Nitrogen addition regulates allometric growth by changing the distribution patterns of endogenous hormones in different organs of Pinus tabuliformis

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Research

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Abstract

**Background:** The mechanisms by which nitrogen (N) affects the allometric growth of plant organs by influencing the distribution of different hormones in plant organs remain unclear. Therefore, this study aimed to examine the effect of soil N levels on soil properties, the hormonal activities and growth parameters of *Pinus tabuliformis* Carr.

**Methods:** Seedlings of *P. tabuliformis* were subjected to 0, 3, 6, 12 g of N m$^{-2}$ year$^{-1}$. Growth parameter and hormone activity data (trans-zeatin riboside (ZR), gibberellin (GA$_{1+3}$), indole acetic acid (IAA), abscisic acid (ABA)) in leaf, stem, total root, coarse root (diameters >2 mm), fine root (1-2 mm) and finest root (0-1 mm) biomass were measured and analysed.

**Results:** The results showed that N addition increased the growth of the leaves; stems; total roots; and coarse, fine, and finest roots of the seedlings, but decreased the ratio of underground to aboveground growth. Additionally, N addition increased the ZR content in stems, while decreasing the ABA content in leaves and whole roots and the GA$_{1+3}$ content in whole roots, but IAA content was not significantly influenced by N addition. Furthermore, the hormones examined significantly influenced the growth of the leaves, stems, roots, and the coarse, fine, and finest roots.

**Conclusions:** N addition affected the trans-zeatin riboside, gibberellin, indole acetic acid, and abscisic acid content in the various organs examined, which influenced the growth of the seedlings. Our research will provide an important reference for forestland governance and forest systems management under global atmospheric N deposition.

**Background**

Over the years, owing to the increase in industrial and human activities, atmospheric N has greatly increased, resulting in changes in the earth’s N cycle (Galloway et al. 2004; Vitousek et al. 1997) and terrestrial ecosystems (Matson et al. 2002). Previous studies have shown that increased atmospheric N may change soil properties by increasing soil available N content and by influencing soil pH (Goulding et al. 1998; Zhao and Zeng 2019). However, there are several uncertainties about the effects of changes in soil available N on the concentrations and activities of hormones in different plant organs. The results of studies on the effect of N on the activity levels and content of plant hormones are conflicting. Wagner and Beck (1993) reported that the type and content of cytokinins varied across different plant organs, and that the response of the cytokinins to changes in N levels also differed. While changes in N levels did not affect the total cytokinin content in nettle stems in the study of Wagner and Beck (1993), ammonium N reduced trans-zeatin riboside content in sweet potato storage roots (Si et al. 2018). Yong et al. (2000) reported that N treatment increased the cytokinin content in the aboveground parts of cotton, especially in the leaves. Similarly, Sakakibara (2006) also reported that N increased the biosynthesis of endogenous cytokinins in plants, and influenced the transport of cytokinins from roots to aboveground parts, thus affecting the content and distribution patterns of cytokinins in different organs of plants (Rahayu 2005; Takei et al. 2001). At present, reports on the effect of soil-available N on the content of endogenous cytokinins, and other endogenous hormones, such as gibberellin (GA), indole acetic acid (IAA), and abscisic acid (ABA) in different organs of plants are conflicting (Liu et al. 2011; Si et al. 2018; Wang et al. 2009).

