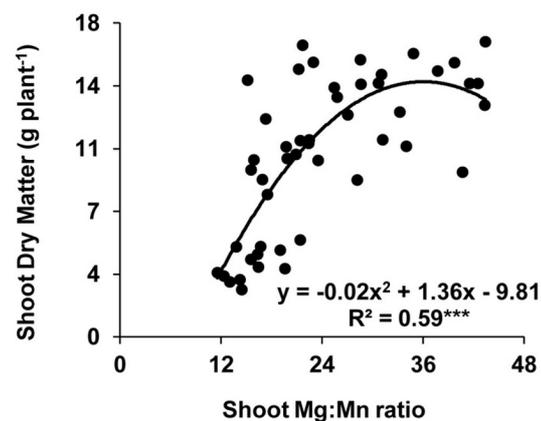


**Fig. 4:** Relationship between shoot P:nutrient ratios (concentration basis,  $\text{g kg}^{-1}$ ) with dry matter production across four sugarcane varieties, foliar P application and two levels of P supply in the nutrient solution ( $n = 48$ ). \*\*\*  $P < 0.001$ .

**Table 1:** Shoot concentration of nitrogen, magnesium, sulphur and manganese in sugarcane as affected by foliar P application and the level of P supply in the nutrient solution. The values are the means of the four sugarcane varieties.

Foliar P	P supply			P values		
	P-deficient	P-sufficient	Average	Foliar P	P supply	Foliar P*P supply
Nitrogen (g kg <sup>-1</sup> )						
no P	14.4 <sup>bB</sup>	20.4 <sup>aA</sup>	17.4			
P spray	17.3 <sup>aB</sup>	21.2 <sup>aA</sup>	19.2	0.0128	<0.0001	0.0312
Average	15.8	20.8				
Magnesium (g kg <sup>-1</sup> )						
no P	2.5	2.9	2.7 <sup>a</sup>			
P spray	2.6	3.1	2.9 <sup>a</sup>	0.2874	<0.0001	0.3829
Average	2.6 <sup>B</sup>	3.0 <sup>A</sup>				
Sulphur (g kg <sup>-1</sup> )						
no P	3.2 <sup>bB</sup>	4.8 <sup>aA</sup>	4.0			
P spray	5.5 <sup>aA</sup>	5.5 <sup>aA</sup>	5.5	<0.0001	<0.0001	<0.0001
Average	4.3	5.1				
Manganese (mg kg <sup>-1</sup> )						
no P	176.3 <sup>aA</sup>	86.8 <sup>bB</sup>	131.5			
P spray	130.6 <sup>bA</sup>	97.0 <sup>aB</sup>	113.8	0.0022	<0.0001	0.0014
Average	153.4	91.9				

Foliar P comparison: means followed by different lowercase letters within columns are significantly different at  $P < 0.05$ . P supply comparison: means followed by different uppercase letters across paired columns are significantly different at  $P < 0.05$ . P spray: three applications of a solution containing 0.16 mol L<sup>-1</sup> of P as ammoniumphosphate - (NH<sub>4</sub>)<sub>3</sub>PO<sub>4</sub> - in 15-day intervals. P-deficient nutrient solution: 0.02 mmol L<sup>-1</sup> of P; P-sufficient nutrient solution: 0.5 mmol L<sup>-1</sup> of P.

**Fig. 5:** Relationship between shoot Mg:Mn ratio (concentration basis, g kg<sup>-1</sup>) with dry matter production across four sugarcane varieties, foliar P application and two levels of P supply in the nutrient solution ( $n = 48$ ). \*\*\*  $P < 0.001$ .

