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Full Length Research Paper

Growth, phosphorus status, and nutritional aspect in common bean exposed to different soil phosphate levels and foliar-applied phosphorus forms

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This study aimed to investigate the effect of the foliar application of phosphite and phosphate on growth, phosphorus (P) status, and nutritional aspect of common bean (*Phaseolus vulgaris* cv. Radiante) plants grown under different soil phosphate levels. Experiment was organized in factorial scheme completely randomized using 2 soil phosphate levels (Pi-starved and Pi-sufficient plants), combined with 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl used as control), and 2 foliar application numbers (single and two applications). In this study were measured root dry weight, shoot dry weight, and root to shoot ratio, as well as shoot P concentration, root P concentration, accumulated P in shoot, accumulated P in root, P uptake efficiency, P utilization efficiency, P translocation, and macro and micronutrients in shoot. Common bean growth under limiting phosphate availability in soil exhibited lower biomass yield and higher concentration of nutrients in shoot tissues. The results exhibit foliar-applied KH_2PO_3 causes harmful effects in phosphate-starved common bean. Either one or two foliar sprays of KH_2PO_4 were not sufficient to affect the growth and nutrition of the common bean plants, regardless of soil P status.

Key words: *Phaseolus vulgaris*, phosphorus, phosphate and phosphite anions, foliar application.

INTRODUCTION

Limited phosphorus (P) availability in Ultisols and Oxisols has been identified as one of the major problems for plant growth in tropical and subtropical regions of the world. High rate P "fixation" and formation of insoluble complexes with aluminum and iron under acid conditions are recognized as an important factor contributing to the low P availability. Thus, application of P-containing fertilizers in these soils is a necessary practice for adequate crop yields in many instances (Vance et al., 2003) and foliar-applied P may increase use efficiency by minimizing soil supply (Girma et al., 2007; Mosali et al., 2006).

Phosphate anions ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}) are considered as the main phosphorus forms assimilated by plants and these can induce adequate growth and development with consequences in yield. However, another P form known as phosphite has been widely marketed either as fungicide or as a superior P source for plant nutrition (McDonald et al., 2001; Thao and Yamakawa, 2009; Deliopoulos et al., 2010). Phosphite anions ($H_2PO_3^-$ and HPO_3^{2-}) are reduced forms of phosphate anions, in which one hydroxyl group is substituted by hydrogen (Danova-Alt et al., 2008).

Several studies conclusively indicate that phosphite is

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effective in controlling some important plant diseases caused by pathogens belonging to the class Oomycetes (phylum Oomycota), such as *Phytophthora* sp. Action of phosphite anion is based on two mechanisms: the first is a direct toxic action on the pathogen and the second in indirect action due to phosphite anion activates plant defence responses (McDonald et al., 2001; Wilkinson et al., 2008; Shearer and Fairman, 2007; Orbovic et al., 2008; Cook et al., 2009; Moor et al., 2009). Thus, phosphite has been used as active ingredient in several fungicides.

In terms of plant nutrition, phosphite-based products have been recommended as fertilizers for foliar application, and number of foliar fertilizers containing the phosphite anion has recently increased (Moor et al., 2009). Phosphite salts are recommended as fertilizer because they contain a cation that may be plant nutrient, such as K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Cu^{2+} or Zn^{2+}, and the P in form of phosphite anion. However, results of studies that investigated nutritional value of phosphite anion as a P nutrient are inconclusive. In the year 1990, it was reported foliar application of potassium phosphite improved set fruit and yield of avocado, and restored normal growth of phosphate-starved citrus (Lovatt, 1990; Lovatt, 1990). Similarly, positive effects of phosphite on plant P nutrition or crop yields also were demonstrated in other works (Albrigo, 1999; Rickard, 2000; Watanabe, 2005). On the other hand, recent studies have indicated phosphite anion may not be used by plants as a P nutrient, even though it is well absorbed by leaves and roots. Moreover, there are indications phosphite supply causes growth depression in phosphate-starved plants (McDonald et al., 2001; Schroetter et al., 2006; Thao et al., 2008, 2009). In this case, it appears that phosphite inhibits the gene expression related to the responses for overcoming P starvation, such as increased phosphatase activity, synthesis of high affinity transporters for P and elongation of the root system (Varadarajan et al., 2002; Ticconi et al., 2001; Lee et al., 2005).

