Effects of Exogenous Ethylene and Cobalt Chloride on Root Growth of Chinese Fir Seedlings under Phosphorus-Deficient Conditions

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Abstract: Studying the effects of different concentrations of ethephon on morphological and physiological changes in the roots of Chinese fir (Cunninghamia lanceolata Lamb. Hook.) seedlings under P deficiency can reveal the internal adaptive mechanisms of these plants under nutrient stress. Herein, we investigated the effects of different ethephon and cobalt chloride concentrations under normal P supply and P deficiency. A significant effect (\(p < 0.05\)) of exogenous additive application was observed on the development of Chinese fir root length, surface area, and volume. These root development indices showed maximum values when the ethephon concentration was 0.01 g kg\(^{-1}\) under normal P supply and P deficiency, and they were significantly different from those under 0.04 g kg\(^{-1}\) ethephon treatment. Similarly, the indices showed maximum values when CoCl\(_2\) concentration was 0.01 g kg\(^{-1}\) under P deficiency and was significantly different (\(p < 0.01\)) from those under 0.2 g kg\(^{-1}\) CoCl\(_2\) treatment. Under normal P supply, an increase in ethephon concentration caused superoxide dismutase (SOD; E.C. 1.15.1.1) activity to decrease and peroxidase (POD; E.C. 1.11.1.X) activity to increase gradually. Conversely, CoCl\(_2\) addition (0.01 g kg\(^{-1}\)) promoted SOD and POD activities under P deficiency. There were no significant differences (\(p > 0.05\)) in malondialdehyde content of seedlings among ethephon or CoCl\(_2\) treatments. In conclusion, ethylene plays a significant role in adaptive mechanisms underlying stress resistance in plants, prompting them to respond to P starvation and improving seedlings’ tolerance to P-deficient conditions.

Keywords: Chinese fir; exogenous ethephon; exogenous cobalt chloride; antioxidant enzymes; root morphology; phosphorus stress

1. Introduction

Plants are subjected to different biotic and abiotic stress conditions during their growth and development. When plants encounter adversity or stress, their hormone levels change; this enables the plants to initiate and regulate the physiological and biochemical processes related to adversity adaptation, thereby coordinating plant growth and development, and inducing the formation of stress resistance [1]. As a part of the hormone regulatory network in plants, the signal transmission of ethylene has an important role in a variety of stress conditions due to its diffusibility and sensitivity; thus, ethylene is a rapid response factor in plants [2]. In addition to having a significant role in the growth and development of plants, many stressful situations can induce the increased secretion of ethylene. Therefore, the role of ethylene in the development of stress resistance in plants is a significant area of research. Numerous studies have shown that ethylene plays an important role in salt [3] and cold tolerance [4] as well as drought [5] and disease resistance [6] of plants. However,
it is not clear whether the production of ethylene is only a symptom of plant damage or an adaptation mechanism to regulate the physiological and biochemical processes related to stress resistance. Therefore, further research needs to be carried out to reveal the relevant mechanism of ethylene action in the regulation of plant stress resistance.

Chinese fir (*Cunninghamia lanceolata* Lamb. Hook.) is an important high-quality afforestation tree species in south China. However, the available phosphorus (P) content of the soil significantly restricts the production and sustainable management of Chinese fir plantations. During the long-term course of evolution, the Chinese fir genotype with an ability of high P utilization has portrayed a series of morphological, physiological, and metabolic adaptive changes in response to P-stress environments. According to a previous study, a P-stress environment, as well as the endogenous plant hormones produced due to stress situations, can prompt changes in the morphology of plant roots [7]. Among the various plant hormones, ethylene plays an important role in root development, including the formation of lateral and adventitious roots, the establishment of the root stem cell microenvironment, and the regulation of root hair development [8]. Phosphorus deficiency-induced ethylene production or changes in ethylene sensitivity are involved in the regulation of root structure [9]. Under nutrient-deficient conditions, increasing ethylene sensitivity and/or yield can increase root elongation, thus enabling plants to forage deeper into the soil in more nutrient-rich areas [10]. Phosphorus stress-induced changes in root geotropism, formation of aeration tissue, and root hair development are regulated by ethylene [11]. Therefore, it is evident that, as a signaling molecule, ethylene participates in the structural and morphological remodeling of roots; moreover, it plays an important role in the acquisition of P resources under P-stress environments. Additionally, ethylene has a role in the mobilization of P resources from aging tissues for use in new tissues and increasing the concentration of available P in rhizosphere soil [12]. In ethylene-sensitive mutants, gene expression related to the induction of P-starvation and increased acid phosphatase (E.C. 3.1.3.2) activity has been reported [13].