Similarly, the result of studies on the effect endogenous hormones on the allometric growth of plant organs differ considerably. Xiao et al. (2018) reported that IAA, GA, and ZT (the same function as ZR) promoted the growth of tea stems and leaves, while ABA inhibited the growth of tea stems and leaves. The content of IAA and ZT increased with root growth, while the content of GA decreased and ABA remained unchanged (Li et al. 2018). Thus, the regulation, content, and activity of plant endogenous hormones are species and organ dependent. Furthermore, the allometric growth of plant organs is regulated by the synergistic activity of multiple hormones (Depuydt and Hardtke 2011). However, studies on how the changes in different endogenous hormones and their activities affect the growth and development of various plant organs is lacking.
Additionally, reports suggest that root growth (coarse, fine, and finest roots) is controlled by the activities of endogenous hormones. The root system of woody plants is a highly heterogeneous hierarchy system. Studies have found that specific root length and specific respiration rate decreased, and plant anatomical structure changed from primary to secondary structure with the increase in root diameter (Jia et al. 2013; Pregitzer et al. 1998). Plant root growth and morphology are closely associated with endogenous hormones, which could regulate plant root configuration and effect changes in root length and diameter (Fan and Yang 2006). However, most studies have focused on the relationship between total root growth and endogenous hormones, with relatively few reports on the relationship between endogenous hormones and the growth of coarse, fine, and finest roots. Some studies reported that plant roots are extremely sensitive to IAA, and that their growth and development are regulated by IAA (Olatunji et al. 2017; Wen et al. 2018) and other plant hormones, such as GA\textsubscript{1+3} and ABA (He et al. 2014). Similarly, researchers (Jing et al. 2017; Wang et al. 2013) have suggested that the differences in the growth rate of roots of different diameters maybe due to the differences in the content and activities of endogenous hormones in these roots.

Taken together, in this study, we examined two hypotheses: 1) changes in soil available N have differing effects on endogenous hormone content and activity rates in different plant organs and that the growth of different plant organs is controlled by the synergistic activities of multiple hormones; 2) total root growth and coarse, fine, and finest root growth are controlled by the synergistic actions of multiple plant hormones.

To verify the above hypotheses, seedlings of Pinus. \textit{tabuliformis} Carr. were subjected to different gradients of N, and the soil properties, growth rates of the leaf, stem, and roots of the seedling were examined. Similarly, the responses of the IAA, GA\textsubscript{1+3}, ZR, and ABA content in the leaves; stems; whole roots; and the coarse, fine, and finest roots to N; and the effects of these hormones on the growth of the different plant organs were examined.

**Materials And Methods**

**Experiment field site**

This research was conducted in the experimental field of the Institute of Soil and Water Conservation in Yangling, Shaanxi Province, China (108°04.27.95″E, 34°16.56.24″N). The site has a mainland monsoon-type climate with a mean annual temperature of 13.2 °C, mean annual precipitation of 674.3 mm, average annual sunshine hours of 1993.7 h, and frost-free days of 225. The experimental soil was collected from the Loess soil surface in Ansai County, Shaanxi Province and it contained 12.92% clay, 63.99% silt, and 23.09% sand. The organic matter content of the soil was 3.63 g kg\textsuperscript{-1}, total soil N content was 0.19 g kg\textsuperscript{-1}, total P content was 0.27 g kg\textsuperscript{-1}, and available N content was 11.55 mg kg\textsuperscript{-1}. The soil moisture content was 7.6%, and soil pH was 8.76.

**Experiment design**

Chinese pine (\textit{Pinus. tabuliformis} Carr.) seedlings were collected from Yangling seedling base, Shaanxi Province, China. All selected samples were biennial seedlings with consistent height and base diameter. Eighty polyvinyl chloride pots of 29.5-cm in diameter and 30-cm in depth were filled with the experimental Loess soil (15 kg in each pot). Three 1.5-cm diameter water outlets were punched around the wall 5 cm from the bottom of each pot. And then 160 seedlings with almost the same height and base diameter were randomly transferred to the pots containing N treated soils treated with 0 (N0), 3 (N3), 6 (N6), and 12 g N m\textsuperscript{-2} year\textsuperscript{-1} (N12) on April 11, 2017. The current level of nitrogen deposition is equivalent to about 2 g N m\textsuperscript{-2} year\textsuperscript{-1} (Wang et al. 2017). There were 20 pots per treatment and 2 seedlings per pot.

