



Original Research Article

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Effects of phosphorus, indolebutyric acid and naphthylphthalamic acid on the lateral root growth of *Poncirus trifoliata*

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Article Info

Abstract

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To explore the influence of phosphorus (P), indolebutyric acid (IBA, Auxin) and Naphthylphthalamic acid (NPA, Auxin transport inhibitor) on plant lateral root (LR) formation, *Poncirus trifoliata* seedlings at two P levels, low P (LP) and control treatment (CK), which was applied with IBA and NPA, and the regulative effects of P level, IBA and NPA on LR formation of trifoliolate orange were investigated. The results showed that LP level significantly reduced the plant biomass, LR number and length. NPA significantly decreased the plant biomass, LR number and length, while IBA did not significantly influence these parameters. These data suggested that auxin signaling pathway could be involved in the regulation of P level on LR formation, and the auxin transportation should be the key factor in LR formation of trifoliolate orange.

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Introduction

Lateral roots are an important component of plant root morphology, and root architecture is largely determined by the length, branching angle and number of lateral roots (Nibau et al., 2008). Lateral root development is influenced by many factors, including biological and abiotic factors (Gruber et al., 2011). Phosphorus was involved in the regulation of lateral root formation, and low phosphorus stress inhibited the increase of the number and length of lateral roots in *Zea mays* maize (Li et al., 2012). Phosphorus increased lateral root density and number of lateral roots in the shallow root system of *Lupinus albus* (Péret et al., 2014).

Auxin plays an important role in plant development and

is an important internal factor regulating lateral root formation (Fukaki and Tasaka, 2009; Lavenus et al., 2013). Activation of pericycsts that form the lateral root primordia is dependent on the accumulation of auxin, with a peak of auxin at the site of lateral root occurrence during subsequent lateral root protrusion and elongation (Nibau et al., 2008). The addition of low concentration exogenous NAA significantly increased the number of lateral roots of white clover *Trifolium repens* cuts, while the addition of auxin transport inhibitors decreased the number of lateral roots (Dinh et al., 2012). Lateral root formation is highly plasticity and complex and is affected by a variety of environmental conditions. However, the regulation model of phosphorus level on lateral root formation and its differences, as well as the role of auxin in the regulation are not clear, and most

studies are based on herbaceous plants, and few studies are based on woody plants.

In this study, phosphorus and exogenous auxin were applied to regulate the formation of lateral roots of *Poncirus trifoliata* seedlings, so as to explore the regulation mode and differences of two factors on the formation of lateral roots.

Materials and methods

Materials

Poncirus trifoliata seeds are purchased from the market. The experimental substrate was red soil and river sand mixed at a volume ratio of 1:1. The basic physicochemical properties of red soil were as follows: pH 4.60 and organic matter. The contents were 1.58%, alkali-hydrolyzable nitrogen 66.1 mg kg⁻¹, available phosphorus 22.2 mg kg⁻¹ and available potassium 56.8 mg kg⁻¹.

The container was a plastic flowerpot (opening width 12 cm, height 14 cm). The red soil and river sand were sterilized by wet heat for 2 h (121 °C, 1.8 kPa, twice, 1 h each time), each basin was filled with 700 g. *Immaturus aurantii* seeds were disinfected with 70% alcohol surface for 20 min, and then washed clean with sterile single steam water. The seeds were accelerated to sprout in sterile peat. When the plant height was 3-4 cm, the seeds were transplanted with 1 plant in each pot.

Methods

A two-factor randomized block design was used, and the two-factor was phosphorus level (low phosphorus LP and high phosphorus HP) and auxin (auxin treated

IBA, auxin transport inhibitor treated NPA, control CK).

Pot each with 200 mg kg⁻¹ N and 50 mg kg⁻¹ K. In addition to the phosphorus nutrition of the substrate itself, different amounts of phosphorus were added to maintain different phosphorus concentrations in the substrate, and 20 mg kg⁻¹ P and 50 mg kg⁻¹ P were added in the low and high P treatments, respectively.

The experiment was carried out on March 10, 2020 in a greenhouse under natural light and at 24-30 °C on July 10, 2020. Each treatment was repeated for 5 times, and samples were collected 4 months after transplanting. The measured indexes included biomass, taproot length, first-grade lateral root length, and the number of lateral roots at each grade.

Data were mean values of 5 repeats. Microsoft Excel software was used to draw the graph, and SAS (6.0) software was used to conduct variance analysis and significance test (P<0.05).