The aim of this study was to investigate (i) interference produced by different soil phosphate levels, to evaluate (ii) action produced by foliar-applied phosphorus forms (phosphite and phosphate), and to measure (iii) as number of foliar application can act on growth, phosphorus status, and nutritional aspect in common bean (*Phaseolus vulgaris* cv. Radiante) plants.

MATERIALS AND METHODS

Growth conditions, substrate, and plant material

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil (21°14' S; 45°00' W; 915 m asl). Plants remained in glasshouse environment under natural conditions day/night. Substrate used was composed by low-fertility Oxisol (Typic Haplustox) placed in plastic pots with capacity of 6 L (Table 1). For plant material, common bean (*Phaseolus vulgaris* cv.Radiante) was used.

Substrate preparation

Surface soil with depth from 0 to 20 cm was collected from a non-cultivated field with natural Brazilian cerrado vegetation, allowed to dry, crushed to pass through a 4-mm sieve and then mixed with $CaCO_3$ and MgO (4:1 stoichiometric ratio of Ca:Mg) to raise soil base saturation to 60% of cation exchange capacity at pH 7.0. After 30 days of incubation, a basal nutrient solution was applied and was thoroughly mixed with the soil. Nutrients without P treatments were supplied at the following rates of 90 N, 80 K, 30 S, 5 Zn, 5 Mn, 2 Cu, 1 B and 0.25 Mo mg dm^{-3} of dry soil.

Experimental application

Experiment was organized in factorial scheme completely randomized using 2 soil phosphate levels (Pi-starved and Pi-sufficient), combined with 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl used as control), and 2 foliar application numbers (single and two applications). For soil phosphate levels, Pi-starved and Pi-sufficient corresponded to 40 and 200 mg of P per dm^3 of dry soil, respectively, applied together with the basal nutrient solution. This study had 3 replicates, and each experimental unit consisted of one pot containing two plants, and all variables measured were expressed as mean of two plants.

Nutrient solutions and foliar applications

Solutions of KH_2PO_3 (monobasic potassium phosphite pa), KH_2PO_4 (monobasic potassium phosphate pa) and KCl (potassium chloride pa) were sprayed at concentration of 40 µM, using a manual backpack sprayer. Concentration of P equals the used dose of approximately 3 L of commercial potassium phosphate to 400 L of water, which is usually recommended for growing beans. And KH_2PO_3 was obtained by reaction of H_3PO_3 (phosphorous acid pa) with KOH (potassium hydroxide pa). Single application was implemented when plants presented fourth trifoliate leaf stage, and two applications was carried out in stage of fourth trifoliate leaf and another application in the beginning of flowering stage.

Fertilization as top dressing, irrigation, and harvest

During the soil pot experiment, fertilizations with 240 N, 210 K, and 45 S mg dm^{-3} of dry soil were supplied as top dressing. These fertilizations were split among into three applications throughout the experiment. Soil moisture was maintained at 60% of the total soil pore space occupied by water through daily irrigation. Plants were harvested at full flowering stage and separated into shoot and root. Both shoot and root were rinsed in deionized water and dried at 60°C for 72 h prior to dry weight determination.

Phosphorus determinations

Shoot and root dry mass were ground and analyzed for total P content colorimetrically (Murphy and Riley, 1962) after nitric-perchloric digestion of the plant material (Johnson and Ulrich, 1959). Data from shoot and root dry wt and total P concentration were used to calculate the P accumulation, P uptake efficiency (Swiader et al., 1994) (P total accumulation in plant / root dry wt), P utilization efficiency (Siddiqi and Glass, 1981) [(plant dry wt)^2/ (P total accumulation in plant)], and P translocation from root to shoot (P total accumulation in shoot / P total accumulation in plant)

Table 1. Chemical, physical and mineralogical compositions of Oxisol.