Atmospheric N is available to plants mainly in the form of ammonium N and nitrate N (Duce et al. 1991). Therefore, to simulate atmospheric N deposition, NH\textsubscript{4}NO\textsubscript{3} was used in this study. N fertilizer was dissolved in water and sprayed into
each pot pending the rains in May, July, and September of 2017. The same amount of water was applied to all the treatments, however, there was no N in the water applied to the N0 treatment (the control) group.

**Soil sampling and properties analysis**

The soils were sampled from the surface layer of 0–10 cm in each pot with a 2.5 cm diameter drill, and four pots were selected randomly for each treatment on August 20, 2018. The soil removed the roots, stones and other impurities was dried in the shade and passed through 0.2-mm and 1-mm sieves to obtain the final soil sample, which was used for further analyses.

Soil organic matter was determined by K$_2$Cr$_2$O$_7$-H$_2$SO$_4$ oxidation and FeSO$_4$ titration, and total N was measured by H$_2$SO$_4$ digestion and H$_2$SO$_4$ titration as described by Wang et al. (2017). Inorganic N was extracted with 2 mol L$^{-1}$ KCl and analysed as described by Zhao and Zeng (2019) with Chemlab Auto-Analyzer (AutoAnalyzer III, Bran+Luebbe GmbH, Germany). The soil pH is determined by that water: soil-1:2.5 ratio slurry with a pH meter (Basic pH meter PB-10, Sartorius Scientific Instruments Co., Ltd, China).

**Seedling biomass and plant hormone analyses**

Four seedlings were harvested randomly from each treatment and separated into leaves, stems, and roots (the roots were divided into three levels, the finest roots (0–1 mm), fine roots (1–2 mm) and coarse roots (2–5 mm) on August 20, 2018. The samples were divided into two parts for analysis. One part was promptly placed into liquid nitrogen and then stored at -80°C for the hormone content assay. The remaining part was deactivated in an oven at 105°C for 30 min, and then dried to constant weight at 40°C for biomass determination.

The ZR, GA$_{1+3}$, IAA and ABA contents of different tissues were determined by enzyme-linked immunosorbent assay (ELISA) at the China Agricultural University of China. The extraction of hormones was followed according to the method described by Zhang et al. (2019) with minor modifications. The 0.5g samples powder grinded in liquid nitrogen of each replicate for each treatment were added with 5ml 80% methanol (containing 10mg L$^{-1}$ -butylhydroxytoluene) to extract hormones at 4°C for 4h. The mixtures were centrifuged at 5000xg at 4 ℃ for 10min. The residues were extracted again as the above method. Then the supernatants were merged together for hormone assay. The analyses of ZR, GA$_{1+3}$, IAA and ABA were carried out as described by Bai et al. (2011) and $w$(ZR)/$w$(ABA), $w$(GA$_{1+3}$)/$w$(ABA), $w$(IAA)/$w$(ABA) and $w$(ZR+GA$_{1+3}$+IAA)/$w$(ABA) ratios were calculated.

**Data analysis**

The data obtained from the study were processed and analysed using Excel 2019 and SPSS 20 statistical analysis software. Data of the soil properties, plant biomass (leaf, stem, root, and total plant growth), and plant endogenous hormone (IAA, GA$_{1+3}$, ZR, and ABA) contents were subjected to an analysis of variance (ANOVA) using SPSS 20 and significant means were compared using least significant difference (LSD) tests using the same software. Pearson correlation analysis was also carried out to determine the relationship between the variables.

**Results**

**Effect of N addition on soil properties**

There was no significant effect of N addition on soil properties (Table 1). With the increases in N, soil organic C, soil total N, and nitrate N contents increased, while soil pH decreased.

| Table 1 | Effect of different gradient concentration of N treatments on soil properties of *Pinus tabuliformis* |
Table 2: Effect of N treatments on plant biomass of leaf, stem, root of *Pinus tabuliformis* seedlings.

