Australian Journal of Crop Science AJCS
AJCS 5(12):1542-1549 (2011) ISSN:1835-2707

Effects of phosphorus fertilization on growth, leaf mineral concentration and xylem-phloem nutrient mobility in two rootstocks of prunus (*Prunus persica* × *Prunus amygdalus*) and (*Prunus insititia*) in the Mediterranean area

R. M. Balal[1*], V. Gimeno[2], M. A. Shahid[3], V. Lidon[4], A. L. Garcia[5], T. Abbas[6], F. Garcia-Sanchez[2] and U. Ghazanfer[1]

[1]University College of Agriculture, University of Sargodha, (Punjab) Pakistan
[2]Dpto. Nutrición Vegetal. Centro de Edafología y Biología aplicada del Segura. CSIC. Campus Universitario de Espinardo. 30100. Espinardo. Murcia. Spain
[3]Department of Horticulture, College of Agriculture and Life Sciences, Cornell University, New York, USA
[4]EPSO (Univ. Miguel Hernández), Ctra. Beniel km 3,2 03312 Orihuela (Alicante), Spain
[5]Dpto. Química Agrícola. Facultad de Química. Universidad de Murcia. P.O. Box 4021 Murcia, Spain
[6]Institute of Horticultural Sciences, University of Agriculture Faisalabad, (Punjab) Pakistan

***Corresponding author. uaf_adnan777@yahoo.com**

Abstract

The study was conducted to investigate the effects of phosphorus fertilization on growth parameters, leaf mineral concentration and xylem and phloem mineral mobility ratio in two clonal *prunus* rootstocks grown in a greenhouse experiment. Trees of hybrid GF677 (*Prunus persica* × *Prunus amygdalus;*) and Pollizo Puebla de Soto 101 (*Prunus insititia*; PP101) were grown in containers with 350 kg of a typical soil from SE Spain (Xeric torriorthen derived from marl). Phosphorous was applied to plants in the form of monoamonic phosphate in five different doses i.e 0.124, 0.248, 0.744, 2.976 and 14.88 g plant^{-1}. Application of P fertilization in the root medium significantly increased the trunk perimeter, total leaf dry weight and total shoot length in GF677 trees but not in PP101 trees. The leaf P, K, Ca, Mg, N and Mn concentrations were also elevated in both rootstocks (GF677 and PP101). However, the leaf Fe, Cu and Zn concentrations were not significantly influenced by P fertilization. In the xylem of both rootstocks the mobility rates were higher for P, N, Mg and K in relation to the phloem mobility rate. On the other hand, the mobility rates of Zn, Mn and Fe were much lower than in the phloem. The higher leaf Ca, Mg and K concentration in GF677 trees was related with both the high mobility of these nutrients in the xylem and the low mobility in the phloem. Based on this experiment, it seems that the *prunus* rootstocks GF677 and PP101 are not sensitive to iron chlorosis by increasing the P in the root zone and in addition, the GF677 improved the growth parameters in response to enhanced P level in soil.

Keywords: plant nutrition, peach, plum, iron chlorosis, micronutrients.
Abbreviations: GF677-Prunus hybrid (PH), PP101-*Prunus insitiata* (PI).

Introduction

In Spain, peach and nectarine occupy the significant position among the cultivated fruit trees and their production is around 29% of the European Union, being the second largest European producer after Italy. Peaches and nectarines trees are principally propagated through budding the desired varieties on various rootstocks (Rom and Carlson, 1987). A wide range of rootstocks is available for grafting and budding of peach and nectarine. All these rootstocks have varying degrees of tree vigour and growth, salt and/or drought tolerance (Fulton et al., 1996; Kramer and Boyer, 1995), resistance to pest and diseases (Rom and Carlson, 1987) and leaf mineral concentration (Boyhan et al., 1995; Rahman et al., 2011; Rokhzadi and Toashih, 2011). In the Mediterranean countries, rootstock breeding programs are also active in the release of new genotypes (De Jong et al., 2004) by crossing various genotypes i.e. almond × peach hybrids. These hybrids give excellent performance because these are more vigorous and compatible with poor dry soil with high $CaCO_3$ concentration which can induce Fe chlorosis (Socias I Company, R., 1995). Beside the effect of rootstock on plant development and foliar levels of mineral nutrients, specific crop systems such as irrigation rate and/or fertilization are also particularly important in the adaptation of peach cultivation to different growth condition (Caboni and Monastra, 1998). The application of phosphorus (P) fertilizer can result in considerable enhancement of crop growth. However, the excessive amount of phosphorus could cause iron (Fe) deficiency in the leaves due to the precipitation of ferric phosphate in the apoplast of the root (Bienfait et al., 1985), Zn deficiency (Cakmak and Marschner, 1987) and alterations in the NO_3^- and Mo status (Villora et al., 2002). By the contrary, if phosphorus availability is scarce in the soil can cause severe P deficiency in plants (<0.14%, Marschner et al., 1995) diminishing aerial plant growth by reducing the leaf photosynthetic activity (Halsted and Lynch, 1996). Despite the importance of P fertilization in crop, the interaction between rootstock and nutrients has been little studied.Root is the unique higher plant organ which is responsible for mineral nutrition. Xylem is the principal pathway for supplying the shoot with the mineral elements essential for growth. The xylem per se is inadequate for this function and a subtle co-operation between root and shoot is required for supplying growing organs with the required nutrient elements (Jeschke and Hartung, 2000). In trees, mineral analysis of the xylem fluid alone does not provide much information due to several reasons: a) the transport and storage of the nutrients distributed through the mature parts of stems and roots (Thomidis and Tsipouridis, 2005; Jeschke and Pate, 1995), b) complex phloem-xylem and xylem-phloem transfers in leaves and stems (Kuhn et al., 1997), and c) fluctuations in the concentration of xylem nutrients related with the soil's water status and transpiration (Dambrine et al., 1995). Thus in this experiment, we studied the interaction effect between P fertilization and prunus rootstocks on growth parameters, leaf mineral concentration and "mobility rates" of the nutrients; the capacity of the nutrients to be taken up and transported within both the xylem and phloem (Khan and Zende, 1977). A study of these mobility rates will permit us to evaluate the processes of nutrient transport to show the propensity of different rootstock to carry out this task and to evaluate the effect of irrigation and fertilization levels, which will be of greater practical significance than the data obtained by the direct analysis in xylem and phloem fluids.