Chemical compositions[1]															
pH	P	K	Zn	Cu	Mn	Fe	EP	Ca	Mg	Al	H+Al	T	m	V	MPAC
		(mg dm^{-3} of soil)					(mg L^{-1})	(cmol$_c$ dm^{-3} of soil)					(%)		(mg kg^{-1})
5.4	0.9	22	0.5	0.7	0.4	27.4	20.5	0.1	0.1	0.1	1.7	2	28	13.3	396

Physical compositions (%)[2]			
Sand	Silt	Clay	OM
60	17	23	0.8

Mineralogical compositions (g kg^{-1} of clay)[3]										
SiO_2	Al_2O_3	Fe_2O_3	TiO_2	P_2O_5	Fe_d	Fe_o	Ct	Gb	Ki	Kr
95.1	97.4	36.2	6.2	0.0	10.8	0.1	752.0	63.0	0.98	0.71

[1] pH in water (1:2.5), P and K by MehlichI extraction, Mg and Al extractable by 1 M KCl solution (Thomas, 1982); P in the equilibrium solution (EP) according to Alvarez et al. (2000); level of organic matter (OM) according to Anne (1945). T = Cation exchange capacity at pH 7.0; m = Aluminum saturation index; V = Base saturation index and MPAC = maximum P adsorption capacity (Ohtake et al., 1996).[2] The soil granulometry was determined by the pipette method of Day (1965). [3] SiO_2, Al_2O_3, Fe_2O_3, TiO_2 and P_2O_5 were determined according to Vettori (1969) with modifications (Embrapa ,1997); Fe_d, according to Mehra and Jackson (1960); Fe_o, according to Schwertmann (1964) and Ct (kaolinite) and Gb (gibbsite) according to Klug and Alexander (1974). Ki = SiO_2 / Al_2O_3 and Kr = SiO_2 / (Al_2O_3 + Fe_2O_3).

Macro and micronutrients

Concentrations of nutrients in shoot were determined after nitric-perchloric digestion as follows: S by turbidimetry; K by flame photometry; Ca, Mg, Cu, Mn, Fe, and Zn by flame atomic absorption spectroscopy. Total N was determined using the Kjeldahl method after sulphuric digestion, and B by colorimetry using the Azomethine-H method after dry digestion, with ash content obtained in muffle furnace by 1 h at 550°C.

Data analysis

Results were submitted to variance analysis (F teste, $p \leq 0.05$), and when significant differences occurred were applied to Tukey test at 5% level of error probability ($p \leq 0.05$), standard errors were calculated in all evaluated points. The statistical analyses were carried out with the Sisvar software (Ferreira, 2008).

RESULTS AND DISCUSSION

Biomass yield

Most variables in this study were not significantly affected ($p > 0.05$) by foliar application numbers during single application timing and two application timings (Figure 1). As expected, common bean plants grown under limiting phosphate availability (Pi-starved) showed considerable reductions in the root and shoot dry wt and increased root to shoot ratio. The increase root to shoot ratio by phosphate-starved plants is a mechanism for overcoming P deficiency (Clarkson, 1985).

Foliar application of potassium phosphite (KH_2PO_3) and potassium phosphate (KH_2PO_4) had no significant effect ($p > 0.05$) on biomass yield of phosphate-sufficient common bean, when compared with the control (foliar application of potassium chloride). However, for plants grown under limiting phosphate availability, shoot and root dry weight were significantly decreased by foliar-applied potassium phosphite. In addition, root to shoot ratio also was increased with two foliar applications of potassium phosphite (an application in the fourth trifoliate leaf stage and another application in the beginning flowering stage) due to the strong inhibition of shoot dry mass yield. This same behavior also occurred for a single foliar application of potassium phosphite (in the fourth trifoliate leaf stage) but in this case there was no significant difference by Tukey's test ($p > 0.05$). Hence, our results showed phosphite may not be used by common bean as a P nutrient, and that this anion inhibits biomass yield

