

Endogenous hormone and nutritional ingredient of pendulous characteristics of *Cunninghamia lanceolata* var. *Luotian*

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Abstract

Cunninghamia lanceolata var. *Luotian* (herein, *Luotian*), a natural variety of *Cunninghamia lanceolata* (Chinese fir, herein *Lanceolata*), shows great potential for agricultural protection short-period and high-density plantations because of its narrow, pointed, tower-shaped crowns, thin braches and older drooping branches that die naturally at 6-7 years old. The physiological mechanisms of the pendulousness and the natural death of the branches are still unclear. To investigate these mechanisms, phytohormone levels and nutritional ingredients of different positions of branches in *Luotian* and *Lanceolata* were determined. Phytohormone analyses showed that IAA and ZR played critical functions on the branch initial growth, and that GA3 was related to bending of branches. Meanwhile, ABA may enhance abscission of the branches in *Luotian*. Nutritional ingredient analyses showed that the accumulation of nutritional ingredients in *Luotian* and *Lanceolata* were consistent with the morphology of their branches. An integrated analysis implied that phytohormones had a strong influence on pendulous characteristics by interacting with other factors. This study helps to elucidate the mechanism governing the pendulous trait and provides theoretical basis and technical support for its cultivation, introduction, plantation management and genetic improvement.

Keywords: *Cunninghamia lanceolata* var. *Luotian*; morphology; nutritional ingredient; pendulous characteristics; phytohormones

Abbreviations: ABA: abscisic acid; BL: branch length; CZ: crown size; DB: downside of base; DBH: diameter at breast height; DINC: branch deep into the crown; DM: downside of middle; DT: downside of tip; F: the first crown layer; GA3: gibberellic acid 3; H: tree height; HCB: height to the lowest live crown base; IAA: indole-3-acetic acid; S: the second crown layer; SP: soluble proteins; SS: total soluble sugars; ST: starch; T: the third crown layer; UB: upper side of the base part of branch; UM: upper side of the middle part of branch; UT: upper side of the tip part of branch; ZR: trans-zeatin-riboside.

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Introduction

Cunninghamia lanceolata (Lamb.) Hook (Chinese fir, denoted as Lanceolata), is the most important forest tree species for plantations in southern China because of its good timber quality, fast growth, and wide distribution. The species has been cultivated in the Yangtze River Basin for more than 1000 years (Zheng, 1983), with a total area of 11.26 million hectares and 734.09 million cubic meters in 2010 (Xu *et al.*, 2015). Chinese fir possesses geographical provenance variations because of its great natural distribution range. Affected by the complex interactions between ecological environment and human factors, Chinese fir populations have formed different genetic characteristics for different ecological environment (Wu, 1984). *Cunninghamia lanceolata* var. *Luotian* (denoted as Luotian), discovered in Luotian County, Hubei Province in the mid-1970s (Hubei Institute *et al.*, 1977), has the novel morphological features, such as narrow, pointed, tower-like crowns, thin branches, and older drooping branches that die naturally at 6-7 years old (Xu *et al.*, 2016, 2019).

Plant architecture is a vital characteristic that can contribute to crops and economically important trees. Crown morphological structure is not only influenced by various factors, such as branch angle and orientation, branch length, plant height, branching pattern, branching cycle, growth rate variation, and plant hormone transport (Srivastava, 2002; Hollender and Dardich, 2015; Wang *et al.*, 2018), but also by environmental factors, such as light, temperature, and moisture (Wang and Li, 2008). Therefore, tree architecture is considered to be a very complex morphology, and the mechanisms involved in tree architecture are not yet clear. In order to well describe and classify types of woody plants, Hallé *et al.* (1978) first proposed 23 general plant types to predict the growth potential of plants under no biological or abiotic stresses, and thought that the growth process of some species could be inferred from these classifications. Additionally, several other nonstandard tree architectures were proposed, such as straight and weeping. A straight tree possesses such a architecture in which the branches grow suberect and upward, named as apogeotropism, while a weeping tree has another crown architecture in which the branches grow prostrate or droop without appearing a straight branch (Halle *et al.*, 1978; Tworkoshi and Scorza, 2001).