Results

Plant growth parameters

Table 1 shows the growth parameters measured in this experiment. For PH trees, P fertilization in the root medium tended to increase the trunk perimeter, total leaf dry weight, and total shoot length. The highest values were obtained with the trees treated with 446.4 g of P, although for the trunk perimeter, the differences among P treatments of 22.32, 89.28 and 446.4 g were statistically non-significant. Meanwhile, for PI trees, the trunk perimeter, total leaf dry weight, and total shoot length were not significantly affected by P fertilization. The number of chlorotic leaves increased with the application of 22.32 and 89.28 g P, for PH trees and with application of 5.7 g P for PI trees.

Leaf mineral concentration

Significant interactions between the rootstock and P fertilization were observed for leaf concentration of K, Zn, Mn, Fe and Cu (Figs. 1 and 2). Application of P fertilizers in the root medium did not affect the leaf K concentration in PI trees, however, in PH trees, application of 5.7g P decreased the K concentration although the difference was not significant in comparison to that of 2.8 and 22.32g P (Fig. 1). The leaf Zn concentration was not affected by P fertilization in PH trees. The prunus PH roosтstock is a mediterranean-adapted tree which can grow in calcareous soil, whereas the antagonism Zn x P only is observed occasionally only when P fertilization is applied in excessive and during a long time period), but in PI trees, the concentration increased with the 5.7 g relative to other P treatments. The highest concentration of Mn was observed with the 89.28 g P application which differs non significantly with 22.32 and 446.4 g dose of P for PH trees and with the 22.32 and 5.7 g P dose for PI trees. The highest concentration of Fe was obtained with the 22.32 g of P (not significant with 446.4 g P application) for PH trees, and with the 5.7 g of P (not significant with 22.32 and 89.28 g of P) for PI trees. In case of the leaf Cu concentration, the highest values were observed in the 2.8, 22.32 and 446.4 g of P treatments (there were not significant differences among them) for PH trees, and with the 2.8 and 89.28 g of P treatments for PI trees (Fig. 2). On the other hand, PH trees had a higher leaf concentration of P, Ca, Mg, Cu and lower leaf concentration of K and Zn, regardless the irrigation rates. Leaf N concentration was not significantly affected by the cultivars or P fertilization (data not shown).

Xylem and phloem mineral mobility rate

The only significant interaction between the rootstock and P fertilization observed in the xylem and phloem macronutrients mobility rate (phloem-P), whereas for PH trees the 5.7 g of P application increased the mobility rate in relation to other P treatments, meanwhile P fertilization did not affect the mobility rate for PI trees. PH trees had higher xylem-Ca, xylem-Mg, phloem-K and lower phloem-Ca, phloem-Mg and xylem-K mineral mobility rate than PI trees, regardless P treatments (Table 2). On the other hand, the xylem-P mobility rate was the highest with 2.8 g P treatment, and the lowest with the 446.4 g of P treatment (not significant with 5.7 and 22.32 g of P) in both PH and PI trees. In the xylem and phloem micronutrient mobility rate significant interactions between rootstock and P fertilization were observed in xylem-Na, phloem-Na, phloem-Zn, xylem-Fe, phloem-Fe, and xylem-Cu. In the case of xylem-Na, phloem-Na, phloem-Zn and xylem-Fe, P fertilization did not affect these mobility rates for PI trees (Table 3). However, for PH trees, the 2.8 g and 446.4 g P treatments increased the xylem-Na mobility rate, 5.7 g P treatment increased the phloem-Na and phloem-Zn mobility rates, and 22.32 g P treatment increased the xylem-Fe (not significantly different with 2.8 and 89.28 g P treatments). The phloem-Fe and xylem-Cu mobility rates were not affected by P fertilization in PH trees, but in PI trees 2.8 g P treatment increased both mobility rates relative to other P treatments.