In recent years, there have been many studies on the relationship between plant endogenous hormones and plant growth and development under P stress. Research on the regulation of plant hormones by exogenous additives is mostly focused on crop plants and *Arabidopsis* sp. [14]. The study of ethylene release under P stress in the Chinese fir will provide information regarding the regulation of the growth of its root system under such adverse conditions; this is of great significance for studying the response mechanisms of plants for adapting to P-deficient environments. Hormones often exhibit an inhibitory effect at high concentrations and a promoting effect at low concentrations. However, the studies conducted to date have not considered the specific regulatory mechanisms of ethylene and the complex effects of varying ethylene concentrations on the development of stress resistance in Chinese fir; therefore, the mechanism of ethylene action in response to P stress in Chinese fir needs further exploration. Under P-deficient conditions, ethylene acts as an important signaling molecule with respect to the adaptation of plants to adversity; however, the exact relationship between the changes in the ethylene concentration and the high efficiency of P utilization in Chinese fir is not clear. Based on this, we propose the following hypotheses: (1) application of low concentrations of ethylene can promote morphological and physiological development of Chinese fir under P-stress conditions, and (2) application of cobalt chloride (CoCl$_2$) can inhibit the synthesis of ethylene under both normal P supply and P stress; at low concentrations, CoCl$_2$ can promote the growth of the root system of Chinese fir under P-stress conditions, whereas, in high concentrations, CoCl$_2$ inhibits root growth.

We used Chinese fir seedlings as the test materials for this study, and indoor potted simulation experiments were used to set the normal P supply (control) and no phosphorus supply (treatment) groups. Thereafter, we added five different concentrations of ethephon and CoCl$_2$ to the plant groups, all of which had different P-supply levels. Subsequently, we measured different root morphological indices as well as the dry matter distribution pattern and antioxidant enzyme activity in the roots of Chinese fir seedlings of the different
treatment groups. We analyzed the relationship between the different morphological and physiological indicators of root growth and the ability of the Chinese fir seedlings to tolerate P stress. The results enabled us to provide a theoretical basis of the mechanism of ethylene action in the physiological responses of Chinese fir to a P-deficient environment.

2. Materials and Methods

2.1. Plant Materials

The experiment was performed in a greenhouse at Fujian Agriculture and Forestry University from July to August 2017. The Chinese fir seedlings for our study were selected from 1.5 generations of a seed orchard at the state-owned forest farm in Zhangping, Fujian Province, SE China; they belonged to family no. 32, and according to a previously conducted study, these seedlings were of the “root proliferative” type, i.e., their root phenotype changed significantly under various experimental conditions [15]. The test seedlings were planted in pots on 21 July 2017, and the test treatments were initiated after 7 days, in the seedlings that were healthy, robust, and uniformly sized.

2.2. Experimental Design

We grew 100 Chinese fir seedlings, each in a round pot (9 cm bottom inner diameter, 18 cm top inner diameter, and 22 cm height). We added 4.5 kg of clean sand, which was passed through a 2 mm sieve, with trace amounts of P to each pot. According to the content of potassium dihydrogen phosphate (KH$_2$PO$_4$) in a modified Hoagland solution, as described by [16], two different P-supply levels were designed, namely P supply (1 mM KH$_2$PO$_4$, P$_1$) and no P supply (0 mM KH$_2$PO$_4$, P$_0$). There were 50 pots in each of the P$_0$ and P$_1$ groups. Potassium chloride (KCl) was used to balance the difference in the K$^+$ levels between the two groups (P$_1$ and P$_0$). To ensure that other nutrient demands of the Chinese fir seedlings were fulfilled, 200 mL of the nutrient solution was added to each pot every 4 days during the entire treatment period.

