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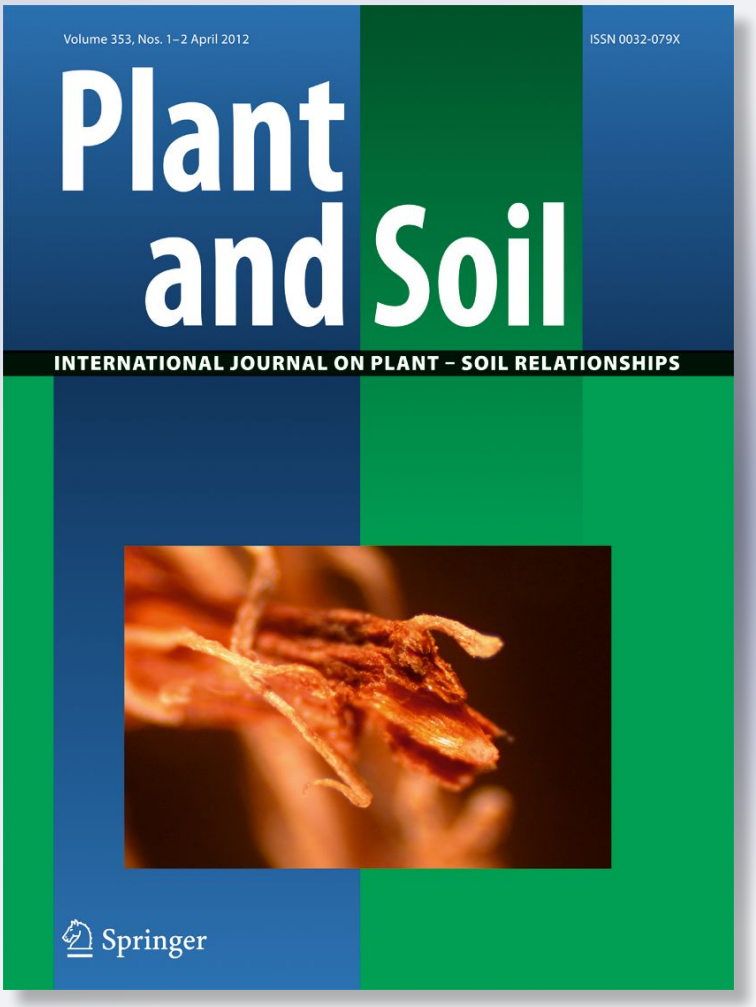

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REGULAR ARTICLE

Contribution of phosphorus (^{32}P) absorption and remobilization for *citrus* growth

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Abstract
Background and aims Phosphorus (P) is a mobile nutrient in the plant so growth depends on its internal remobilization and a plant's ability to respond to its availability in the growing media. This study was conducted to evaluate the influence of P status and rootstocks on the patterns of P uptake and remobilization in orange trees.
Methods Sweet orange trees on Cleopatra mandarin (CM) or Rangpur lime (RL) rootstocks were grown for nine months in nutrient solution (NS) that was either P-deficient (DNS) or was P-sufficient (SNS). After this period, half of the trees were reciprocally transferred between DNS and SNS (from D to S and S to D), while the others remained in their initial P availability.
Results Trees on RL had more shoot and root growth, accumulated more P and had greater efficiency of P absorption and transport to the shoot (PAE) than those on CM. The major source of P for growth was previously stored P even with an adequate current P supply to the roots. This suggested the dominance of P remobilization over P uptake and the requirement that trees had sufficient stored P to meet P demand of new growth. Trees on CM had greater concentrations of remobilized P in new shoots than trees on RL.
Conclusion Trees grafted on rootstocks less able to take up P (CM) were more dependent on the internal reserves of P for new growth than rootstocks with higher PAE (RL).

Keywords Nutritional stress · Nutrient solution · Rootstock varieties · Root growth · Uptake efficiency

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Introduction

Phosphorus (P) is remobilized and then retranslocated in the phloem to meet P demand of new tissues in response to plant growth and/or to limited P availability in the soil (Lauer and Blevins 1989; Peng and Li 2005; Fife et al. 2008). Thus, enhanced remobilization of internal P in plants is considered to be an important mechanism to overcome transient P

deficiency stress and to improve P utilization efficiency (Schachtman et al. 1998; Hammond et al. 2004). On the other hand, high remobilization rates of P can contribute to leaf senescence (Usuda 1995; Lim et al. 2007), resulting in defoliation and reduced photosynthetic potential.

