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Research Article
Responses of Plant Growth, Root Morphology, Chlorophyll and Indoleacetic Acid to Phosphorus Stress in Trifoliate Orange

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Abstract

Background and Objective: In citriculture, citrus trees are often exposed to low soil phosphorus (P) levels, resulting in low fruit production. The present study aimed to analyze the changes in plant growth, biomass production, root morphology, chlorophyll and root indoleacetic acid (IAA) of trifoliate orange (*Poncirus trifoliata* L. Raf.) in response to different substrate P stresses. **Methodology:** Five-leaf-old trifoliate orange seedlings were grown in plastic pots supplied with sands, accompanied with the Hoagland solution with no-P (0 mM P), low-P (0.1 mM P), adequate-P (1 mM P) and high-P (10 mM P) treatments for 8 weeks. **Results:** No-P, low-P and high-P treatments significantly decreased plant height, stem diameter, leaf number and shoot and root biomass as compared with adequate-P. An increased trend in root morphological traits (total length, surface area, projected area, volume, taproot length and No. of 1st and 2nd order lateral roots) was from 0-1 mM P levels and a decreased trend was from 1-10 mM P levels. Compared with adequate-P treatment, no-P, low-P and high-P treatments significantly decreased chlorophyll a and carotenoid level. In addition, low-P and high-P treatments significantly increased root IAA concentration relative to adequate-P. **Conclusion:** The results of this study suggested that low-P and high-P considerably inhibited plant growth and root development, which may be closely related with chlorophyll changes but not with root IAA levels.

Key words: Chlorophyll, citrus, high-P, P deficiency, IAA, lateral root, root morphology, trifoliate orange

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Roots are the interface between plants and soils and have the functioning on absorption of water and nutrients from soils. Root morphology is the spatial modeling and distribution of roots in soils, whose characteristics can directly affect the capacity of root absorption, further regulating growth of shoots[1]. Root development can be affected by lots of factors, including soil texture, soil nutrient status and soil microorganisms[2].

Phosphorus (P) is the necessary nutrient element in the process of plant growth. However, soil available P is relatively low. As a result, crops are often exposed to low soil P conditions[3]. In fact, plants also develop a large amount of adaption mechanisms to enhance P absorption, including root modification[4,5], release of root exudates and the establishment of mycorrhizal symbiosis[6,7]. In tobacco plants, low-P treatment (31.50 kg hm^{-2}) significantly increased the No. of 1st order lateral roots and adventitious roots[8]. Yan *et al.*[9] observed that low-P application resulted in shallow roots distributed in soils. In barley, root length reduced with the increase of soil P levels[10]. Such results suggested that P levels in substrates will strongly affect root responses in field crops. However, information about the response of citrus to P stress is poorly known.

In citriculture, trifoliate orange (*Poncirus trifoliata* L. Raf.) is the main rootstock used in Eastern Asian. The objective of this study was to analyze the effects of P stress on plant growth, root morphology, chlorophyll and root indoleacetic acid (IAA) levels of trifoliate orange seedlings.

MATERIALS AND METHODS

The five-leaf-old trifoliate orange without mycorrhization which grew in autoclaved sands under the condition of 28/20°C (day/night temperature) and 80% relative air humidity were transplanted into a plastic pot (upper diameter: 15.5 cm, bottom diameter: 11.0 cm, height: 13.0 cm). The pot was supplied with autoclaved sands ($\leq$ 4 mm size).

During 1 week of seedlings transplanting, 100 mL deionized water was supplied into each pot. Subsequently, 100 mL Hoagland solution with different P levels was added in the pot every 2 days. Phosphorus levels consisted of 0, 0.1, 1 and 10 mM KH_2PO_4, respectively. Herein 0, 0.1, 1 and 10 mM P were designed as no-P, low-P, adequate-P and high-P, respectively.

After 8 weeks of P treatments, all the seedlings were harvested. Before harvested, plant height, stem diameter and leaf number per seedling were determined. The collected roots from each pot were scanned with the Epson Perfection V700 Photo Dual Lens System (Seiko Epson Corp, Japan) and the images were then analyzed with the WinRHIZO software (Regent Instruments Incorporated, Canada) to obtain length, area, volume and diameter. And then, the roots were placed in laboratory table and counted for No. of 1st and 2nd order lateral roots.

Leaf chlorophyll concentration was determined using the method of Lichtenthaler and Wellburn[11] with 80% acetone extraction. Root IAA concentration was extracted with the protocol of Chen *et al.*[12] and was determined by the ELISA kits. These ELISA kits were provided by the Crop Chemical Control Center, the Engineering Research Center of Plant Growth Regulator, China Agricultural University.

Data were analyzed using one-way analysis of variance followed by DMRT test for comparisons among treatment means with a significance level of 5%.