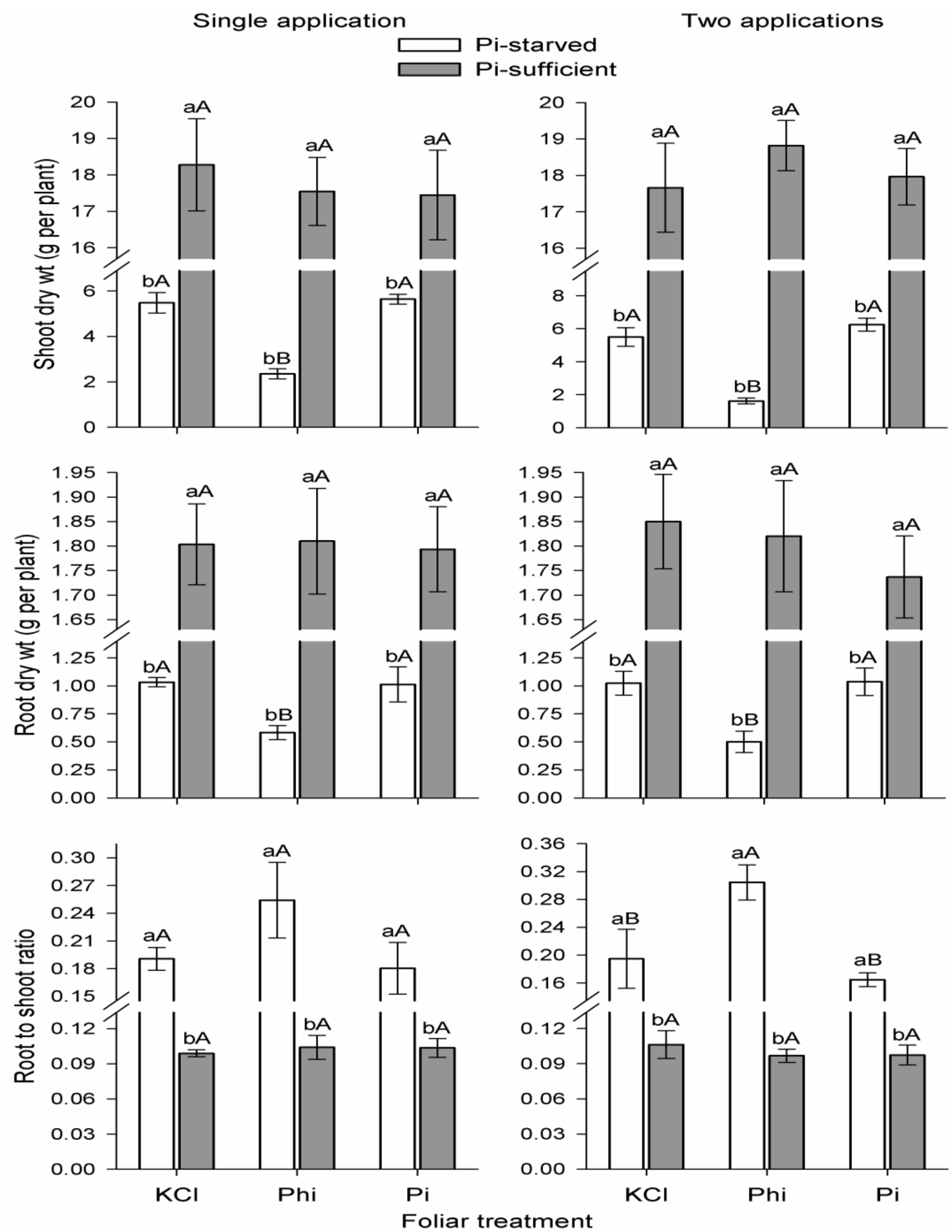

Figure 1. Root dry weight, shoot dry weight, and root to shoot ratio in common bean grown in Oxisol under 2 phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar application numbers (single and two applications). Averages followed by the same lowercase letter within soil phosphate levels and uppercase letter among foliar application for each phosphate level, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

under phosphate-deficient conditions.

The inhibiting effect of the phosphite anion on growth of phosphate-starved plants has been reported by different workers (Thao and Yamakawa, 2009). The causes of this effect are not well understood. The most plausible hypothesis to date is that plants do not metabolize phosphite anion, which, after uptake, remains stable in the cell compartments. Furthermore, phosphite anion inhibits some mechanisms involved in overcoming of phosphate deficiency, such as increased synthesis of phosphatases, phosphodiesterases, nucleases, and high-affinity P transporters. Most likely, the molecular mecha-