Since the P status of plants affects the rates of nutrient uptake by the roots (Jungk et al. 1990; Morcuende et al. 2007), there are different patterns of nutrient remobilization in P deficient plants compared to well-nourished plants (Marschner et al. 1996). In citrus trees, P is stored mainly in leaves (<6-month-old), new twigs (<1.5 cm diameter) and roots (Mattos et al. 2003) and an important fraction of P can be remobilized to the flowers (Sanz et al. 1987). Thus, there can be a strong association between P remobilization rates and the total amount of P accumulated in the trees with adequate fruit yields. Under limited P availability, trees develop leaves with increased size, and dull brownish-green color which might fall off prematurely from stems beginning from the base to the apex. In tree fruit crops, rootstock varieties are an additional factor that defines P accumulation since they differ in nutrient acquisition capability and ability to transport P to shoots (Wutscher 1989; Taylor and Dimsey 1993; Quaggio et al. 2004). This results in rootstock differences in dependency on mycorrihizae for P nutrition (Graham and Syvertsen 1985) and also in distinct responses of trees to P fertilization.

Trees on Rangpur lime had greater fruit yield at low P rates than those on Cleopatra mandarin in a P deficient soil (Mattos et al. 2006) indicating that these rootstocks differed in their adaptation to low-P availability. However, the patterns of P remobilization under varying P availability of trees on contrasting rootstocks have not been evaluated. Such data would be useful to improve our understanding of P demand and P use efficiency in citrus and other long-lived subtropical evergreen trees.

We hypothesized that rootstock varieties with contrasting P acquisition capacity would also differ in their dependence on P remobilization to meet new growth demand. We tested if the P nutritional status of the trees would affect the pattern of remobilization and uptake of P using the radioisotope ^{32}P. Trees were grown in P deficient or P sufficient nutrient solution and then labelled with ^{32}P to distinguish the fraction of P coming from nutrient solution (current uptake) from that previously stored in tree tissues.

Materials and methods

Trees were grown for a total of approximately 11 months, from October 2007 to September 2008, in an unshaded greenhouse with average day/night temperatures of 36°C/23°C. Maximum photosynthetically active radiation (PAR) was approximately 1500 μmol m^{-2} s^{-1} and relative humidity varied from 30% to 100%. Uniform, 1 year-old nursery trees of 'Pêra' sweet orange [*Citrus sinensis* (L.) Osbeck] on either Cleopatra mandarin (*Citrus reshni* hort. ex Tanaka, CM) or Rangpur lime (*Citrus limonia* Osbeck, RL) rootstocks were obtained from a commercial nursery, bare-rooted and supported in non-draining pots of 8 L of ¼ strength Sarruge's complete basic nutrient solution (BNS, Sarruge 1975) but without P for establishment. Each pot with solution contained one tree and was equipped with a tube extending to the bottom through which air was continuously bubbled for aeration. Solution pH was adjusted to 5.5–5.7 using 0.1 mol L^{-1} KOH and hydroponic solutions were replaced every 7–10 days. Ten days after transplant, the ¼ BNS was replaced by full strength BNS [in mmol L^{-1}, 9.6 N (11% as NH_4^+), 3.0 K, 4.5 Ca, 1.2 Mg, 1.2 S and, in μmol L^{-1}, 41.6 B, 54.0 Fe, 8.2 Mn, 2.5 Zn, 1.1 Cu and 1.0 Mo] that was either P deficient (D)=0.05 mmol L^{-1} or P sufficient (S)= 1.0 mmol L^{-1} of P. There were 6 replicate trees of each rootstock for each P treatment, 24 trees total. Trees were grown under these conditions for 180 days when the P concentration in the D treatment was reduced to 0.005 mmol L^{-1} while the 1.0 mmol L^{-1} P concentration in the S treatment was maintained for an additional 90 days. This low P adjustment was adopted to impose a moderate P deficiency stress but to avoid severely stunting P deficiency stress that could limit citrus responses to P (Misson et al. 2005).

After 255 days of P treatments, trees were slightly pruned to stimulate new shoot growth. Fifteen days after pruning, half of each D or S group of trees was maintained in D or S treatments, whereas the other half was changed to D or S nutrient solutions. Thus, the P treatments were DD, DS, SS and SD on both rootstocks and were arranged in a randomized design (four P treatments x two rootstocks combination) with three replicate trees. At the same time, solutions were labelled with ^{32}P using 14.8 MBq per pot. Thereafter, hydroponic solutions were not replaced but resupplied only once after 24 days with BNS nutrients

(without P) to bring electric conductivity to original values (1.7–1.8 mS cm^{-1}). The experiment was terminated 24 days later after a total ^{32}P labeling of 48 days.

Five- to six-new flush shoots were allowed to grow on each tree during the ^{32}P treatments. Trees were harvested and separated into new leaves, new twigs, flowers and fruits developed after ^{32}P labeling, and old leaves, old twigs, trunk that were present before ^{32}P labeling and entire root system. Tissues were rinsed with deionized water, oven-dried at 60°C for at least 72 h, dry weight (DW) determined, and subsequently ground and digested in nitric-perchloric acid for the colorimetric determination of total P (Bataglia et al. 1983). A 20 mL aliquot of the nitric-perchloric acid extract was used to determine the specific activity of ^{32}P in tree parts (P derived from nutrient solution) using the Cerenkov effect in a liquid scintillation counter with quenching correction by channel relation (Nascimento Filho and Lobão 1977).