Results

Effects of different factors on *Poncirus trifoliata* seedling growth

The effects of each treatment on biomass and root-shoot ratio were shown in Table 1. Auxin treatments significantly affected the biomass and root-shoot ratio of *Poncirus trifoliata*, which is that NPA significantly inhibited the biomass and root-shoot ratio of *Poncirus trifoliata*, while IBA had no significant effect on these two indexes. Furthermore, LP level had negative effect on biomass and root-shoot ratio, compare that with CK.

Table 1. The influence of P and auxin on the growth of *Poncirus trifoliata* seedlings.

Treatments	Biomass (g)	Root-shoot ratio
CK	2.1±0.2a	1.2±0.1a
CK	IBA	2.0±0.1a
	NPA	1.5±0.1c
LP	IBA	1.8±0.2b
	NPA	1.0±0.1d

Note: Data (means ± SD, n = 5) followed by different letters indicate significant differences (LSD, p < 0.05) among treatments.

Effects of Different Factors on root length of *Poncirus trifoliata* Seedling

The effect of each treatment on root length was shown in Table 2. Auxin treatments significantly affected the

tap root and lateral root length of *Poncirus trifoliata*, which is that NPA significantly induce the tap root and lateral root length of *Poncirus trifoliata*, while IBA increased it. Furthermore, LP level had negative effect on the tap root and lateral root length.

Effects of Different Factors on lateral root number of *Poncirus trifoliata* Seedling

The effect of each treatment on lateral root number was shown in Table 3. Auxin treatments significantly affected

the number of primary and secondary lateral roots of *Poncirus trifoliata*, which is that NPA significantly induce the tap root and lateral root length of *Poncirus trifoliata*, while IBA increased it. Furthermore, LP level had negative effect on the tap root and lateral root length.

Table 2. The influence of P and auxin on the root length of *Poncirus trifoliata* seedlings.

Treatments	Tap root length (cm)	Lateral root length (cm)
CK	10.8±1.1b	6.1±0.5b
CK	IBA	11.2±1.2a
	NPA	8.5±0.7c
LP	IBA	9.8±0.8c
	NPA	7.0±0.3d

Note: Data (means ± SD, n = 5) followed by different letters indicate significant differences (LSD, p < 0.05) among treatments.

Table 3. The influence of P and auxin on lateral root number of *Poncirus trifoliata* seedlings.

Treatments	Primary lateral roots (no.)	Secondary lateral roots (no.)
CK	31.2±2.1b	102.3±9.5b
CK	IBA	35.4±3.2a
	NPA	25.5±2.7c
LP	IBA	24.9±2.9c
	NPA	20.1±1.9d

Note: Data (means ± SD, n = 5) followed by different letters indicate significant differences (LSD, p < 0.05) among treatments.

Discussion

The Auxin can affect the growth and development of plants in many aspects. In particular, auxin can promote the formation of lateral roots by activating pericycle cells in roots and thus cause the change of root architecture (Teale et al., 2006). The formation of lateral roots was concentration dependent on auxin. Low concentration promoted the increase of the number and length of lateral roots, while high concentration inhibited them (Dinh et al., 2012; Mayzlish-Gati et al., 2012). In addition, application of auxin transport inhibitors can significantly reduce the number and density of lateral roots (De Dorlodot et al., 2007). In this experiment, 10 mg kg⁻¹ NPA significantly reduced the number and length of lateral roots while 20 mg kg⁻¹ IBA observably increased its, indicating that NPA could effectively reduce auxin transport, inhibit the formation of lateral roots and reduce the number of lateral roots, which revealed the important role of auxin transport and metabolism in the process of lateral root formation.

The regulation mode of low P on lateral root formation in plants is controlled by genetics, and the effects of low P on different plants are not consistent. Low

phosphorus induced an increase in the number of lateral roots in *Arabidopsis thaliana* (Mayzlish-Gati et al., 2012). However, some studies indicated that low P treatment increased the number of lateral roots in the short term and had significant inhibitory effects on various root indexes over time (Nacry et al., 2005). It has also been reported that low phosphorus treatment reduced the number of lateral roots of maize (Li et al., 2012). In this experiment, P level had a significant effect on each index. Specifically, low P reduced the biomass, root-shoot ratio, root length and number of *Poncirus trifoliata*, which also showed that P played an important role in plant growth.

Conclusions

Auxin signaling pathway could be involved in the regulation of P level on LR formation, and the auxin transportation should be the key factor in LR formation of *Poncirus trifoliata*. Phosphorus is the environmental factor regulating the formation of lateral roots, while auxin is the internal factor affecting the formation of lateral roots. In addition, auxin was involved in the regulation of phosphorus on lateral root formation.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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