RESULTS AND DISCUSSION

Plant growth: In this study, plant growth of trifoliate orange seedlings was dependent on substrate P levels (Table 1). Compared with adequate-P, no-P, low-P and high-P treatments significantly decreased plant height, stem diameter, leaf number and shoot and root biomass, respectively. In general, significantly higher plant growth among treatments ranked as adequate-P>no-P ≈ low-P ≈ high-P in plant height, adequate-P>low-P ≈ high-P>no-P in stem diameter, adequate-P>high-P$\geq$low-P>no-P in leaf number, adequate-P>high-P ≈ low-P ≈ no-P in shoot biomass and adequate-P>low-P>high-P ≈ no-P in root biomass in the decreasing order. This result implied that 1 mM P is the best P level in the substrate for plant growth of trifoliate orange

Table 1: Effects of substrate P levels on plant growth and biomass production of trifoliate orange seedlings

P treatments (mM)	Plant height (cm)	Stem diameter (mm)	Leaf number per plant	Fresh weight (g FW $plant^{-1}$)	
				Shoot	Root
0	11.02±0.70[b]	2.08±0.08[c]	9±0.91[c]	0.447±0.019[d]	0.161±0.007[d]
0.1	11.08±0.60[b]	2.29±0.04[b]	10±0.76[b]	0.515±0.005[c]	0.284±0.015[b]
1	13.98±0.69[a]	2.48±0.10[a]	14±0.50[a]	0.759±0.013[a]	0.387±0.020[a]
10	11.80±1.04[b]	2.28±0.10[b]	12±0.58[b]	0.598±0.018[b]	0.222±0.006[c]

Data (Means±SD, n = 4) followed by different letters indicated significant differences (p<0.05) between treatments

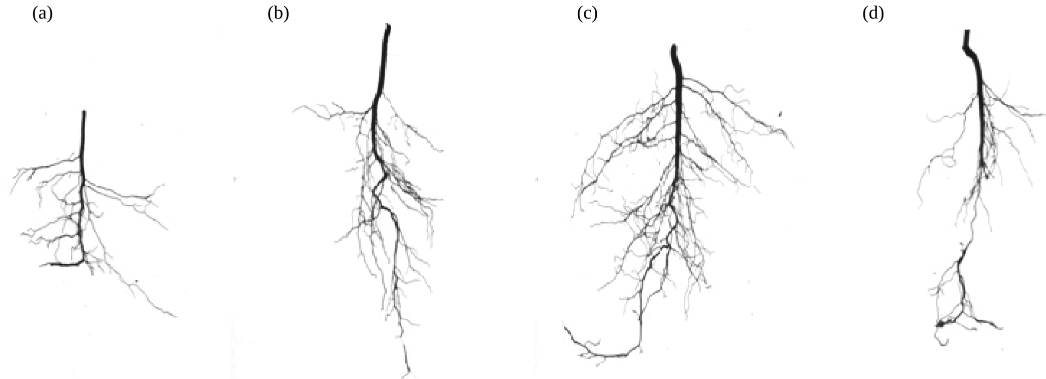

Fig. 1(a-d): Effects of substrate P levels on root morphology of trifoliate orange seedlings, (a) 0 mM P, (b) 0.1 mM P, (c) 1 mM P and (d) 10 mM P

Table 2: Effects of substrate P levels on root morphological traits and lateral root number of trifoliate orange seedlings

P treatment (mM)	Root morphological traits: Length (cm)	Project area (cm^2)	Surface (cm^2)	Average diameter (mm)	Volume (cm^3)	Taproot length (cm)	No. of lateral root: 1st order	2nd order
0	68 ± 3^{d}	3.05 ± 0.12^{d}	9.6 ± 0.39^{d}	4.50 ± 0.01^{b}	0.108 ± 0.004^{c}	6.42 ± 0.28^{c}	17 ± 2^{d}	38 ± 2^{c}
0.1	104 ± 3^{b}	4.70 ± 0.04^{b}	14.8 ± 0.13^{b}	4.50 ± 0.15^{b}	0.167 ± 0.007^{b}	10.72 ± 0.35^{b}	27 ± 1^{b}	45 ± 2^{b}
1	146 ± 11^{a}	6.48 ± 0.49^{a}	20.4 ± 1.53^{a}	4.44 ± 0.22^{b}	0.226 ± 0.023^{a}	12.23 ± 0.88^{a}	33 ± 2^{a}	67 ± 5^{a}
10	87 ± 2^{c}	4.16 ± 0.07^{c}	13.1 ± 0.23^{c}	4.81 ± 0.04^{a}	0.156 ± 0.002^{b}	11.50 ± 0.35^{b}	23 ± 1^{c}	35 ± 4^{c}

Data (Means±SD, n = 4) followed by different letters indicated significant differences ($p<0.05$) between treatments

seedlings. And, low-P or high-P heavily inhibited plant growth and biomass of trifoliate orange seedlings. Possibly, low-P and high-P do not provide the energy source for synthesis of membrane lipids and nucleic acids, which is associated with plant growth[13]. High-P also down-regulated the Pi transporters involved in net P uptake from rhizosphere[13].