^{32}P concentration (μg g^{-1}) in different parts of the trees was calculated by dividing the total ^{32}P accumulated by tissue DW. Efficiency of P absorption and transport to the shoot (PAE) was calculated as total ^{32}P accumulated in the shoot: PAE (mg g^{-1}) = (total ^{32}P accumulated in the shoot, mg)/(root DW, g). P remobilization was either expressed as absolute amount of P remobilized to the new shoots (mg $tree^{-1}$), or based on the total P previously stored in the tree (original shoot plus roots) before ^{32}P labeling, and calculated as fraction remobilized (%) = [(total remobilized P, mg $tree^{-1}$)/(total previously stored P, mg $tree^{-1}$)]×100. The contribution of remobilized P per unit of DW produced in new shoots (remobilized P concentration, mg g^{-1}) was also calculated.

Data were analyzed using a two-way variance analysis with 4 P treatments×two rootstocks and three replicates trees per treatment. When interaction terms were significant, the effects of P treatments within rootstocks and the effects of rootstocks within P treatments were compared using Duncan's multiple range and F test at the 0.05 probability level, respectively. When interaction term was not significant and main factors (P treatments and/or rootstocks) were significant, Duncan's multiple range and F test at the 0.05 probability level were run to separate means of the main factors. Linear correlation was used to describe the relationships between the selected variables.

Results

Growth and phosphorus nutrition

A significant interaction ($p<0.05$) between P treatments and rootstocks on tree growth at tree harvest (48 days after ^{32}P labeling) was only observed for new shoot DW (sum of new leaves, new twigs, flowers and fruits DW) (Table 1). The DD treatment limited new shoot growth by 31–56% compared to SS trees. The RL trees produced 1.9–3.1 fold more new shoot DW than CM trees across all P treatments. The DW of old shoots (sum of old leaves, old twigs and trunk DW) did not differ among P treatments, but trees on RL had 1.8 fold more old shoot DW than trees on CM (Table 1). Furthermore, root growth of trees on RL was 31% greater than those on CM when averaged across P treatments. Root:shoot DW ratio, except for the DD treatment, was up to 67% higher for CM trees, and the DD trees had root:shoot DW ratio 25–50% greater compared to SS trees (data not shown).

The effects of P treatments on total P concentration (mg g^{-1}) in the new growth (new leaves, new twigs, flowers and fruits) and roots differed between rootstocks as indicated by the significant interactions ($p<0.05$) between P treatments and rootstocks (Table 2). On the other hand, this significant interaction was not observed for old leaves. The lowest P concentrations were found for lowest P treatment, the DD trees. Since P availability was diminished in SD treatment, except for flowers and old leaves in both rootstocks and fruits for trees on RL, P concentration decreased in relation to SS treatment. The opposite occurred to DS trees in which P concentration was increased compared to DD treatment (Table 2). Similar effects of P treatments on total P concentration in old twigs and trunk were also observed, with the greatest difference ($p<0.05$) between DD and SS trees (data not shown). For instance, using the average of rootstocks, total P concentration ranged from 0.6 to 1.4 and 0.4 to 1.0 mg g^{-1} for old twigs and trunk, respectively. Furthermore, comparisons between new leaves vs. old leaves and new twigs vs. old twigs, demonstrated that new tissues had higher P concentrations.

In the same treatment, reproductive tissues (flowers and fruits) had higher total P concentrations than vegetative tissues (new leaves and new twigs) especially for flowers, which had a higher P concentration than fruits (Table 2). When averaged across P treatments, trees on RL accumulated 1.5 fold more total P than those on

Table 1 Growth of sweet orange trees on Cleopatra mandarin (CM) or Rangpur lime (RL) rootstocks at harvest time under varying P availability in the nutrient solution

P treatments/rootstocks	Dry weight (g tree^{-1})[a]			
	New shoot		Old shoot	Roots
	CM	RL	Rootstocks average	
DD	18.3±$^{0.2}$ bB	35.3±$^{4.8}$ bA	100.3±$^{13.3}$ a	60.4±$^{5.7}$ ab
DS	26.6±$^{1.7}$ aB	57.9±$^{2.0}$ aA	104.7±$^{12.5}$ a	67.4±$^{3.6}$ a
SD	15.3±$^{0.3}$ bB	47.7±$^{3.7}$ aA	105.9±$^{14.4}$ a	44.8±$^{2.9}$ c
SS	24.0±$^{1.4}$ aB	54.9±$^{2.1}$ aA	108.3±$^{13.9}$ a	51.1±$^{3.3}$ bc
			P treatments average	
CM			75.7±$^{2.3}$ b	48.5±$^{2.4}$ b
RL			133.9±$^{2.6}$ a	63.3±$^{3.0}$ a