Discussion

It is well known that the rootstock type can determine the size, production and fruit quality in peach trees since the rootstocks differ in their ability to uptake water and nutrients (Giorgi et al., 2005). According to previous results, it has been shown that the GF677 variety (almond × peach hybrid) has a great potential like rootstock in peach trees since trees grafted on this rootstock gave higher yields, good fruit quality and girth expansion than the other rootstocks (Tsipouridis and Thomidis, 2005). The screening techniques for tolerance to iron chlorosis have also showed that the plum Puebla de Soto 101 and the hybrid GF677 have a low degree of chlorosis (Romera et al., 1991). In our experiment, we have demonstrated that P fertilization can interact with the less available micronutrients in calcareous soil, although the effects of the P fertilization depend on rootstock nature. Although the low available micronutrients in the calcareous soil resulted in very low leaf and root Fe, Mn and Zn concentrations, regardless of the P fertilization supply and rootstock, the growth parameters was increased by P fertilization in PH trees but not in PI trees. This data indicated that under calcareous soil PH trees had a best skill

Table 1. Effect of phosphorus fertilization on the trunk perimeter (cm), total leaf dry weight (g), total shoot length (cm) and the number of chlorotic leaves in PH (GF677) and PI (Puebla de Soto) trees at the end of experimental period.

Treatment		Trunk perimeter	Total leaf D.W.	Total shoot L.	Number of chlorotic leaves
Rootstock	P Fertilzer				
PH	2.8	9.7 cd	200.2 cd	1787 c	421 d
	5.7	6.9 d	91.3 d	738 c	145 d
	22.32	13.2 ab	558.3 b	6673 b	2582 b
	89.32	13.3 ab	609.7 b	6337 b	103 d
	446.4	14.6 a	835.6 a	9092 a	4360 a
PI	2.8	11.5 abc	232.1 c	1892 c	2546 b
	5.7	11.1 bc	180.3 cd	1485 c	4574 a
	22.32	10.0 bcd	128.9 cd	1311 c	1612 bc
	89.32	11.3 abc	215.6 c	1782 c	1115 cd
	446.4	11.5 abc	197.3 cd	1681 c	1575 bc
ANOVA	R	ns	***	***	**
	P Fertilzer	**	***	***	***
	R × P Fertilzer	**	***	***	***

Different letters in each rootstock × P fertilization interaction represent significant difference at P = 0.05 level based on Duncan's multiple range test. ns, **, *** indicate non-significant differences and significant differences at P < 0.01 and 0.001, respectively.

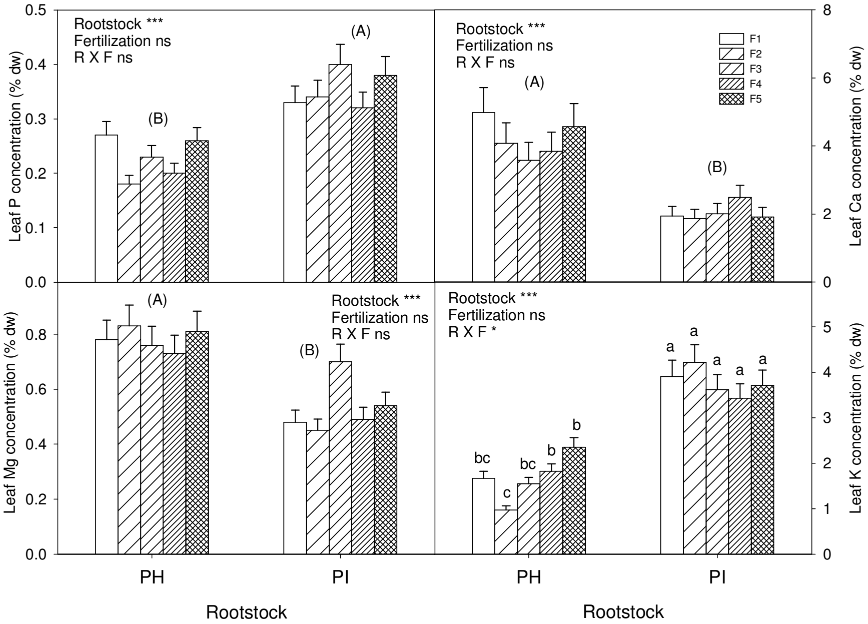

Fig 1. Effect of phosphorus fertilization on the leaf macronutrient (P, Ca, Mg and K) concentration in leaves of PH (GF677) and PI (Puebla de Soto) trees at the end of experimental period. Each value is the mean of 4 repetitions. ns, *, *** indicate non-significant differences or significant differences at P < 0.05 and 0.001, respectively. Significant differences (P < 0.05) between rootstocks x P treatments interaction are denoted with different lower case letters. Differences between rootstocks are indicated by different upper case letters, regardless nitrate supplementation treatment.