Ethephon and CoCl$_2$ were added to both treatment groups, P$_1$, and P$_0$. Ethephon is a precursor of ethylene synthesis and signal transduction, and it functions as a plant growth regulator. Depending on the environment, ethylene signaling pathways can lead to the inhibition or activation of a series of genes, thereby inducing differential morphological developments in plants [17,18]. In contrast, CoCl$_2$ promotes the synthesis of auxin and cytokinin and directly inhibits the synthesis of ethylene; therefore, it can reduce the effects of ethylene, and thus, significantly affect plant growth and development as well as the stress resistance physiology [19]. Therefore, we used exogenous ethylene and CoCl$_2$ as the additives to treat the two experimental plant groups. Five rates of ethephon (0, 0.01, 0.1, 0.2, and 0.4 g kg$^{-1}$ sand) and five rates of CoCl$_2$ (0, 0.01, 0.05, 0.1, and 0.2 g kg$^{-1}$ sand) were set up [20]. The ethephon ($\geq$90.0%, Macklin Reagent, Shanghai, China) and CoCl$_2$ were mixed into the sand in each pot and applied at three intervals (4, 8, 12) days separately. Each treatment group had five replicates; therefore, 25 P$_1$ group seedlings were treated with ethephon, 25 P$_1$ group seedlings were treated with CoCl$_2$, 25 P$_0$ group seedlings were treated with ethephon, and 25 P$_0$ group seedlings were treated with CoCl$_2$.

2.3. Harvest and Data Collection

The test seedlings were harvested after 16 days of cultivation. The plants were divided into aboveground and belowground parts. The plant samples were heated at 108 °C for 30 min and subsequently dried at 80 °C for approximately 2 days to obtain a constant weight. The biomasses of the aboveground and belowground parts were accurately weighed. Vanadium molybdate yellow colorimetric analysis was used to determine the P content of the aboveground and belowground parts of the plant. The fresh, white root system was identified as the newly grown roots of the harvested seedlings. Root morphology was scanned with a Canadian digital scanner (STD1600, Epson, Tokyo, Japan), and the root length, root surface area, and root volume were quantitatively analyzed using WinRHIZO (version 4.0b, Quebec, Canada) root analysis system software. Additionally, the specific
root length (cm·g⁻¹) was determined by dividing the root length (cm) by the root dry mass (g), and the root tissue density (g·cm⁻³) was calculated by dividing the root dry mass (g) by the root volume (cm³) [21]. Subsequently, the fresh root tips were accurately weighed, cleaned with 0.1 g ultrapure water, and ground into a homogenate for testing. Superoxide dismutase (SOD; E.C. 1.15.1.1), peroxidase (POD; E.C. 1.11.1.X), and malondialdehyde (MDA) assay kits provided by the Nanjing Jiancheng Institute of Biological Engineering were used to determine the root SOD activity, POD activity, and MDA content, respectively.

2.4. Statistical Analyses

Microsoft Excel (2007) (Microsoft Corporation, Redmond, AL, USA) was used for data processing, and SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used to conduct a two-factor analysis of variance (ANOVA) on the root morphological indices of the Chinese fir seedlings cultivated with different types and concentrations of exogenous additives under normal P supply and P-stress conditions. As the two-way interactive effect was not significant (p > 0.05), one-way ANOVA and the least significant difference method for multiple comparisons were also performed for each factor separately by excluding the other factors, after assessing the homoscedasticity of datasets using Levene’s test. The data obtained for the multiple treatment plant groups were analyzed for significant differences (p = 0.05). All the data were expressed as average values ± standard error. The relevant charts for this article were drawn with Origin 9.0 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Two-Factor Analysis of the Influence of P Supply Level and Exogenous Additive Concentration on the Determination Indices

When ethephon was added, the interaction between the two factors, that is, the P supply level and the exogenous additive concentration, was significant for root tissue density (p < 0.05) and SOD activity (p < 0.01) of Chinese fir seedlings. With respect to the individual factors, both the exogenous additive concentration and the P supply level had a significant effect (p < 0.05) on root length, root surface area, root volume, and root average diameter. When CoCl₂ was added, the interaction between the two factors, that is, the P supply level and the CoCl₂ additive concentration, was significant for root tissue density (p < 0.01) and POD activity of Chinese fir seedlings. With respect to the individual factors, both the exogenous additive concentration and the P supply level had a significant effect (p < 0.01) on root length, root surface area, the root volume. Additionally, the exogenous additive concentration and P supply level had a significant effect (p < 0.05 and p < 0.01, respectively) on SOD (Table 1).