[a] Significant interaction ($p<0.05$) between P treatments and rootstocks only occurred for new shoot. D and S are P deficient and sufficient nutrient solution (NS), respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. P treatments comparison: means ($n=3\pm$SE or $n=6\pm$SE) followed by different lowercase letters within columns are significantly different by the Duncan's multiple range test ($p<0.05$). Rootstocks comparison: means ($n=3\pm$SE or $n=12\pm$SE) followed by different uppercase letters across paired columns or by lowercase within columns are significantly different by the F test ($p<0.05$)

CM (307.7 mg tree^{-1} vs. 199.9 mg tree^{-1}), and SS trees accumulated 2.6 fold more P than DD trees (349.9 mg tree^{-1} vs. 136.1 mg tree^{-1}) for averaged rootstocks treatments. The pattern of total P accumulation for all tree

Table 2 Total phosphorus (P) concentration in different parts of sweet orange trees on Cleopatra mandarin (CM) or Rangpur lime (RL) rootstocks at harvest time under varying P availability in the nutrient solution

P treatments	P concentration (mg g^{-1})[a]					
	CM	RL	CM	RL	CM	RL
	New leaves		New twigs		Flowers	
DD	1.6±$^{0.06}$ cA	1.0±$^{0.03}$ dB	1.3±$^{0.02}$ cA	0.8±$^{0.02}$ dB	2.3±$^{0.15}$ bA	1.8±$^{0.09}$ dB
DS	2.3±$^{0.06}$ bA	2.3±$^{0.03}$ bA	1.9±$^{0.02}$ bA	1.5±$^{0.05}$ cB	3.5±$^{0.29}$ aA	3.2±$^{0.12}$ cA
SD	2.4±$^{0.06}$ bA	2.1±$^{0.03}$ cB	1.8±$^{0.11}$ bA	2.1±$^{0.15}$ bA	3.4±$^{0.09}$ aB	4.2±$^{0.11}$ aA
SS	2.6±$^{0.09}$ aA	2.4±$^{0.06}$ aA	2.9±$^{0.04}$ aA	2.6±$^{0.16}$ aA	3.9±$^{0.20}$ aA	3.7±$^{0.09}$ bA
	Old leaves		Roots		Fruits	
	Rootstocks average					
DD	0.7±$^{0.05}$ c		0.8±$^{0.02}$ dA	0.8±$^{0.08}$ dA	2.0±$^{0.05}$ cA	1.3±$^{0.02}$ bB
DS	1.1±$^{0.07}$ b		1.1±$^{0.04}$ cA	1.3±$^{0.04}$ cA	2.7±$^{0.14}$ bA	2.5±$^{0.05}$ aA
SD	1.4±$^{0.09}$ ab		1.6±$^{0.11}$ bA	1.9±$^{0.08}$ bA	2.6±$^{0.01}$ bA	2.4±$^{0.10}$ aA
SS	1.6±$^{0.11}$ a		2.0±$^{0.07}$ aB	2.7±$^{0.07}$ aA	3.0±$^{0.11}$ aA	2.6±$^{0.02}$ aB
	P treatments average					
	1.4±$^{0.10}$ A	1.0±$^{0.07}$ B				

[a] Significant interaction ($p<0.05$) between P treatments and rootstocks occurred for all tree parts, except old leaves. D and S are P deficient and sufficient nutrient solution (NS), respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. P treatments comparison: means ($n=3\pm$SE or $n=6\pm$SE) followed by different lowercase letters within columns are significantly different by the Duncan's multiple range test ($p<0.05$). Rootstocks comparison: means ($n=3\pm$SE or $n=12\pm$SE) followed by different uppercase letters across paired columns are significantly different by the F test ($p<0.05$)

parts was well-correlated with DW (r=0.82 to 0.99; p< 0.0001; n=24).

Accumulation of ^{32}P

Significant interactions (p<0.05) between P treatments and rootstocks were found for total ^{32}P accumulated (mg tree^{-1}) (Fig. 1a), efficiency of P absorption and transport to the shoot (PAE, mg of ^{32}P in the shoot g^{-1} root DW) (Fig. 1b) and ^{32}P concentration (μg g^{-1}) in various trees tissues, except for old leaves (Table 3).

DS trees and trees on RL had 62–85% and 119–151% more currently taken up P (labelled ^{32}P) than SS trees and trees on CM, respectively (Fig. 1a). Furthermore, based on P applied per pot (248 mg) in DS and SS treatments, CM and RL trees took up 8–16% and 21–35%, respectively, of the total amount of the P in the nutrient solution during labeling period. The pattern of ^{32}P accumulation in the entire tree was also proportional to growth as indicated by correlation between DW and ^{32}P accumulated for pooled data of DS and SS treatments (r=0.90; p<0.0001; n=12). The PAE in DS trees increased 55–60% compared to SS trees and trees on RL had 105–112% higher PAE than those on CM (Fig. 1b).