of adaptation since they were more vigorous than PI when they were treated with a high range of P (446 g in the two years). In addition, PH trees had a lower leaf Zn and Cu concentration than PI trees, however, as indicated above, the growth parameters were higher; therefore, these trees showed a lesser degree of sensitivity to this soil conditions. Moreover, chlorosis susceptibility, measured by counting the leaves with the visual symptoms, was affected by P fertilization, although there was not a clear trend as the highest chlorotic leaf number was found with the 5.7 g and 89.28 g of P treatment for PH trees and in 5.7 g P treatment for PI trees. In other experiments, phosphate has also been considered as a factor inducing chlorosis, but this seems to be dependent on the species or the cultivars. Romera et al. (1991) did not observe effects of the phosphate concentration on the chlorosis induction in GF677 and Puebla de Soto trees growing in a hydroponic system. Samar et al. (2007) also observed in apple trees under calcareous soil that high available P in the soil did not increase the chloloris symptoms. Leaf analysis showed high values for the elements of P, K, Ca, Mg, N and Mn in both PH and PI rootstocks. In contrast, there was little Zn, Fe, Cu in leaves (Thomidis et al., 2007), regardless the rootstock or the P fertilization level, indicating that calcareous soil cause micronutrients deficiency in the leaves even when the P fertilization is very low (Bienfait et al.,1985; Cakmak and Marschner, 1987). On the other hand, the leaf P concentration was not affected by the P fertilization level for both PH and PI trees. In PH trees,

Table 2. Effect of phosphorus fertilization on xylem and phloem P, N, Ca, Mg and K mobility ratio in trees of PH (GF677) and PI (Puebla de Soto) at the end of experimental period.

		P		N		Ca		Mg		K	
Main Factor	Treatments	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem
Rootstock	PH	2.14 b	1.04 a	1.60	0.61	2.22 a	0.84 a	2.32 a	0.22 a	3.86 a	0.48 a
	PI	4.00 a	0.59 b	1.61	0.55	0.75 b	1.38 b	1.53 b	0.35 b	7.92 b	0.27 b
Fertilization	2.8	5.07 a	0.83	1.66	0.58	1.78	1.07	1.92	0.28	5.80	0.36
	5.7	2.39 b	1.14	1.41	0.62	1.23	1.18	1.76	0.30	5.62	0.45
	22.32	2.76 b	0.71	1.69	0.60	1.43	1.04	2.07	0.26	5.73	0.39
	89.32	1.96 b	0.75	1.77	0.51	1.26	1.18	1.84	0.34	5.70	0.38
	446.4	3.19 b	0.67	1.50	0.62	0.85	1.09	2.04	0.25	6.59	0.32
Interaction Rootstock × Fertilization											
PH	2.8	3.8	0.91 b	1.77	0.52	2.77	0.75	2.41	0.16	3.85	0.45
	5.7	1.14	1.78 a	1.3	0.67	1.68	0.92	2.11	0.33	1.71	0.65
	22.32	2.36	0.86 b	1.62	0.66	2.23	0.9	2.25	0.19	3.52	0.48
	89.32	1.45	0.82 b	1.82	0.52	1.68	0.93	2.25	0.22	4.67	0.44
	446.4	1.97	0.84 b	1.48	0.7	2.72	0.69	2.59	0.20	5.53	0.37
PI	2.8	6.33	0.74 b	1.55	0.64	0.79	1.38	1.43	0.39	7.74	0.26
	5.7	3.64	0.49 b	1.52	0.56	0.78	1.44	1.41	0.27	9.53	0.24
	22.32	3.16	0.56 b	1.75	0.53	0.62	1.18	1.89	0.32	7.94	0.29
	89.32	2.46	0.68 b	1.71	0.5	0.84	1.42	1.43	0.46	6.73	0.32
	446.4	4.41	0.49 b	1.52	0.54	0.72	1.48	1.48	0.30	7.65	0.26
ANOVA	R	***	***	ns	ns	***	***	***	***	***	***
	P Fertilzer	***	**	ns	ns	ns	ns	ns	ns	ns	ns
	R × P Fertilzer	ns	**	ns	ns	ns	ns	ns	ns	ns	ns

Different letters in each rootstock x P fertilization interaction represent significant difference at P = 0.05 level based on Duncan's multiple range test. ns, **, *** indicate non-significant differences or significant differences at $P < 0.01$, 0.001, respectively.