Root morphology: The present study showed the increased trend in root morphological traits (total length, surface area, projected area, volume, taproot length and No. of 1st and 2nd order lateral roots) 0-1 mM P levels and the decreased trend 1-10 mM P levels (Table 2, Fig. 1). As reported by Li *et al.*[14], substrate P availability can considerably improve root morphology. The change in root morphology is an indicator to judge P response in plants[15]. Pan *et al.*[16] observed that low P could stimulate root growth and development but the stimulation is dependent on plant genotypes and substrate P concentrations. In this study, low-P and high-P treatments strongly reduced root morphology, indicating that trifoliate orange did not have the capacity to adapt low or high-P. It was also suggested that plant carbohydrates have less distributed into root systems for its growth under low-P and high-P conditions than under adequate-P[13]. This is consistent with the results of Wu *et al.*[17] in trifoliate orange.

Chlorophyll levels: Compared with adequate-P treatment, no-P, low-P and high-P treatments significantly decreased chlorophyll a level by 11.3, 27.7 and 18.7% and carotenoid level by 20.2, 31.1 and 15.5%, respectively (Fig. 2). For chlorophyll b, low-P treatment induced a significant decrease relative to adequate-P. Such changes in chlorophyll in this study are in agreement with *Larix olgensis* plants reported by Wu *et al.*[18] Generally, P participates in the process of photosynthesis. Low-P induced the increase in PSII close in maize plants and the decrease in both light energy conversion and electron transfer[19], which can explain the decrease of chlorophyll by low-P.

Root IAA concentrations: Compared with adequate-P treatment, low-P and high-P treatments significantly increased root IAA concentration by 32.8 and 13.7%, respectively (Fig. 3). There was no significant difference in root IAA level between no-P and adequate-P treatments. Marchant *et al.*[20] reported the positive effects of exogenous auxin on stimulating the formation and No. of lateral roots in *Arabidopsis*. On the other hand, when plants are subjected to environmental stresses, plants could develop a self-protection to adapt the stress[16]. Hence, in the present study, low-P and high-P resulted in the increment of root IAA, which is due to the transfer of shoot IAA into roots for its development. However, the best

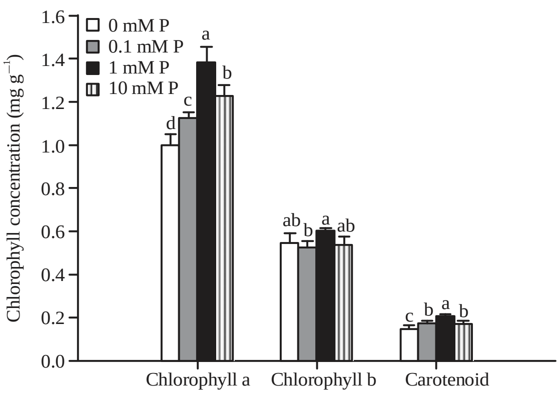

Fig. 2: Effects of substrate P levels on leaf chlorophyll concentration of trifoliate orange seedlings
Data (Means±SD, n = 4) followed by different letters indicated significant differences (p<0.05) between treatments

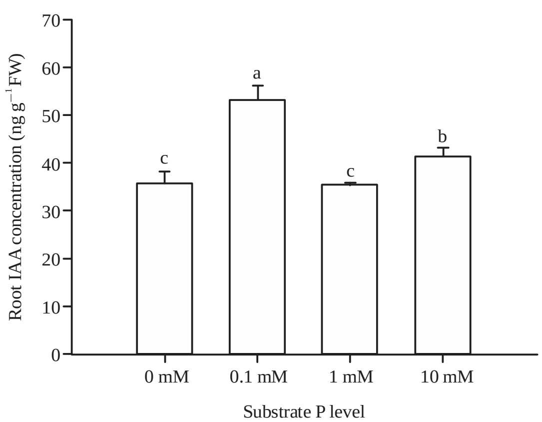

Fig. 3: Effects of substrate P levels on root IAA contents of trifoliate orange seedlings
Data (Means±SD, n = 4) followed by different letters indicated significant differences (p<0.05) between treatments

root morphology was found in the adequate-P-grew trifoliate orange seedlings. It seems that, besides IAA, other mechanisms, such as ethylene, might be involved in the root adapt to low and high P in trifoliate orange.

CONCLUSION

Trifoliate orange plants heavily depended on substrate P level for plant growth and root development. In general, trifoliate orange represented better plant growth and root morphology under 1 mM P level conditions than under the other P levels, which is closely related with chlorophyll changes but not with root IAA levels. As a result, in citriculture, citrus trees need to provide adequate soil P supply but not low or high P supply, for its growth, which is the key factor for the greater yield and quality in citrus fruits.

SIGNIFICANCE STATEMENTS

- This study tried to analyze the changes in plant growth, root morphology, chlorophyll and root IAA in trifoliate orange subjected to P stresses
- No-P, low-P and high-P treatments significantly decreased plant growth and root morphology, compared with adequate-P
- Compared with adequate-P treatment, no-P, low-P and high-P treatments significantly decreased chlorophyll a and carotenoid level
- Low-P and high-P treatments significantly increased root IAA concentration relative to adequate-P
- The inhibition of plant growth and root morphology by low or high P may be closely related with chlorophyll changes but not with root IAA levels

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