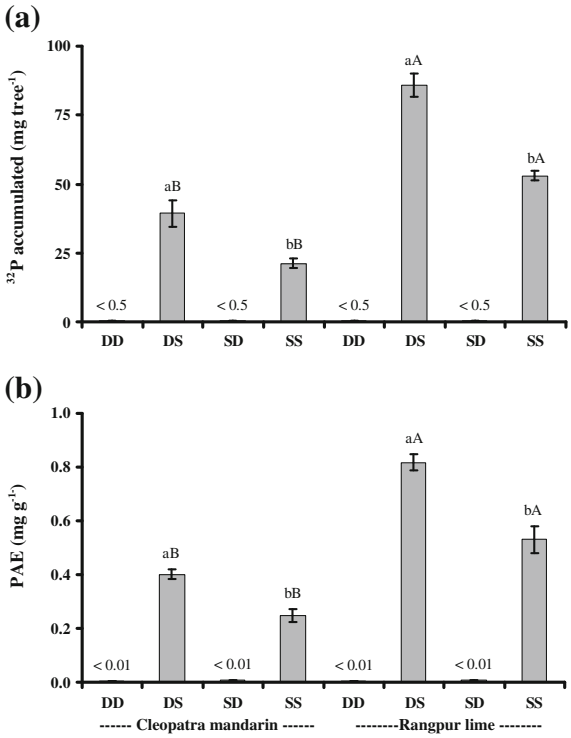

Fig. 1 ^{32}Phosphorus (^{32}P) accumulated **a** and efficiency of P absorption and transport to the shoot (PAE) **b** of sweet orange trees on Cleopatra mandarin or Rangpur lime rootstocks subjected to varying P availability in the nutrient solution (NS). D and S are P deficient and sufficient NS, respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. Significant interaction (p<0.05) between P treatments and rootstocks occurred for both ^{32}P accumulated and PAE. P treatments comparison: columns followed by different lowercase letters within the same rootstock are significantly different by the Duncan's multiple range test (p<0.05). Rootstocks comparison: columns followed by different uppercase letters for the same P treatment are significantly different by the F test (p<0.05). The bars indicate the standard-error (n=3)

Except for old leaves and fruits, trees on CM had higher ^{32}P concentration in the lowest P (DD) treatment, whereas trees on RL had greater values in the highest P (SS) treatment (Table 3). With the exception of the roots, ^{32}P concentration for trees on both rootstocks was much higher in the DS than in the SS treatment. ^{32}P concentration was greater in new leaves and new twigs than in old leaves (Table 3) and old twigs (data not shown). Furthermore, this currently taken up P was preferentially allocated to flowers rather than to new leaves after resupplying P in the DS treatment. The differences in ^{32}P concentration between new leaves and flowers corresponded to 70% and 32% for trees on CM and RL, respectively (Table 3). The trunk represented the least important tissue for currently taken up P accumulation, as indicated by its lowest ^{32}P concentration (data not shown) compared to other tree parts.

Phosphorus remobilization in trees on different rootstocks and of differing P status

The fraction of P remobilized (%), total P remobilization (mg tree^{-1}) and the contribution of remobilized P per unit of DW produced in new shoots (mg g^{-1}) showed significant interactions (p<0.05) between P treatments and rootstocks (Figs. 2a-b, 3).

Approximately 20–31% and 24–38% of the total amount of P previously stored in CM and RL trees, respectively, was remobilized to new shoot growth (Fig. 2a). Total P remobilization was higher in RL than CM trees except for the comparison in the lowest P (DD) treatment (Fig. 2b). This remobilization was greater in SD and SS than DD and DS treatments for trees on RL and greatest in the SS treatment for trees on CM.

The contribution of remobilized P per unit of DW produced in the new shoots was higher for trees on CM than those on RL except for new twigs in SD

Table 3 ^{32}Phosphorus (^{32}P) concentration in different parts of sweet orange trees on Cleopatra mandarin (CM) or Rangpur lime (RL) rootstocks at harvest time under varying P availability in the nutrient solution

P treatments	^{32}P concentration (μg g^{-1})[a]					
	CM	RL	CM	RL	CM	RL
	New leaves		New twigs		Flowers	
DD	4.8±$^{0.1}$ cA	3.3±$^{0.3}$ cB	4.1±$^{0.2}$ cA	1.9±$^{0.2}$ cB	5.6±$^{0.6}$ cA	3.6±$^{0.2}$ cB
DS	340.5±$^{35.6}$ aB	698.1±$^{9.1}$ aA	515.2±$^{80.5}$ aA	497.6±$^{19.0}$ aA	579.8±$^{41.7}$ aB	921.4±$^{28.0}$ aA
SD	5.9±$^{0.7}$ cA	3.4±$^{0.3}$ cB	5.6±$^{0.4}$ cA	3.3±$^{0.1}$ cB	8.4±$^{0.1}$ cA	5.1±$^{0.2}$ cB
SS	133.5±$^{7.8}$ bB	295.4±$^{24.2}$ bA	158.2±$^{10.8}$ bB	384.4±$^{65.5}$ bA	148.0±$^{19.9}$ bB	231.0±$^{28.8}$ bA
	Old leaves		Roots		Fruits	
	Rootstocks average					
DD	1.4±$^{0.1}$ c		5.5±$^{0.2}$ cA	4.0±$^{0.4}$ cB	4.9±$^{0.5}$ cA	4.4±$^{0.4}$ cA
DS	224.9±$^{9.7}$ a		208.3±$^{8.4}$ bB	335.1±$^{3.7}$ bA	508.5±$^{31.2}$ aB	706.3±$^{49.6}$ aA
SD	2.3±$^{0.4}$ c		3.9±$^{0.5}$ cA	3.0±$^{0.3}$ cA	6.1±$^{0.5}$ cA	3.7±$^{0.2}$ cB
SS	136.0±$^{13.0}$ b		299.8±$^{0.8}$ aB	398.1±$^{38.4}$ aA	242.1±$^{24.5}$ bA	256.3±$^{50.1}$ bA
	P treatments average					
	88.7±$^{24.5}$ A	93.6±$^{27.0}$ A				