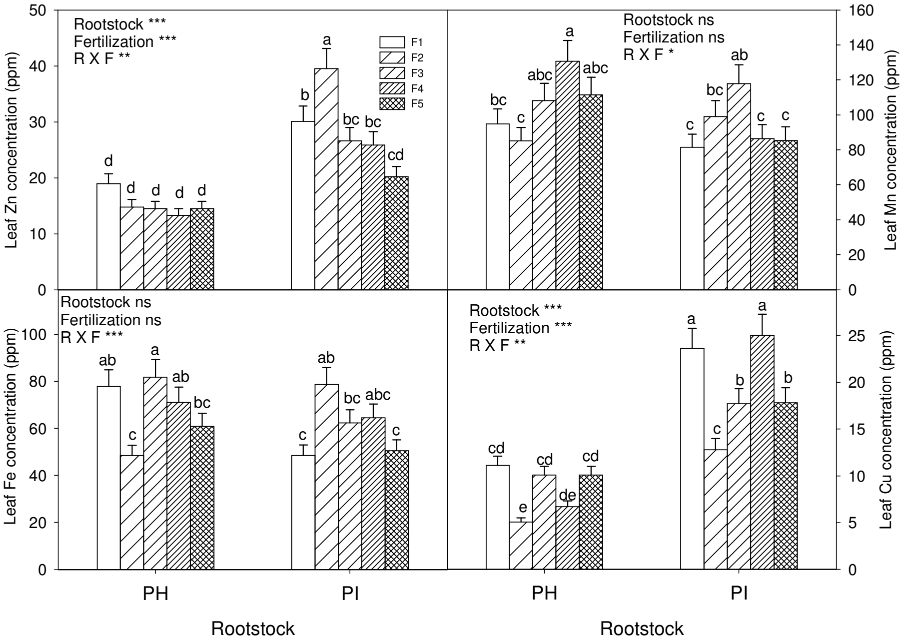

Fig 2. Effect of phosphorus fertilization on the leaf micronutrient (Zn, Mn, Fe and Cu) concentration in leaves of PH (GF677) and PI (Puebla de Soto) trees at the end of experimental period. Each value is the mean of 4 repetitions. ns, *, **, *** indicate non-significant differences or significant differences at P < 0.05, 0.01, 0.001, respectively.

maybe this could be due to that the increase in the growth parameters, especially total leaf dry weight, by P fertilization treatments caused a decrease in P concentration by dilution effect. In PI trees, low Fe deficiency in leaves and roots could have affected physiological and biochemical processes (Fernandez et al., 2008) including alterations in P acquisition by the roots. Moreover, our data also confirmed that *prunus* rootstocks have different capacities for the mineral uptake, as PI trees had higher leaf P, K, Zn and Cu concentration but lower leaf Ca and Mg concentration than PH trees. In citrus, it has also been observed that their rootstocks differ in the fertilizer use efficiency (Mattos et al., 2006), whereas trees on 'Rangpur' lime demonstrated the ability to use N, P, and K more efficiently and consequently produced more fruits than trees on 'Cleopatra' mandarin and 'Swingle' citrumelo rootstocks. Although phloem/xylem partition processes reflect changes in the availability of water and nutrients (Warren and Adams, 200), the greater range of mobility of P in the phloem (Table2) shows the greater effectiveness of PH rootstock for the translocation of this element that compensated its low mobility through the xylem (Bienfait et al., 1985; Pate et al., 1998). The nitrogen shows significantly lower values of mobility in the xylem than the phosphorus and of the same order of magnitude in the phloem. Similar results were obtained in *Ecualiptus* by Pate et al. (1998) when studying the spatial distribution of nitrogen in the phloem fluid and also the role of its transport in the xylem and subsequent storage in woody plants (Jescchke and Pate, 1995). The greater mobility of calcium in the xylem in PH plants contrasts with the findings of Kuhn et al. (1997) in *Picea*, in which calcium levels were higher in the phloem fluid. The calcium levels in leaves and roots confirmed that the low concentration of this element in the leaves of PH was not due to the selective control of root absorption, but to the difficulties involved in its transport through the xylem and its great capacity for retranslocation (Kuhn et al., 1997). Magnesium mobility via the xylem was significantly greater in PH although the transpiration current in both rootstocks moved a greater quantity of nutrients than is strictly necessary for the plant (Mengel and Kirby, 2001). It has been remarked that in *Picea* the Mg content increases in young shoots and that there is a xylem-phloem interchange (Kuhn et al., 1997). The phloem of PI showed greater Mg mobility than that of PH, which coincides with the finding of three-fold higher levels in the phloem of Eucalyptus than in the xylem, the opposite occurring with Ca (Pate et al., 1998). As in the case of Ca the lower concentration of Mg in the leaves of the PI rootstock can be attributed both to the control of xylem transport (Westgate and Boyer, 1984) and to the greater translocation that occurs in the phloem (Hocking and Pate, 1978). Of all the nutrients, only K showed greatest mobility in the xylem, although this was significantly affected by the rootstocks type, being much higher in PI than in PH (Mengel and Kirby, 2001). The opposite occurred in the phloem (low levels but greater in PH). Both findings explain because the concentration of K in PI leaves was higher than in PH leaves, unlike observed for Ca and Mg. In the case of Fe, the mobility in the phloem was extremely low for PI trees, probably due to low loading as a result of the precipitation with the phosphate (Bienfait et al., 1985). However, its mobility in the phloem is not significantly affected by fertilization treatment. The higher values for phloem-Fe than xylem-Fe, demonstrating that there is absence of any transportation for this element (Hocking and Pate, 1978); Pate et al., 1998). Manganese mobility in the phloem showed values higher in PH than in PI trees, indicating the greater efficiency of translocation in PH (Pate et al., 1998). Also, the low mobility of Na in the xylem is not affected by fertilization treatment, but so by the rootstock; furthermore, the lower Na content in the leaves of PI rootstock in comparison to that of PH trees can only be explained by its control in the xylem countertransport. The

Table 3. Effect of phosphorus fertilization on xylem and phloem Na, Zn, Mn, Fe and Cu mobility ratio in trees of PH (GF677) and PI (Puebla de Soto) at the end of experimental period.