Table 1. Two-factor analysis of the influence of phosphorus-supply level and the concentrations of exogenous ethephon and CoCl₂ on the development indices of Chinese fir seedlings.

| Seedling Traits          | Ethephon F Value | CoCl₂ F Value |
|--------------------------|------------------|---------------|
|                          | a     | b   | a × b | a     | b   | a × b |
| Root length              | 6.030 * | 3.702 * | 0.807 | 10.347 ** | 8.367 ** | 0.794 |
| Root surface area        | 5.660 * | 3.457 * | 0.668 | 10.701 ** | 8.146 ** | 0.955 |
| Root volume              | 5.196 * | 3.230 * | 0.57  | 9.982 **  | 7.329 **  | 1.054 |
| Root diameter            | 4.573 * | 3.556 * | 0.986 | 1.75  | 1.805  | 0.537 |
| Root tissue density      | 14.051 ** | 4.703 ** | 3.62 * | 30.672 ** | 34.790 ** | 15.723 ** |
| Specific root length     | 2.003  | 1.424 | 1.406 | 1.706  | 1.643  | 1.593 |
| POD                      | 0.978  | 12.027 ** | 6.015 ** | 0.359  | 2.978 *  | 9.380 ** |
| SOD                      | 0.411  | 2.461 | 0.695 | 7.190 *  | 5.738 ** | 2.075 |
| MDA                      | 0.041  | 1.901 | 0.366 | 2.269  | 0.788  | 1.746 |

a: Exogenous additive concentration; b: Phosphorus supply level; * Significant at p < 0.05; ** Significant at p < 0.01.
3.2. Root Morphology

For both the P₁ and P₀ groups, root length, root surface area, and root volume under the 0.01 g kg⁻¹ ethephon treatment were significantly different (p < 0.05) from those under the 0.2 and 0.4 g kg⁻¹ ethephon treatments. The average root diameter of the plants under the 0.01 g kg⁻¹ ethephon treatment was significantly different from that of the plants under the 0.4 g kg⁻¹ ethephon treatment for both the P₁ and P₀ groups. Additionally, under the 0.01 g kg⁻¹ ethephon treatment, root tissue density was lower than that of the plants under the 0.4 g kg⁻¹ ethephon treatment, for both the P₁ and P₀ groups (Figure 1).

Figure 1. Differences in the root length (A), root surface area (B), root volume (C), root average diameter (D), root tissue density (E), and specific root length (F) between the two groups, namely phosphorus supply (1 mmol KH₂PO₄, P₁) and no phosphorus supply (0 mmol KH₂PO₄, P₀), treated with different concentrations of exogenous ethephon. Bars (mean ± standard error) accompanied by the same lowercase letter(s) are not significantly different at the 5% probability level. Different capital letters indicate significant differences between different additive concentrations under normal P supply conditions, while different lowercase letters indicate significant differences between different additive concentrations under low-P stress. * Indicates that the same additive concentration is significantly different under different P supply levels.

For the P₁ treatment group, there were no significant differences (p > 0.05) in the root indices among the varying CoCl₂ treatments. However, for the P₀ treatment group, the plants under the 0.01 g kg⁻¹ CoCl₂ treatment had significantly different (p < 0.05) root
lengths, root surface areas, and root volumes compared to the plants grown under the 0.1 and 0.2 g kg\(^{-1}\) CoCl\(_2\) treatments (Figure 2).