[a] Significant interaction ($p<0.05$) between P treatments and rootstocks occurred for all tree parts, except old leaves. D and S are P deficient and sufficient nutrient solution (NS), respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. P treatments comparison: means ($n=3\pm$SE or $n=6\pm$SE) followed by different lowercase letters within columns are significantly different by the Duncan's multiple range test ($p<0.05$). Rootstocks comparison: means ($n=3\pm$SE or $n=12\pm$SE) followed by different uppercase letters across paired columns are significantly different by the F test ($p<0.05$)

treatment and flowers in SD and SS treatments (Fig. 3). Within each rootstock, this contribution was higher in the SD and SS than in the DD and DS treatments. Remobilized P concentration was 42–58% and 44–105% greater for flowers than new leaves for trees on CM and RL, respectively (Fig. 3). Based on the data of Table 2 and Fig. 3, remobilized P represented 91–97% and 87–92% of the total P contained in each unit of DW produced in new shoot parts (new leaves, new twigs, flowers and fruits) of trees on CM and RL, respectively, when averaged across P treatments.

Discussion

Sweet orange trees on RL had more vigorous growth than those on CM (Table 1), supporting the idea that rootstock varieties can markedly affect tree growth (Georgiou 2000, 2002; Giorgi et al. 2005). The greater DW produced in the new shoot of trees on RL resulted in its higher P demand than those on CM as RL trees accumulated greater amounts of ^{32}P (Fig. 1a). Similarly, differences in P uptake by citrus rootstock seedlings (Syvertsen and Graham 1985) and N uptake by grapefruit trees on two contrasting rootstocks (Lea-Cox and Syvertsen 2001) have been attributed to variations in the vigor of vegetative and fruit development that resulted in distinct nutrient demands. The greater root growth of RL trees also contributed to the increased P uptake, since there was positive correlation between ^{32}P accumulated and root DW when DS and SS treatments were pooled ($r=0.95$; $p<0.001$; $n=12$). Furthermore, trees on RL had a more efficient root system to meet the shoot demand for P than trees on CM as demonstrated by the greater PAE of RL trees (Fig. 1b) and lower root:shoot ratio. This higher P uptake per unit of root DW also led to greater capacity of RL trees for acquiring P as supported by the correlation between PAE and ^{32}P accumulated in the DS and SS treatments ($r=0.91$; $p<0.001$; $n=12$). Thus, the more vigorous root growth of RL trees associated with its greater PAE probably relates to better adaptation of these trees to low-P soils compared to CM trees. This has been demonstrated previously by

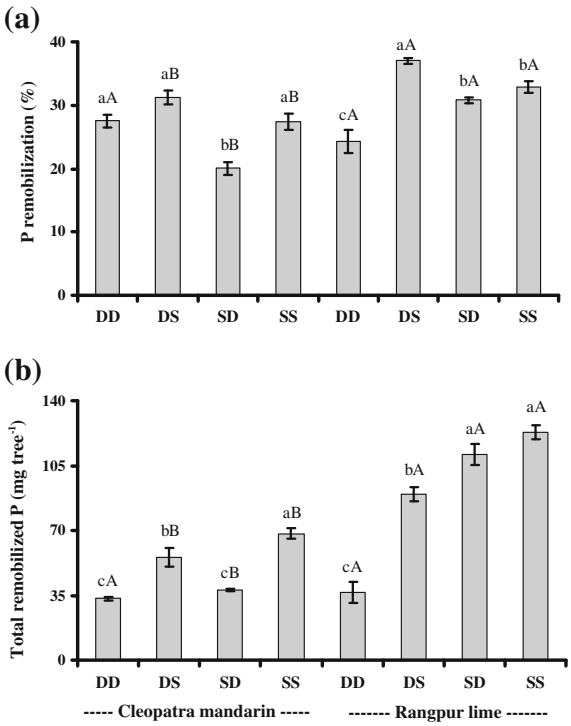

Fig. 2 Phosphorus (P) remobilization **a** and total remobilized P **b** of sweet orange trees on Cleopatra mandarin or Rangpur lime rootstocks subjected to varying P availability in the nutrient solution (NS). D and S are P deficient and sufficient NS, respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. Significant interaction ($p<0.05$) between P treatments and rootstocks occurred for P remobilization and total remobilized P. P treatments comparison: columns followed by different lowercase letters within the same rootstock are significantly different by the Duncan's multiple range test ($p<0.05$). Rootstocks comparison: columns followed by different uppercase letters for the same P treatment are significantly different by the F test ($p<0.05$). The bars indicate the standard-error ($n=3$)