| N treatment | Leaf biomass | Stem biomass | Root biomass g plant \(^{-1}\) | Root Shoot | Total plant biomass g plant \(^{-1}\) |
|-------------|--------------|--------------|--------------------------------|------------|----------------------------------|
| g N m \(^{-1}\) year \(^{-1}\) | g plant \(^{-1}\) | g plant \(^{-1}\) | Finest root | Fine root | Coarse root | Whole | Shoot | Plant |
| N0          | 12.9±0.9c    | 13.9±1.3b    | 5.9±0.05b | 3.6±0.3ab | 5.8±0.6bc | 15.3±0.9b | 0.58±0.02a | 42.1±3.2b |
| N3          | 13.9±1.1bc  | 16.9±2.2b    | 5.6±0.3b  | 3.2±0.2b  | 5.6±0.3c  | 14.4±0.6b | 0.47±0.03b | 45.2±3.1b |
| N6          | 17.8±0.9b   | 15.6±1.1b    | 5.9±0.05b | 3.9±0.4ab | 6.9±0.1b  | 16.7±0.9b | 0.50±0.01b | 50.1±2.7b |
| N12         | 38.2±2.3a   | 30.9±1.1a    | 8.5±1.3a  | 4.8±0.7a  | 8.5±0.1a  | 21.8±2.1a | 0.31±0.02c | 90.9±3.2a |

Note: Data were the means ± SE of 4 seedlings. Values in a column followed by the same letter are not significant different (p=0.05) among N treatments.

**Effect of N on the biomass of plant organs**

The biomass accumulation of the seedlings increased with an increase in N level and the maximum value was recorded in N12 (90.9 ± 3.2 g plant \(^{-1}\)) and the minimum value was recorded in N0 (42.1 ± 3.2 g plant \(^{-1}\)). Similarly, the maximum organ biomass (leaves; total root; stems; and coarse, fine, and finest roots) was recorded in N12, which was significantly different from the values obtained in the other treatments (Table 2). The leaf, stem, whole root, finest root, fine root, and coarse root biomass increased with any increases in N, while the ratio of underground to aboveground biomass in seedlings decreased.

**Effect of N addition on plant endogenous hormones**

The ZR content of stems increased with an increase in N level, and the maximum value was obtained in N12, which was significantly different from N0 treatment. The maximum value was 10.117 ng g \(^{-1}\) FW and the minimum value was 7.643 ng g \(^{-1}\) FW (Fig.1). There were no significant differences in the ZR content of leaves; whole roots; nor the coarse, fine, and finest roots. With increases in soil N, the content of GA\(_{1+3}\) in whole roots and the coarse, fine, and the finest roots decreased, and the value in N0 was significantly higher than that in other treatments (Fig. 2). The lowest values of GA\(_{1+3}\) content in whole roots and the coarse, fine, and the finest roots were 4.829, 4.628, 4.662, and 4.802 ng g \(^{-1}\) FW, respectively; which was significantly different from those in N0. There were no significant differences in IAA content of the leaves; stems; whole roots; nor the coarse, fine, and finest roots (Fig. 3). The ABA content of the leaves, whole roots, and the coarse and fine roots reduced with increases in soil N (Fig. 4).

**Correlation analysis between endogenous hormone level and the biomass of different organs of *P. tabuliformis***

Note: Data were the means ± SE of 4 pots. Values in a column followed by the same letter are not significant different (p=0.05) among N treatments.
The growth rate of the leaves, stems, whole roots, fine roots, and finest roots were positively correlated with ZR content, while the growth of coarse roots was negatively correlated with GA\textsubscript{1+3} content (Table 3). There was a significant positive correlation between leaf growth and \(\frac{w(ZR)}{w(ABA)}\) ratio (\(P = 0.667^*\)), and a significant positive correlation between stem growth and \(\frac{w(ZR)}{w(ABA)}\) (\(P = 0.762^{**}\)) and \(\frac{w(ZR + GA_{1+3} + IAA)}{w(ABA)}\) ratios (\(P = 0.604^*\)). The growth rates of leaves; stems; whole roots; and coarse, fine, and finest roots were significantly positively correlated with \(\frac{w(ZR)}{w(IAA)}\). Additionally, the growth rates of the leaves, stems, and total roots were positively correlated with \(\frac{w(ZR)}{w(GA_{1+3})}\).