![Figure 2](image-url)

**Figure 2.** Differences in the root length (A), root surface area (B), root volume (C), root average diameter (D), root tissue density (E), and specific root length (F) between the two groups, namely phosphorus supply (1 mmol KH\(_2\)PO\(_4\), P\(_1\)) and no phosphorus supply (0 mmol KH\(_2\)PO\(_4\), P\(_0\)), treated with different concentrations of exogenous CoCl\(_2\). Bars (mean ± standard error) accompanied by the same lowercase letter(s) are not significantly different at the 5% probability level. Different capital letters indicate significant differences between different additive concentrations under normal P supply conditions, while different lowercase letters indicate significant differences between different additive concentrations under low-P stress. * Indicates that the same additive concentration is significantly different under different P supply levels.

### 3.3. Protective Enzyme Activities

For the P\(_1\) treatment group, the POD activity was the lowest under the 0.01 g kg\(^{-1}\) ethephon treatment, and it gradually increased with the increase in the ethephon concentration. In contrast, there were no significant differences (\(p > 0.05\)) in the POD activities of the P\(_0\) seedlings grown under varying ethephon concentrations (Figure 3A).
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Figure 3. Differences in the POD activity (A,B) and SOD activity (C,D) between the two groups, namely phosphorus supply (1 mmol·KH₂PO₄, P₁) and no phosphorus supply (0 mmol·KH₂PO₄, P₀), treated with different concentrations of exogenous additives (ethephon and CoCl₂). Bars (mean ± standard error) accompanied by the same lowercase letter(s) are not significantly different at the 5% probability level. Different capital letters indicate significant differences between different additive concentrations under normal P supply conditions, while different lowercase letters indicate significant differences between different additive concentrations under low-P stress. * Indicates that the same additive concentration is significantly different under different P supply levels.

For the P₁ treatment group, the addition of 0.05 g kg⁻¹ CoCl₂ caused the highest POD activity in the Chinese fir seedlings, but higher CoCl₂ concentrations gradually decreased the POD activity. For the P₀ treatment group, POD activity was the highest in the seedlings grown under 0.01 g kg⁻¹ CoCl₂ treatment (Figure 3B).

For the P₁ treatment group, SOD activity gradually decreased with increasing ethephon concentration, and its value in the seedlings grown under 0.4 g kg⁻¹ ethephon treatment was significantly different (p < 0.05) as compared to the SOD activity in the seedlings grown under 0.01 g kg⁻¹ ethephon treatment. For the P₀ treatment group, the plants grown under the 0.01 g kg⁻¹ ethephon treatment showed higher SOD activity than those grown under the other ethephon concentration treatments (Figure 3C).

For the P₁ treatment group, there were no significant differences in the SOD activities of the seedlings grown under varying CoCl₂ concentrations (p > 0.05); however, for the P₀ treatment group, SOD activity was highest in the seedlings grown under the 0.01 g kg⁻¹ CoCl₂ treatment, and its value was significantly different (p < 0.05) as compared to the SOD activities of the seedlings grown under 0.2 and 0.4 g kg⁻¹ ethephon treatments (Figure 3D).

3.4. MDA Content

The MDA content reached a maximum concentration in the seedlings grown under 0.01 g kg⁻¹ ethephon treatment in both the P₁ and P₀ treatment groups; however, there were no significant differences among the various ethephon treatment groups (p > 0.05, Figure 4).
forests supply (1 mmol·KH₂PO₄, P₁) and no phosphorus supply (0 mmol·KH₂PO₄, P₀), treated with different concentrations of exogenous additives (ethephon and CoCl₂).

Figure 4. Differences in the MDA activity (A,B) between the two groups, namely phosphorus supply (1 mmol·KH₂PO₄, P₁) and no phosphorus supply (0 mmol·KH₂PO₄, P₀), treated with different concentrations of exogenous additives (ethephon and CoCl₂).

For the P₁ group, MDA content reached a maximum concentration in the seedlings grown under 0.01 g kg⁻¹ CoCl₂ treatment, whereas for the P₀ group, the MDA content was the highest in the seedlings grown under 0.1 g kg⁻¹ CoCl₂ treatment (Figure 4).