the superior performance of RL trees in low-P soils during the first years after planting in the field (Mattos et al. 2006), when P deficiency symptoms in the foliage are more frequent. The DS trees had higher ^{32}P concentration (Table 3) as well as higher accumulated ^{32}P (Fig. 1a) and PAE (Fig. 1b) than SS trees underscoring the effect of P nutritional status on the pattern of P uptake. The increased capacity of P uptake in P deficient plants is an important adaptation response to low P stress and probably resulted from an increased density of high affinity transporters at the root surface (Liu et al. 1998; Raghothama 1999, 2000; Karthikeyan et al. 2002).

Currently taken up P (labelled ^{32}P) was preferentially allocated to the new shoots (greater ^{32}P concentration) rather than to old shoots especially in DS trees (Table 3). This suggests that in P deficient trees in which the P availability was increased, the proportional allocation of recently taken up P to young tissues increased as a result of greater growth demand of these tissues and the high mobility of P into the trees (Jeschke et al. 1997; Fife et al. 2008). Despite the preferential allocation of labelled ^{32}P to the new shoots, there was large dependence on previously stored P to meet new growth demand, reflecting the elevated rates of P reallocation in these citrus trees. Up 20% of total P previously accumulated was remobilized from old tissues to new shoot growth (Fig. 2a). Even in SS trees with continuously high P, the participation of remobilized P to the total amount of P accumulated per unit of new shoot DW (vegetative and eproductive organs), corresponded to 92-97% and 85-95% for trees on CM and RL, respectively. Moreover, in low P DD and SD trees, this participation of remobilized P in the new shoot growth accounted for up to 99%. Under these conditions, the restricted supply of P from roots to shoot was compensated for by increased P remobilization which likely involved the mobilization of inorganic P stored in the vacuoles and/or the solubilization of P contained in organic forms and its subsequent translocation to new tree parts (Mimura 2001; Rausch and Bucher 2002).

Trees on RL had higher absolute P remobilization (mg tree^{-1}) than those on CL (Fig. 2b), probably as a result of more vigorous new shoot growth of the former (Table 1), since the total amount of P remobilized per tree was proportional to new shoot DW ($r=0.83$; $p=0.001$; $n=24$). The sink strength acts as a primary control on the nutrient remobilization to new growth (Nambiar and Fife 1991) and is probably related to the reduction in P concentration in old tissues (Helmisaari 1992). In the present study, when citrus trees were subjected to P deficiency stress after being grown with sufficient P (SD treatment), P concentration of old tissues (old twigs, trunk and roots) decreased 20-42% compared to SS trees, with the highest difference occurring in roots (Table 2). In addition, the amount of remobilized P is strongly related with nutrient content (nutritional status) immediately before the remobilization (Saur et al. 2000; Milla et al. 2005). For instance, the total P concentration in old leaves at tree harvest was positively