		Na		Zn		Mn		Fe		Cu	
Main Factor	Treatments	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem	Phloem
Rootstock	PH	0.13 a	2.00 a	0.45 b	2.25 a	0.82	0.43 a	0.06	0.82 a	0.12 b	1.34
	PI	0.07 b	1.51 b	0.89 a	0.86 b	0.77	0.21 b	0.05	0.56 b	0.35 a	0.82
Fertilization	2.8	0.12	1.79	0.64	1.62	0.67	0.33	0.06	0.87	0.33	0.74
	5.7	0.10	2.48	0.68	2.38	0.73	0.30	0.05	0.69	0.10	1.28
	22.32	0.09	1.58	0.82	1.28	0.85	0.35	0.06	0.56	0.31	1.40
	89.32	0.08	1.62	0.58	1.19	0.96	0.27	0.07	0.57	0.23	0.91
	446.4	0.13	1.32	0.63	1.30	0.76	0.35	0.05	0.79	0.21	1.10
Interaction Rootstock × Fertilization											
PH	2.8	0.18 a	1.58 bc	0.43	2.33 b	0.63	0.45	0.07 ab	0.65 abcd	0.15 cd	0.74
	5.7	0.10 b	3.89 a	0.33	4.10 a	0.59	0.40	0.03 d	0.96 abc	0.04 d	1.65
	22.32	0.10 b	1.60 bc	0.49	1.80 bc	0.85	0.47	0.08 a	0.6 abc	0.14 cd	1.92
	89.32	0.08 b	1.91 bc	0.54	1.33 bc	1.16	0.35	0.07 ab	0.79 abcd	0.08 d	1.34
	446.4	0.19 a	1.02 c	0.48	1.67 bc	0.85	0.48	0.05 bcd	1.11 a	0.17 cd	1.06
PI	2.8	0.06 b	2.00 b	0.85	0.90 c	0.70	0.21	0.04 cd	1.08 ab	0.50 a	0.73
	5.7	0.10 b	1.07 bc	1.03	0.66 c	0.87	0.20	0.06 abc	0.42 d	0.15 cd	0.9
	22.32	0.07 b	1.55 bc	1.15	0.75 c	0.85	0.23	0.04 cd	0.51 cd	0.47 ab	0.88
	89.32	0.08 b	1.32 bc	0.62	1.05 c	0.76	0.19	0.06 abc	0.35 d	0.37 b	0.47
	446.4	0.06 b	1.61 bc	0.78	0.93 c	0.67	0.21	0.05 bcd	0.46 d	0.24 c	1.14
ANOVA	R	***	*	***	***	ns	***	ns	*	***	**
	P Fertilzer	ns	**	ns	*	ns	ns	ns	ns	***	ns
	R × P Fertilzer	*	***	ns	**	ns	ns	**	**	**	ns

Different letters in each rootstock x P fertilization interaction represent significant difference at P = 0.05 level based on Duncan's multiple range test. ns, *, **, *** indicate non-significant differences or significant differences at P < 0.05, 0.01, 0.001, respectively.

highest mobility values in the phloem were observed for Zn, which showed low mobility in the xylem in PH. Both factors determining the low concentration measured in leaves and corroborating the antagonistic relation of this element with phosphorus (Cakmak and Marschner, 1987).

Material and methods

Plant material and growth condition

Two-year-old *Prunus*, Hybrid GF677 (*Prunus persica* x *Prunus amygdalus*; PH) and Pollizo Puebla de Soto (*Prunus insititia*; PI), based on the current Spanish legislation concerning varietal purity and sanitary state, were used in this experiment. In winter 2007, the trees were planted in 250 L plastic containers filled with 350 kg of an uncultivated soil, Xeric torriorthent derived from marl, having: 32.6% sand (0.05-2mm), 27% clay (<0.002mm), 92.1 mmol kg^{-1}CEC, 0.04% N_{total}, 1.71 $mgKg^{-1}$ available P (Watanabe and Olsen, 1965), 58.3% total $CaCO_3$, 10.2% active $CaCO_3$, 1.40 $g{\cdot}cm^{-3}$ bulk density. This volume of the containers was sufficient for the roots development during the two years of experimental period. The trees were grown in a plastic greenhouse placed in the Segura River valley, 38º5'N, 1º4'W, with a climatic characteristic of 36/16 ºC T max./min. and 70/50% RH max./min.