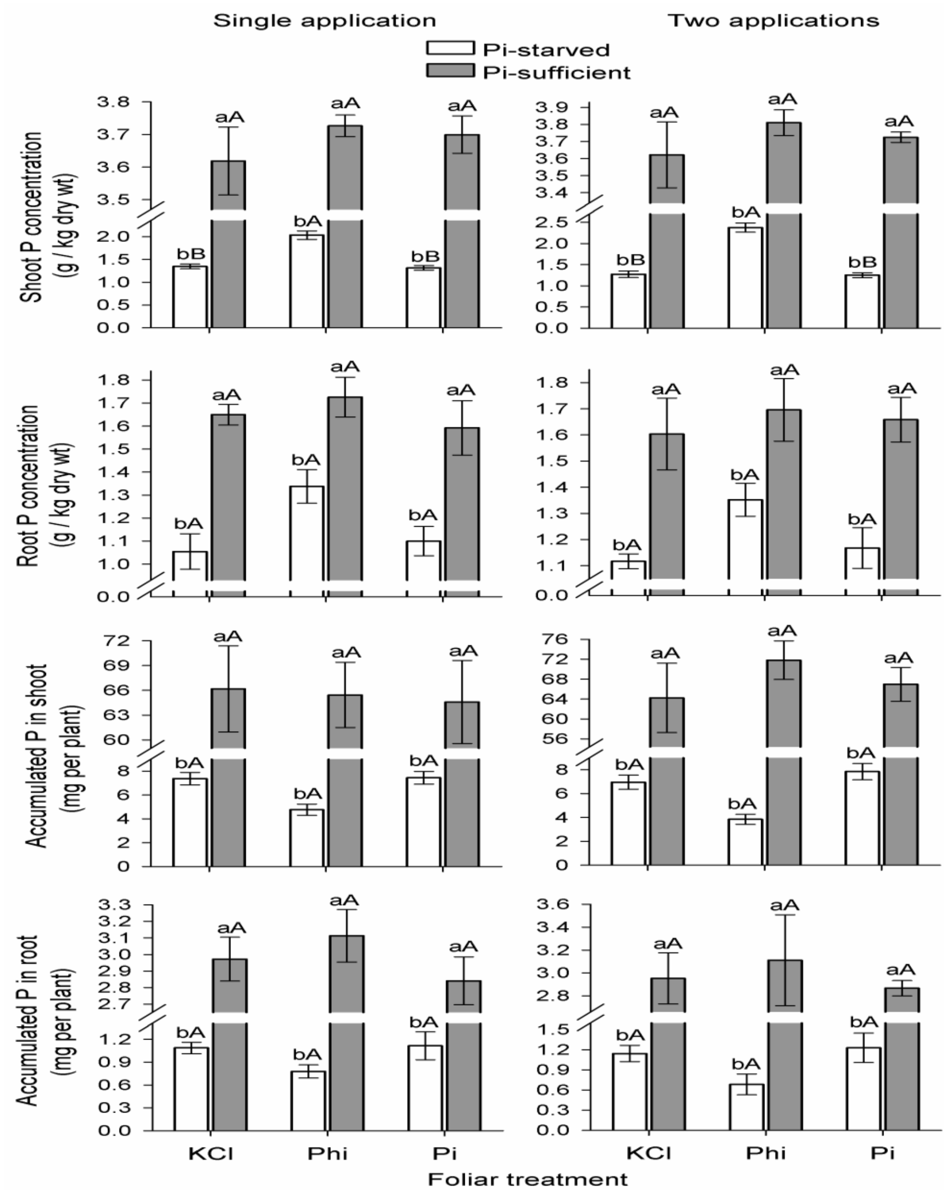

Figure 2. Shoot P concentration, root P concentration, accumulated P in shoot, and accumulated P in root in common bean grown in Oxisol under 2 phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar application numbers (single and two applications). Averages followed by the same lowercase letter within soil phosphate levels and uppercase letter among foliar application for each phosphate level, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

nisms responsible for signaling P deficiency do not discriminate phosphate from phosphite. Thus, there is no expression of genes responsible for proteins involved in P starvation responses (Varadarajan et al., 2002; Ticconi et al., 2001; Lee et al., 2005).

We also found that one and two foliar applications of potassium phosphate had no significant effect ($p > 0.05$) on biomass yield of phosphate-starved common bean, when compared with the control. Thus, these results suggest that several foliar applications of phosphate may be necessary to adequately correct a P deficiency, impractical in most cases.

Concentrations and accumulations of P in shoot and root

Common bean plants grown under limiting phosphate availability (Pi-starved) showed decreased concentrations and accumulations of P in shoot and root (Figure 2).

Foliar-applied potassium phosphite did not affect P nutrition of phosphate-sufficient plants, but increased concentration of P in shoot of phosphate-starved plants. However, accumulation of P was not significant varied among foliar application treatments ($p > 0.05$), showing that this increased concentration of P was not due directly to the P from the foliar-applied phosphite, but likely to concentration effect, which is confirmed by the lower shoot dry weight. When biomass yield decreases, this concentration effect for some nutrients may occur (Crusciol et al., 2008; Marschner, 1995), which is the elevation of their concentration in the tissues without there being an alteration in the quantity of nutrient taken up.