4. Discussion

During the development of plant roots, the remodeling of root structures can enable plants to forage P resources more effectively. Changes in root length, root surface area, and root volume will affect the ability of the roots to explore the soil for P [22]. The development of the root system is extremely sensitive to nutrition, moisture, and other environmental parameters, and ethylene is an important signaling molecule in this process. Studies have shown that exogenous ethylene can affect the content of endogenous aminocyclopropane-1 carboxylic acid synthase (ACC synthase; E.C. 4.4.1.14), thereby inducing the biosynthesis of endogenous ethylene; moreover, when the ethylene concentration in the plant reaches a certain threshold, it can promote root growth, but if it exceeds this threshold, then it will inhibit the growth of the root system [23]. In this study, we found that when Chinese fir seedlings were treated with 0.01 g kg⁻¹ ethephon, their endogenous ethylene concentration reached the optimal value for stimulating the growth of the root system; the root length, root surface area, root volume, and average root diameter of the seedlings reached their respective maximum values under these conditions. However, with higher ethephon concentrations, the growth of the root system was inhibited. This observation indicates that ethephon concentration is closely related to root configuration remodeling. Root growth is determined by cell division in the root meristem and cell elongation in the root elongation zone; ethylene mainly inhibits the latter. Ethylene primarily acts upon the epidermis to control the growth of plant roots and stems; additionally, it inhibits the growth of plants by inhibiting the effect of auxin in the outermost plant cells [24]. Studies have shown that low concentrations of ethylene trigger the synthesis and transport of auxin. Moreover, the accumulation of auxin in the roots is partly dependent on ethylene metabolism, and this may promote the emergence of secondary roots, thereby affecting the ultimate root configuration [25]. On the contrary, the stimulation of auxin transport mediated by high concentrations of ethylene reduces the accumulation of auxin in the primary wood sheath, and this may inhibit root formation. Recent studies have verified that the homeobox protein 52 gene (HB52) is an important node in the ethylene and auxin signaling pathways, and it participates in the ethylene-mediated inhibition of root elongation by regulating the expression of auxin transport components downstream of the ethylene-insensitive 3 genes (EIN3). Inhibition of root elongation may be controlled by inhibiting the activities of auxin-dependent plasma membrane H⁺-ATPase and reactive oxygen species (ROS) that modify the cell wall characteristics [26]. In addition, an increase in the ethylene concentration can prevent cell division and promote cell differentiation by interacting with jasmonic acid, thereby affecting the activity of the taproot meristem [27].
According to the optimization theory of plants, mass release of root volatiles will cause rapid consumption of the stored nutrients in the plants; this is not conducive to the adaptation of plants to a P-stress environment [28]. Under P-stress conditions, a low concentration of CoCl\(_2\) inhibits aminocyclopropanecarboxylate oxidase (ACC oxidase; E.C. 1.14.17.4), thereby reducing the endogenous ethylene production caused by the stress condition. Therefore, the plants can save the energy that would otherwise go towards ethylene production and use this energy to expand their range of foraging for P sources through root proliferation. The root length, root surface area, and root volume of the Chinese fir seedlings reached their maximum values in the P\(_0\) plants grown under the 0.01 g kg\(^{-1}\) CoCl\(_2\) treatment, and in comparison, to the growth of the P0 plants without any exogenous additives, these values were increased by 72.78%, 102.56%, and 138.18%, respectively. However, there were no significant differences in these root morphology indicators among the P1 group of Chinese fir seedlings grown under varying CoCl\(_2\) concentrations. Therefore, low concentrations of CoCl\(_2\) can reverse the inhibitory effects of ethylene and low P on the taproot, but as the concentration of CoCl\(_2\) increases, root growth is inhibited. Hence, it is evident that only low concentrations of CoCl\(_2\) can stimulate root growth in Chinese fir seedlings. Additionally, the root system produces ethylene gas in a P-stress environment; this increases the ethylene sensitivity of the root system of the Chinese fir seedlings, leading to changes in their root morphology. Studies have shown that under P-deficiency conditions, the anac044 gene regulates the activation of cell wall P remobilization through the transduction of ethylene signals; this, in turn, increases the re-utilization efficiency of the P pool in the root system, improves the reabsorption of acquired P in the plant, and hence, maximizes the allocation of P to the developmental organs [29]. Moreover, ethylene-mediated changes in the expression of some Pi starvation-induced (PSI) genes directly affect the absorption of P from the soil and the redistribution of P within the plants as well as in the environment [13]. Therefore, it is evident that under P-stress conditions, the changes in the sensitivity and signal transduction of ethylene in the plants can adjust the source–sink relationship of P resources in Chinese fir, thereby ensuring that the internal resources are continuously transported and reused, and the plants adapt completely to the P-stress environment.