Treatments and experimental design

Plastic containers of 250 L capacity were arranged in 12 rows (each row comprised of 10 containers), with sufficient room between them to allow the canopy to grow. A drip line was run across all the containers with one self-compensating emitter in the centre of each container where the emitters provided 4 $L{\cdot}h^{-1}$. The flow rates were periodically checked throughout the experiment with coefficients in excess of 92% always being obtained. Four tensiometers of 40 cm length were installed at random to assess soil humidity. When the tensiometers reached values above 25 cb, all the plants were watered until the tensiometers readings were 0 cb. Water used in this experiment had the following characteristics : pH of 7.63, electrical conductivity of 0.88 $dS{\cdot}m^{-1}$, Cl^- of 1.50 $mmol{\cdot}L^{-1}$, SO_4^{-2} of 2.60 $mmol{\cdot}L^{-1}$, HCO_3^- of 2.15 $mmol{\cdot}L^{-1}$, Ca^{+2} of 1.80 $mmol{\cdot}L^{-1}$, Mg^{+2} of 2.20 $mmol{\cdot}L^{-1}$, Na^+ of 0.80 $mmol{\cdot}L^{-1}$ (where potassium and carbonates were not detected). After an adaptation period (February-April, 2003), during which the plants only received water, P fertilization treatments were started where monoamonic phosphate fertilizer ($NH_4H_2PO_4$) were applied as soil application in the followings five treatments: 0.124, 0.248, 0.744, 2.976 and 14.88 g of P per plant. During the first year each P treatment received 12 applications of fertilizer, which was increased to 18 applications during the second year in the case of 0.744, 2.976 and 14.88 g per plant. However, 0.124 and 0.248 g per plant treatments received 11 applications in an attempt to ensure that the leaf concentrations of phosphorus in the trees of these treatments were not as high as the levels recorded in the periodic controls carried out during the first year of the experiment. Therefore, the trees received a total of 2.8, 5.7, 22.32, 89.28 and 446.4 g of P per tree in two consecutive years. P application in each treatment was done periodically distributed equally throughout the year.

Growth parameters

At the end of the experimental period, the growth parameters of trunk perimeter (cm) was measured at the height of 10 cm above the ground, total fresh weight of leaf (g), total dry weight of leaf (g), total length of shoots (cm), and number of chlorotic leaves were measured in all the trees.

Mineral concentration

At the end of the experiment, the mineral concentration of N, P, Na, K, Ca, Mg, Fe, Mn, Zn and Cu in leaves, root and branch bark was determined. The N concentration was determined by the Kjeldahl method modified at a semimicro scale with 50 mg of dry leaf sample. Phosphorus was determined colorimetrically by measuring the yellowness of the phosphovanadate complex (Chapman and Parker, 1961). The remaining nutrients were determined by atomic absorption spectrometry (Haswell, 1991) (Ca, Mg, Fe, Mn, Zn and Cu; Perkin Elmer) or emission spectrometry (Allen, 1973) (Na and K; Perkin Elmer) after humid mineralization with a nitric-perchloric mixture of dry leaf material. Mineral concentration of chlorotic leaves was also measured at the end of the experiment. Nutrient mobility rate in the xylem was calculated as leaf nutrient concentration/root nutrient concentration and nutrient mobility rate in the phloem was calculated as bark nutrient concentration/leaf nutrient concentration (Khan and Zende, 1977).

Statistical analysis

As the design of this experiment was two factor factorial so data were subjected to analysis of variance using a two-way ANOVA (SPSS statistical package, Chicago, IL) with two rootstocks x five P treatments and twelve replicate plants per combination. When interaction term were significant ($P < 0.05$), treatment means were separated using Duncan's multiple range test. When interaction term was not significant and, main factors (P supplementation and/or rootstock) were significant, Duncan's multiple range test was run to the main factor regardless each other (Little and Hills, 1987).

Conclusions

This experiment shows that calcareous soil typical from the Region of Murcia cause a decrease in leaf Fe, Cu and Zn concentration in the prunus rootstocks GF677 and Puebla de Soto. Increasing P fertilization did not tend to decrease the leaf micronutrient concentrations but even increased the growth parameters in PH trees. Therefore, in these conditions, we can tell that PH trees are more tolerant to chlorosis induced by calcareous soil when treated with high P fertilization. High leaf Ca, Mg and low K concentration in PH trees was related with both the high mobility of these nutrients in the xylem and the low mobility in the phloem.

Acknowledgements

The authors want to thank Dr. Philip Thomas, English Associate Professor of the Murcia University, for the English correction of the manuscript.

References

Allen CW (1973) Astrophysical Quantities, 3rd edition. 109-172

Bienfait HF, Van den Briel W, Mesland-Mul NT (1985) Free space iron pool in roots. Plant Physiol 78: 596-600

Boyhan GE, Norton JD, Pitts JA (1995) Establishment, Growth, and Foliar Nutrient Content of plum trees on various rootstocks. HortSci 30: 219-221

Caboni E, Monastra F (1998) New almond selection as putative rootstocks for peach and almond. Second International Symposium on Pistachios and Almonds. Acta Hortic 470: 188-194

Cakmak I, Marschner H (1987) Mechanism of Phosphorus-Induced Zinc-Deficiency in Cotton. III. Changes in Physiological Availability of Zinc in Plants. Physiol Plant 70: 13- 20.

Dambrine E, Martin F, Carisey N, Granier A, Hällgren, JE (1995) Xylem sap composition: a tool for investigating mineral uptake and cycling in adult spruce. Plant Soil 7: 233-241

Chapman, H. D and F. Parker. 1961. Determination of NPK method of analysis for soil, plant and waters. Pvt. Div. Agri. Univ. California, U.S.A., pp. 150-179.