In this study, foliar-applied potassium phosphate did not significantly affect ($p > 0.05$) concentrations and accumulations of P in common bean, when compared with the control. This shows that either one or two foliar applications of phosphate were not sufficient to affect the plant P status. Several attempts to use foliar-applied phosphate in plant nutrition are known, but results are inconclusive. It was recorded that phosphate uptake by leaves after the foliar spray is about 50% (Kannan, 1990). A previous study reported that P concentration in grain of common bean grown under field conditions was not affected by three foliar application timing of phosphate anion (Contee Castro and Boaretto, 2001). On the other hand, Girma et al. (2007) found effect of foliar-applied phosphate on forage and grain P concentrations of maize varied with both applied P levels and plant growth stage. Another study by Mosali et al. (2006) on winter wheat indicated foliar application of phosphate generally increased grain yield, P uptake and P use efficiency, suggesting the authors that low rates of foliar-applied phosphate might correct mid-season P deficiency.

Effects on P uptake efficiency, P utilization efficiency, and P translocation

Either one or two foliar applications of potassium phosphate and phosphite had no significant effects ($p > 0.05$) on P uptake efficiency such as ability to take up P from soil, and also P translocation such as ability to transporter P from root to shoot (Figure 3). Nevertheless, regardless of the foliar-applied treatments, limiting phosphate availability in soil reduced P uptake efficiency and P translocation by common bean. This was principally due to decreased P uptake by phosphate-starved plants according to the Figure 2, although the root dry weight (Figure 1) also was reduced, but in lower magnitude. To reiterate, in this study the P uptake efficiency represent the P-uptake amount per root dry weight unit, and the P translocation from root to shoot represent the ratio between accumulated P in shoot and accumulated P in plant. When the phosphate availability to plants is insufficient, P translocation from root to shoot decreases, increasing the root growth rate to the detriment of shoot growth rate. Thus, phosphate-starved plants commonly have higher root dry weight to shoot dry weight ratio, a response that enhances the P uptake efficiency (Raghothama, 1999; Schenk, 2006).

Foliar-applied treatments did not affect P utilization efficiency (that is, ability to yield biomass for a given plant P concentration) (Siddiqi and Glass, 1981) of phosphate-sufficient plants, whereas under limiting phosphate availability in soil, foliar-applied phosphite decreased P utilization efficiency of common bean. This result was due to the inhibitory effect of phosphite on biomass yield of the phosphate-starved plants (according to the Figure 1), since accumulated P of the plants was not affected by the treatments. In contrast, when compared with the control, either one or two application timings of foliar potassium phosphate had no significant effect ($p > 0.05$) on P utilization efficiency. Other studies had shown phosphate-starved plants exhibit more P utilization efficiency, as response to soil phosphate deficiency, but this more P utilization efficiency varies among cultivars of the same species. Akhtar et al. (2008) found *Brassica* cultivars differ substantially in P utilization efficiency when grown with sparingly soluble P-forms ($Ca_3(PO_4)_2$ and Jordan rock-P). These investigators suggested the existence of useful genetic differences among cultivars for mobilization of P from sparingly soluble P-sources. In spite of phosphate-starved plants tend to increase the P utilization efficiency; the results of this study may have been a reflection of the strong P uptake decrease of the plants grown under phosphate deficient Oxisol.

Concentrations of nutrients in shoot

Likewise for shoot P concentration, concentrations of other nutrients in plant shoot tissues were not significantly affected ($p > 0.05$) by foliar application numbers (Table 2). Apart from the K, common bean grown under limiting phosphate availability exhibited higher concentrations of nutrients in shoot, regardless of the foliar-applied treatments. Foliar application of potassium phosphite also increased concentrations of N, K, Mg, B, Cu, Mn and Fe in shoot tissues of phosphate-starved plants. However, this increased concentration of nutrients coincides with the decreased shoot dry weight (Figure 1), which may suggest that it is involved with the concentration effect, as mentioned above in the presentation of P concentration. Indeed, the applied treatments did not increase accumulation of nutrients in shoot (data not shown), supporting the suggestion above. On the contrary, plants grown under limiting phosphate availability exhibited lower accumulations for all nutrients measured in shoot, regardless of the foliar-applied treatments, due the strong inhibition of shoot biomass yield. Likewise, foliar application of potassium phosphite decreased the accumulation for the majority of nutrients