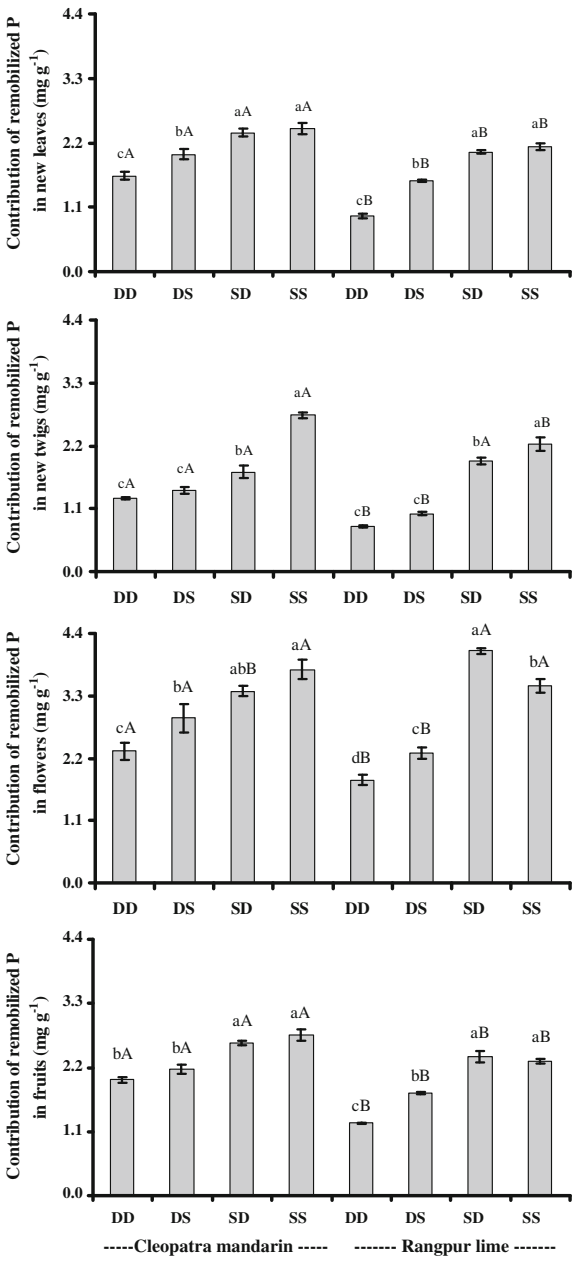

Fig. 3 Contribution of remobilized P per unit of dry weight produced in new shoot parts of sweet orange trees according to P treatments in the nutrient solution (NS) and rootstocks. D and S are P deficient and sufficient NS, respectively. DD, DS, SD and SS are management of P supply, in which the first and second letter refers to P availability in the NS before and after labeling with ^{32}P, respectively. Significant interaction ($p<0.05$) between P treatments and rootstocks occurred for the contribution of remobilized P in all new shoot parts. P treatments comparison: columns followed by different lowercase letters within the same rootstock are significantly different by the Duncan's multiple range test ($p<0.05$). Rootstocks comparison: columns followed by different uppercase letters for the same P treatment are significantly different by the F test ($p<0.05$). The bars indicate the standard-error ($n=3$)

correlated with the amounts of remobilized P when DS and SS treatments were pooled ($r=0.45$; $p=0.03$; $n=12$).

Although trees on RL had higher amounts of remobilized P, trees on CM tended to have a greater concentration of remobilized P in the new shoot parts (Fig. 3). Taking into account that RL trees had higher amounts of stored P in old parts and also more vigorous growth, this smaller contribution of remobilized P per unit of new shoot DW in RL trees than in CM trees was probably related to a greater capacity of RL roots to take up P to meet new shoot demand for P (higher PAE, Fig. 1b). Therefore, trees on CM appear to be more dependent on the pool of previously stored P to support new shoots than trees on RL at the same regime of P supply. In support of this, we confirmed that acid phosphatase activity in mature leaves of trees on CM grown in a low-P soil was significantly greater than the activity determined in the leaves of trees on RL (Zambrosi and Mattos unpublished). This reflects the greater need of P remobilization through the action of acid phosphatase activity in the leaves in response to the relatively low ability of CM roots to acquire P and to meet shoot demand. There was also more remobilized P concentration in the new shoots of DS trees than in SS trees (Fig. 3) indicating that increased PAE in the roots of P deficient DS trees resulted in reduced dependence of previously stored P to meet new shoot demand when adequate P supply was re-established. Furthermore, these differences might have also been a consequence of the greater amount of total P accumulated in the old tissues of SS trees in relation to DS throughout the experiment, which maximized the participation of remobilized P in the construction of new shoots in SS trees.

Since there was a greater participation of remobilized P than currently taken up P in the formation of new shoots, our results suggested that there is a need for citrus trees to be previously well-nourish to meet P demand of new growth even with adequate current P supply. This would explain the observations that severe symptoms of P deficiency in young citrus orchards diminished only after one to two years of fertilizer P application in the field. Thus, currently taken up P was less important than the pool of previously stored P in the tree to meet the current demand of new flushes. In the case of reproductive organs, even when there was sufficient current P supply (DS and SS treatments), the remobilized P represented 82-97% and 72-95% of the P present per unit of DW

in flowers and fruits of trees on CM and RL, respectively. These results along with the high total P concentration in reproductive organs (Table 2) emphasize the high P demand for flower and fruit formation (Guardiola et al. 1984) and the dependence on adequate levels of previously stored P to support reproductive growth. This dependence was also supported by the fact that during the period of new growth (48 days of ^{32}P treatments), CM and RL trees with sufficient P (DS and SS treatments) took up a maximum of 16% and 35%, respectively, of the total supplied P.

Conclusions

Trees on Rangpur lime (RL) were more efficient in meeting P shoot growth demand than those on Cleopatra mandarin (CM), which supports previous observations for a higher requirement of *Citrus* on CM than on RL for P fertilization during the first years after planting in the field. Thus, any fine tuning of *Citrus* fertilization should account for differences in P efficiency of rootstock varieties. This difference in P efficiency was also reflected in the pattern of P remobilization, since trees on CM were more dependent on remobilized P to meet the demand of new shoots than were trees on RL. Overall, based on the dependence of remobilized P for to the construction of new flushes, trees must have sufficient amounts of stored P to meet P growth demand, even with adequate current supply of the nutrient to the roots. Our results suggest also that remobilization of P might be more important than P uptake to meet the demands of new vegetative and reproductive growth. As a result, P supply management should enable trees to accumulate adequate amounts of P in order to sustain spring/summer flushes growth during the next season.

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