## 4 Discussion

It is well known that an improved nutritional status in sugarcane at early growth enhances plant vigour which can significantly increase stalk yield in the field (Muchow *et al.*, 1996). Accordingly, the present research aimed at improving our understanding of the effects of P spray on nutritional status of plants under P stress to explore the potential of mechanisms by which such practice affects initial shoot growth. As the influence of foliar P application on growth and nutritional status of sugarcane under contrasting P availability in nutrient solution was not genotype-dependent (data not shown), the averaged values of all tested varieties were used to study the responses of sugarcane plants to different regimes of P supply in the foliage and rooting medium. Our data clearly showed that foliar P application influences the mineral composition of shoot tissue of sugarcane plants at early growth. For instance, foliar P spray increased shoot concentration of N and S in PD plants (Table 1), most likely because the processes of nutrient absorption by the root system and transport to the shoot are impaired in P deficient plants (Jeschke *et al.*, 1997). This finding has major practical relevance since sugarcane cultivated in P-deficient field soils may be more prone to nutrient stresses (such as N deficiency) and has lower nutrient use efficiency (Zambrosi, 2012). Furthermore, the greater shoot P concentration of PD plants under foliar P spray (Fig. 2) improved the balance of P with other nutrients in the shoot tissue and therefore enhanced sugarcane performance, as suggested by the positive relationships between shoot DM and P:nutrient ratios (Fig. 4).

The data also showed that shoot Mn in PD plants were higher than in PS plants, but this effect was diminished with foliar P application (Table 1). The higher shoot Mn concentration in the PD treatment may be associated with the fact that under such condition there is less interference of P on Mn absorption by the roots (Pedas *et al.*, 2011). Even though we did not note typical symptoms of Mn toxicity in the leaves and Mn concentrations were not likely in the toxic range (Macnicol & Beckett, 1985; van Raij *et al.*, 1997), it was previously demonstrated that plant growth is impaired at shoot Mg:Mn below 20.0 (Goss & Carvalho, 1992). Therefore, despite the presence of adequate levels of Mg and Mn in the nutrient solution, P deficiency favoured an imbalance between these nutrients, as revealed by a Mg:Mn ratio of 14.0 in PD plants compared to 33.0 in PS plants. However, foliar P application increased the Mg:Mn ratio to 20.0 by reducing shoot Mn concentration of PD plants (Table 1), which may help to secure more stable

Mg functions in plants (Hermans *et al.*, 2010). This argument about the beneficial effects of an improved balance between Mg and Mn following foliar P spray and also sufficient P supply in the rooting medium is supported by the fact that the Mg:Mn ratio was positively related to shoot DM whereby maximum plant growth may be achieved at a ratio of 34.0 (Fig. 5).

The P deficiency imposed in the present study corresponded to a condition of severe nutrient limitation, since the relative shoot growth of PD plants was less than 30 % of that obtained for plants grown with adequate P in the rooting medium (Fig. 1b). However, shoot DM was increased by 156 % with foliar P application, reflecting its efficacy in alleviating severe P deficiency in sugarcane. The improved P nutritional status also enhanced leaf growth of sugarcane plants grown in the P deficient rooting medium (Fig. 1a), which may be the result of enhanced cell division and expansion (Chiera *et al.*, 2002). The greater leaf area formation favoured dry matter production of shoot and root (Figs. 1b and 1c), most likely due to enhanced photosynthate production and subsequent utilisation for biomass production (Zambrosi *et al.*, 2015, 2016). This is supported by the positive correlation between leaf area and DM production of PD plants ( $r = 0.82$ ,  $n = 24$  and  $P < 0.001$ ). However, it is noteworthy that despite the positive effect of P spray on plant growth under low P condition, this approach was not able to sustain the production of similar leaf area and shoot DM to those in PS plants, that is relative yields were lower than 70 % (Figs. 1a and 1b). Such result may reflect the fact that foliar P spray, even with three applications over the experimental duration, did not increase shoot P tissue concentration to the required  $3.4 \text{ g kg}^{-1}$  to attain maximum growth (Figs. 2 and 3). Indeed, this finding is in accordance with the concept that foliar P application should be used as a complementary management strategy to supply the nutrient when the roots are unable to meet plant's demand (Fageria *et al.*, 2009; Noack *et al.*, 2010).

In conclusion, our data show that foliar P application can enhance early growth of P deficient sugarcane in nutrient solution by improving nutritional balance and also leaf area formation in the plants. Accordingly, field studies should be undertaken to further investigate these effects and the feasibility of this management practice under on-farm conditions.

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