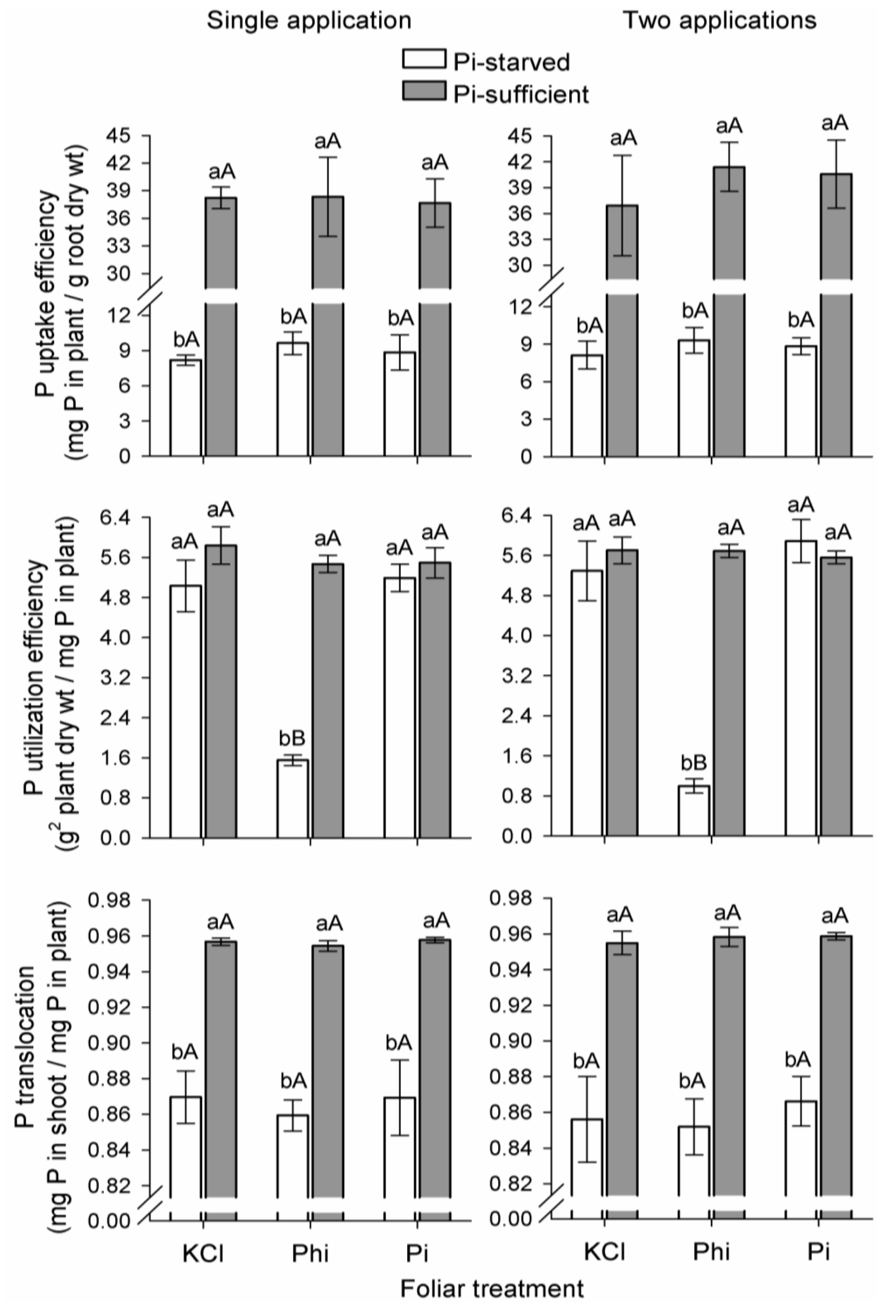

Figure 3. P uptake efficiency, P utilization efficiency, P translocation in common bean grown in Oxisol under 2 phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar supplied via foliar application application numbers (single and two applications). Averages followed by the same lowercase letter within soil phosphate levels and uppercase letter among foliar application for each phosphate level, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

in shoot of phosphate-starved plant, whereas for others nutrients the foliar treatment effects were not significant ($p > 0.05$). In general, foliar-applied phosphate has no effect on nutrient concentrations and accumulations in shoot. Hu et al. (2008) also did not find alterations in leaf P concentrations of maize plants submitted to foliar NPK applications. In this study the concentrations of nutrients in roots (data not shown) were not affected by application of foliar potassium phosphate and phosphite as well as by foliar application numbers, whereas the limiting phosphate availability in soil increased concentration and decreased accumulation of some nutrients.