Table 3 Correlation analysis between endogenous hormone and biomass in different organs of \textit{Pnus tabuliformis}

| Endogenous hormone | Leaf biomass | Stem biomass | Root biomass | Finest root | Fine root | Coarse root | Whole root |
|--------------------|--------------|--------------|--------------|-------------|-----------|-------------|------------|
| ZR                 | 0.672*       | 0.679*       | 0.744**      | 0.715**     | 0.437     | 0.718**     |
| GA\textsubscript{1+3} | -0.297       | -0.3         | 0.093        | 0.084       | -0.611*   | -0.611      |
| IAA                | -0.096       | 0.11         | 0.202        | 0.167       | -0.294    | 0.033       |
| ABA                | -0.475       | -0.57        | -0.173       | -0.174      | -0.467    | -0.305      |
| \(\frac{w(ZR)}{w(ABA)}\) | 0.667*       | 0.762**      | 0.434        | 0.407       | 0.562     | 0.530       |
| \(\frac{w(GA_{1+3})}{w(ABA)}\) | 0.250        | 0.384        | 0.236        | 0.212       | -0.017    | 0.166       |
| \(\frac{w(IAA)}{w(ABA)}\) | 0.368        | 0.570        | 0.181        | 0.154       | 0.291     | 0.238       |
| \(\frac{w(ZR+GA_{1+3}+IAA)}{w(ABA)}\) | 0.419        | 0.604*       | 0.233        | 0.205       | 0.319     | 0.288       |
| \(\frac{w(ZR)}{w(GA_{1+3})}\) | 0.718*       | 0.738**      | 0.437        | 0.435       | 0.792     | 0.626*      |
| \(\frac{w(ZR)}{w(IAA)}\) | 0.848**      | 0.702*       | 0.677*       | 0.672*      | 0.739**   | 0.787**     |
| \(\frac{w(ZR)}{w(IAA+GA_{1+3})}\) | 0.866**      | 0.744**      | 0.660*       | 0.653*      | 0.794**   | 0.794**     |

Note: One and two asterisks mean the significance level at 0.05 level and 0.01 level, respectively.

**Discussion**

As atmospheric N increases with the increase in human and industrial activities, it is expected that available soil N in the form of ammonium N will also increase. N has been reported to play significant roles in plant growth through the actions of several plant hormones, such as GA\textsubscript{1+3}, ABA, IAA, and ZR. In the present study, increases in soil N had various effects on the contents of ZR, GA\textsubscript{1+3}, IAA, and ABA in the leaves, stems, whole roots, finest roots, fine roots, and coarse roots of \textit{Pnus tabuliformis}. Additionally, the synergistic regulatory activities of the various hormones influenced the growth of the leaves, stems and roots, thus confirming the first hypothesis of this study. We noticed that N addition increased the ZR content in stems and decreased the ABA content of the leaves and whole roots and the GA\textsubscript{1+3} content of the whole roots (Fig. 1,2 and 4), while the IAA content was not significantly affected by N addition in various organs (Fig. 3), and this was not completely consistent with the results obtained in dwarf apple trees (Li et al. 2015). Growth-promoting hormones, ZR, IAA, and GA\textsubscript{1+3} are beneficial for plant growth, but their mechanisms are different in different plant organs (Ciura and Kruk 2018). N application was reported to increase ZT content of the leaves of Green tea, while the concentration of ABA, a
growth inhibiting hormone, decreased with N application (Xiao et al. 2018). The reported differences on the effect of N on hormones in different plant organs may be attributed to differences in plant species and organ types.