Under nutrient-stress conditions, plants produce a large amount of ROS, such as O\(_2^–\) and OH\(^–\). These ROS cause denaturation of proteins and other biological macromolecules, leading to an increase in the peroxidation of cell membranes. When the severity of the environmental stress decreases, the antioxidant enzyme system of the plant initiates an adaptive mechanism to remove the excess ROS and maintain them at a normal dynamic level to improve the plant’s resistance to stress [27]. Although ROS destroy biological macromolecules, a minimum level of ROS needs to be maintained because they are essential for signal transduction and many biological processes such as plant growth, the cell cycle, and programmed cell death [30]. Research shows that a burst of intracellular oxidation can prompt the activation of the mitogen-activated protein kinase (MAPK) cascade and induce the activation/phosphorylation of ACC synthase, thereby increasing the production of ethylene [9]. Recently, it has been discovered that the connection between ROS, ethylene, and sugar signals is a key mediator of stress responses. They integrate information from the endoplasmic reticulum, chloroplast, and mitochondria, thereby facilitating a coordinated stress response. Hence, it is evident that ROS participate in various ethylene-mediated plant responses [31]. In this study, we observed that under normal P supply conditions, with increasing exogenous ethephon concentration, the activity of SOD was inhibited, and the activity of POD was enhanced. However, under P-stress conditions, the addition of 0.01 g kg\(^{-1}\) CoCl\(_2\) promoted the activities of both SOD and POD. Hence, it is evident that ethylene stimulates the plant’s antioxidative enzyme system, which, in turn, makes the redox state of the root meristem unbalanced. Among these antioxidant enzymes, SOD is a natural superoxide radical scavenging enzyme in cells that can convert unstable O\(_2^–\) into H\(_2\)O\(_2\), and POD can convert the H\(_2\)O\(_2\) produced by the SOD disproportionation reaction into non-toxic H\(_2\)O [32]. Moreover, H\(_2\)O\(_2\) is an intermediate in the pathway
of ethylene synthesis from methionine, and O$_2$ is related to the activity of ethylene synthase; hence, CoCl$_2$ may affect the production of endogenous ethylene by regulating the production of ROS in response to the P-stress conditions of fir [33]. Therefore, CoCl$_2$ plays an important role in the remodeling of plant roots, thereby prompting plants to respond to the P starvation environment and improving the seedlings’ tolerance to P-deficient conditions. The accumulation of H$_2$O$_2$ also affects related stress signals including ROS synthesis and various plant hormones. Hence, these endogenous factors interact with the signaling pathways that are promoted by oxidative stress to form a complex transduction network that regulates cell division, expansion, and ultimately the development of the root system of the plants [34].

5. Conclusions

Under different P-supply conditions, with increasing exogenous ethylene concentration, root growth was gradually inhibited. However, low concentrations of CoCl$_2$ promoted root growth in Chinese fir seedlings under P-stress conditions, thereby enhancing the ability of the roots to forage for P resources deep in the soil. With respect to the changes in the physiological indicators of stress, under a normal P supply, with increasing exogenous ethephon concentration, the activity of SOD was inhibited, and the activity of POD was enhanced. Under P-stress conditions, low concentrations of CoCl$_2$, such as 0.01 g kg$^{-1}$ CoCl$_2$, promoted the activity of both POD and SOD in the Chinese fir seedlings, but their activities decreased with the gradual increase in the concentration of exogenous CoCl$_2$. Exogenous ethephon and CoCl$_2$ have been found to be closely related to root remodeling, which provides a theoretical basis for future research on the internal regulation mechanism of exogenous hormones on woody plants adapting to nutrient stress.

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Abbreviations

SOD—Superoxide dismutase; POD—Peroxidase; MDA—Malondialdehyde; ROS—Reactive oxygen species; ACC synthase—Aminocyclopropane-1 carboxylic acid synthase; ACC oxidase—Aminocyclopropanecarboxylate oxidase.

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