Table 2. Concentrations of nutrients in common bean shoot grown in Oxisol under 2 phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar application numbers (single and two applications).

Application numbers	Soil P status	Foliar treatments	Macronutrients (g kg^{-1})					Micronutrients (mg kg^{-1})				
			N	**K**	**Ca**	**Mg**	**S**	**B**	**Zn**	**Cu**	**Mn**	**Fe**
A single foliar application timing	Pi-starved	KCl	47^{bA}	19^{bA}	13^{aA}	6^{bA}	2^{aA}	32^{abA}	57^{aA}	6^{bA}	61^{bA}	347^{bA}
		Phi	57^{aA}	22^{aA}	15^{aA}	9^{aA}	2^{aA}	37^{aA}	61^{aA}	8^{aA}	77^{aA}	511^{aA}
		Pi	46^{bA}	18^{bA}	14^{aA}	6^{bA}	2^{aA}	30^{bA}	58^{aA}	5^{bA}	58^{bA}	381^{bA}
	Pi-sufficient	KCl	33^{aB}	17^{aA}	9^{aB}	4^{aB}	1^{aB}	17^{aB}	30^{aB}	4^{aB}	39^{aB}	131^{aB}
		Phi	33^{aB}	17^{aB}	9^{aB}	4^{aB}	1^{aB}	17^{aB}	29^{aB}	3^{aB}	42^{aB}	183^{aB}
		Pi	31^{aB}	18^{aA}	8^{aB}	3^{aB}	1^{aB}	16^{aB}	35^{aB}	4^{aB}	37^{aB}	167^{aB}
Two foliar application timings	Pi-starved	KCl	46^{bA}	20^{bA}	14^{aA}	6^{bA}	2^{aA}	31^{abA}	52^{aA}	5^{bA}	55^{bA}	334^{bA}
		Phi	55^{aA}	23^{aA}	16^{aA}	8^{aA}	2^{aA}	36^{aA}	53^{aA}	7^{aA}	73^{aA}	559^{aA}
		Pi	45^{bA}	19^{bA}	13^{aA}	6^{bA}	2^{aA}	29^{bA}	51^{aA}	5^{bA}	62^{abA}	310^{bA}
	Pi-sufficient	KCl	29^{aB}	18^{aA}	9^{aB}	3^{aB}	1^{aB}	17^{aB}	32^{aB}	4^{aB}	40^{aB}	186^{aB}
		Phi	34^{aB}	19^{aB}	8^{aB}	4^{aB}	1^{aB}	16^{aB}	34^{aB}	3^{aB}	38^{aB}	176^{aB}
		Pi	32^{aB}	19^{aA}	7^{aB}	4^{aB}	1^{aB}	16^{aB}	36^{aB}	3^{aB}	41^{aB}	148^{aB}
Source of variation:												
Foliar application numbers (A)			ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns
Soil P status (B)			***	***	***	***	***	***	***	***	***	***
Foliar treatments (C)			***	*	ns	***	ns	**	ns	***	*	***
A × B			ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns
A × C			ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns
B × C			*	*	ns	**	ns	*	ns	***	*	***
A × B × C			ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns

In each number of foliar application (a single application timing and two application timings), lower case compare the foliar application products (KCl, Phi and Pi) for each soil phosphate level (Pi-starved and Pi-sufficient), and upper case compare the soil phosphate levels for each foliar application product. Means followed by same letter are not different by Tukey's test ($p \leq 0.05$). *, **, ***, and ns corresponding to $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and non-significant, respectively, by F test.

Conclusion

Common bean growth under limiting phosphate availability in soil exhibited lower biomass yield and higher concentration of nutrients in shoot tissues, which may be due to concentration effect since accumulation of nutrients in shoot was not increased. Either one or two foliar sprays of potassium phosphate were not sufficient to affect the growth and nutrition of the common bean, regard-less of soil P status. However, the results exhibit foliar-applied potassium phosphite causes harmful effects in phosphate-starved common bean, but no effect is observed in phosphate-sufficient common bean, confirming earlier investigations with other plant species. Our results indicate phosphite anion may not be recommended as a P source for nutrition of common bean, but it is suitable to be used for other purposes that requires an optimum soil phosphate status.

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