In the present study, an increase in soil N promoted plant, leaf, stem, and root growth (Table 2), which was consistent with the results of Zhao and Zeng (2019). Several studies have shown that N can promote plant growth, however, excessive N is inhibitory to plant growth (Wang et al. 2019; Xu et al. 2018). In the present study, N addition did not inhibit growth and this may be due to the low soil available N content of the Loess soil used for the study and the duration of the N treatment. We also observed that N addition reduced the ratio of underground to aboveground biomass of the seedlings of Pinus tabuliformis (Table 2), which is consistent with the functional balance hypothesis (Li et al. 2016; Wang and Liu 2014), indicating that the mechanism of underground and aboveground growth may be related to the pattern of plant hormone distribution. Grechi et al. (2007) reported that plants may preferentially distribute photosynthetic products to the organs that absorb the resources during times of resource scarcity. Additionally, it has been reported that apart from the direct impact of some hormones on plant growth, the interaction of several hormones may also affect growth, such as the ratio of ZR:ABA (Depuydt and Hardtke 2011). In the present study, the growth of leaves, stems, and whole roots was prominently promoted by ZR content and the synergistic regulatory activities of the hormones. The stem growth was mainly influenced by the $w(ZR)/w(GA_{1+3})$ ratio, while the leaf and whole root growth were influenced by $w(ZR)/w(IAA)$ ratio (Table 3), which was similar to the findings of Li et al. (2015), implying that the synergistic regulatory activities of growth-promoting hormones may be largely responsible for growth differences among plant organs.

Similarly, the growth of the coarse, fine, and finest roots of P. tabuliformis increased with an increase in N addition (Table 2), however, their growth rates were affected differentially by hormone levels and this result partially supports our second hypothesis. The plant root system is a highly heterogeneous hierarchy system, and the growth of the coarse, fine, and finest roots are affected differently by plant endogenous hormones. Trans-zeatin ribose promoted the growth of the finest roots and fine roots, while coarse roots were negatively regulated by GA$_{1+3}$ (Table 3), which is similar to the findings of Li et al. (2018). Several studies have reported that ZR, a cytokinin is involved in root growth, while GA, a plant hormone, regulates stem growth and is also involved in root growth; additionally, root elongation is initiated at low GA concentrations (Tanimoto 2012). Furthermore, in the present study, $w(ZR)/w(IAA)$ ratio was correlated with the growth rates of the coarse, fine, and finest roots; indicating that ZR and IAA play a balanced role in promoting root growth. The increase in ZR led to a decrease in IAA content, which affected the growth of the coarse, fine, and finest roots and this is similar to the findings of Mercier et al. (1997), suggesting that there is a balance between IAA and cytokinins in plant roots. In this study, more attention was paid to the effect of N addition on the endogenous hormone content in different organs and root systems of different diameters to understand plant growth, but little attention was paid to the molecular mechanism behind it. In future studies, we need to further elucidate how changes in endogenous hormone levels affect plant growth across molecular, genetic, and other levels.

**Declarations**

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**Authors’ contributions**

All authors contributed to the study. Material preparation and data collection were performed by Keli Chen and Furong Wei. Data analysis and the draft were performed by Yuhang Peng. Research design, paper framework and subsequent revision were all completed under the guidance of Yanping Ma and Guoliang Wang. All authors already read the manuscript and approved it.
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Availability of data and material
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests

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**Figures**
Figure 1

Changes of ZR contents in different organs of seedlings under different concentrations of nitrogen treatments. Note: Data are the means ± SE of 3 seedlings. Different letters mean significant differences among N treatments at 0.05 level.
Figure 2

Changes of GA1+3 contents in different organs of seedlings under different concentrations of nitrogen treatments. Note: Data are the means ± SE of 3 seedlings. Different letters mean significant differences among N treatments at 0.05 level.
Figure 3

Changes of IAA contents in different organs of seedlings under different concentrations of nitrogen treatments. Note: Data are the means ± SE of 3 seedlings. Different letters mean significant differences among N treatments at 0.05 level.
Figure 4

Changes of ABA contents in different organs of seedlings under different concentrations of nitrogen treatments. Note: Data are the means ±SE of 3 seedlings. Different letters mean significant differences among N treatments at 0.05 level.