De Jong WS, Eannetta NT, De Jong DM, Bodis M (2004) Candidate gene analysis of anthocyanin pigmentation loci in the Solanaceae. Theor Appl Genet 108: 423-432

Fernandez V, Del Río V, Pumarino L, Igartua E, Abadía J, Abadía A (2008) Foliar fertilization of peach (*Prunus persica* (L.) Batsch) with different iron formulations: Effects on re-greening, iron concentration and mineral composition in treated and untreated leaf surfaces. Sci Hortic 117: 241-248

Fulton AE, Wildman WE, Begg EL, Huntington GL (1996) The evaluation and modification of physical soil problems. En Almond Production Manual. Div. Agric Nat Resour Public 3364, Univ. of California. USA.

Giorgi M, Capocasa F, Scalzo J, Murri G, Battino M, Mezzetti B (2005) The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach (cv. 'Suncrest'). Sci Hortic 107: 36-42

Halsted M, Lynch J (1996) Phosphorus responses of C-3 and C-4 species. J Exp Bot 47: 497-505

Haswell, SJ (1991) Atomic Absorption Spectrometry; Theory, Design and Applications. Elsevier, Amsterdam.

Hocking PJ, Pate JS (1978) Accumulation and distribution of mineral elements in annual lupins (*Lupinus albus* and *Lupinus angustifolius* L.). Aus J Agri Res 29: 267-280

Jeschke WD, Hartung W (2000) Root-shoot interactions in mineral nutrition. Plant Soil226: 57-69

Jeschke WD, Pate JS (1995) Mineral nutrition and transport in xylem and phloem of Banksia pronotes and proteaceous trees of dimorfic root morphology. J Exp Bot 46: 895-905

Khan AA, Zende GK (1977) The side for Zn-P interactions in plants. Plant Soil 46: 259-262

Khan AA, Zende GK (1977) The site for Zn-P interaction in plants. Plant Soil 46: 259-262

Kramer PJ, Boyer JS, (1995) Water relations of plants and soils. Academic Press, San Diego, California, USA. pp 495.

Kuhn AJ, Schroder WH, Bauch J (1997) On the distribution and transport of mineral elements in xylem, cambium and phloem of spruce (*Picea abies* [L.] Karst.). Holzforschung, 51: 487-496

Little, T.M., Hills, F.J., 1987. Metodos estadisticos para la investigacion en la agricultura. Trillas, Mexico, p. 270.

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press, London. pp. 299– 312

Mattos D, Quaggio JA, Cantarella H, Alva AK, Graetz DA (2006) Response of young citrus trees on selected rootstocks to nitrogen, phosphorus, and potassium fertilization. J. Plant Nutr 29: 1371-1385

Mengel K, Kirkby A (2001) Principles of Plant Nutrition. 5th Edition. Kluwer Academic Publishers. Dordrecht, The Netherlands. pp 849

Pate JS, Shedley E, Arthur D, Adams M (1998) Spatial and temporal variations in phloem sap composition of plantation-grown Eucalyptus globulus. Oecologia, 117: 312-322

Rahman, MM, Abdou AS, Fareed H, Al Darweesh, Sofian-Azirun M (2011) Responses of sulfur, nitrogen and irrigation water on *Zea mays* growth and nutrients uptake. Aus J Crop Sci 5: 347-357

Rokhzadi A, Toashih V (2011) Nutrient uptake and yield of chickpea (*Cicer arietinum* L.) inoculated with plant growth promoting rhizobacteria. Aus J Crop Sci 5: 44-48

Rom CR, Carlson RF (1987) Rootstocks for fruit crops. Wiley, New York.

Romera FJ, Alcantara E, Delaguardia MD (1991) Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. Plant Soil 130: 115-119

Samar SM, Shahabian M, Fallahi E, Davoodi MH, Bagheri YR, Noorgholipoor F (2007) Iron deficiency of apple tree as affected by increasing soil available phosphorous. J Plant Nutr 30: 1-7

Socias I Company R (1995) El germoplasma frutal (1ª Parte). Razones para una conservación. Fruticultura Profesional 74: 6-12

Thomidis T, Sotiropoulos T, Karagiannidis N, Tsipouridis C, Papadakis I, Almaliotis D Boulgarakis N (2007) Efficacy of three calcium products for control of peach brown rot. Horttechnol 17: 234-237

Thomidis T, Tsipouridis C (2005) Influence of rootstocks, pH, iron supply (in nutrient solutions) and Agrobacterium radiobacter on chlorophyll and iron concentration in leaves of a peach variety. J Plant Nutr 28: 1833-1842

Tsipouridis C, Thomidis T (2005) Effect of 14 peach rootstocks on the yield, fruit quality, mortality, girth expansion and resistance to frost damages of May Crest peach variety and their susceptibility on Phytophthora citrophthora. Sci Hortic 103: 421-428

Villora G, Moreno DA, Romero L (2002) Phosphorus supply influences the molybdenum nitrate and nitrate reductase activity in eggplant. J Horti Sci Biotech 77: 305-309

Watanabe FS, Olsen SR (1965) Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Proc Soil Sci Soc Am 26: 677–678

Westgate ME, Boyer JS (1984) Transpiration-Induced and Growth-Induced Water Potentials in Maize. Plant Physiol 74: 882-889

Warren CR, Adams MA (2000) Capillary electrophoresis for the determination of major amino acids and sugars in foliage: application to the nitrogen nutrition of sclerophyllous species. J Exp Bot 51: 1147-157