Pendulous tree architecture has been found in many woody plants, not only in broad-leaved species, such as *Betula pendula* (Salojärvi *et al.*, 2017), *Prunus mume* (Lv and Chen, 2003; Zhang *et al.*, 2015), *Prunus persica* (Werner and Chaparro, 2005) and *Morus alba* (Yamanouchi *et al.*, 2009), but also in coniferous species, such as *Araucaria cunninghamii* 'Pendula', *Cedrus deodara* 'Pendula', *Larix deciduas* 'Varied Directions', *Picea glauca* 'Pendula', *Taxodium ascendens* 'Nutans' (Lv, 2001). For woody plants, the secondary xylems in the vascular bundles are the key supporting structures, and the formation of secondary xylem is often involved in the co-action of various phytohormones. Phytohormones play important roles in initiating primary wall expansion and secondary wall formation in procambium differentiation (Mellerowicz *et al.*, 2001; Sundberg *et al.*, 2000). Among these phytohormones, auxin is the most important hormone that controls wood formation, which is involved in many physiological processes, including apical potential, stem elongation and lateral root formation, and is also recognized as a stimulant of wood formation (Davies, 2010). Other phytohormones are also found to be involved in xylem development, with synergism (gibberellin, cytokinin, ethylene) or antagonism (abscisic acid) with IAA (Plomion *et al.*, 2001). It was found that the change in GA concentration inside the branches, the timing of reaction wood formation and the response to gravitation might be the factors for the formation of pendulous branch habits. However, the relationship between these factors and the experimental results is not clear at present (Hollender and Dardich 2015). Nugroho *et al.* (2012) suggested that GA had a strong influence on the initial stages during the formation of tension wood and stem gravitropism in *Acacia mangium*. Similarly, GA could restore the upward and upright growth trend of the branches in weeping peach (*Prunus persica*) and weeping cherry (*Prunus spachiana*) (Nakamura *et al.*, 1994, 1995). Israelsson *et al.* (2005) showed that GA was mainly localized and synthesized in the xylem cell extension area, and the main function of GA in wood formation was to regulate early xylem differentiation, including cell elongation. Liu *et al.* (2017) suggested an important role of GA and auxin in regulating the weeping trait in

Salix matsudana. Li *et al.* (2020) also indicated that GA synthesis and signal transduction pathways play a role in weeping traits in crape myrtle (*Lagerstroemia indica*).

Compared with common Chinese fir, under the same site conditions, height, diameter at breast height (DBH), and volume of single tree of Luotian was 30%~ 60%, 10%~40%, 30%~70% higher than that of common Chinese fir, respectively, (Xu *et al.*, 2005). In addition, Luotian has a straight trunk with little junctions and high economic productions (Zheng, 1983; Xu *et al.*, 2005). Since short-period plantations provide small and medium size timber, higher densities in Luotian plantations might maintain higher yield. Additionally, Luotian is a good variety as agricultural protection forest species for its narrow and small crown, with less effect on crops in aspect of light. Previous studies mainly focused on its propagation (Huang *et al.*, 2016; Xu *et al.*, 2017), photosynthetic physiology (Huang *et al.*, 2015), morphology (Xu *et al.*, 2005, 2020), anatomy (Yang *et al.*, 2018), and genetics (Li *et al.*, 2012). However, the physiological mechanism involved in the formation of pendulous branches in Luotian and its natural pruning are not clear. In order to explore the mechanisms, this study investigated the changes in the concentrations of phytohormones, the total soluble sugars and proteins, and starch in different developmental stages of *Cunninghamia lanceolata* var. *Luotian* and *Cunninghamia lanceolata*. This paper attempts to reveal the formation process of pendulous branches and its relationship with phytohormones and nutrients, and provides theoretical basis and technical support for its cultivation, introduction, management and genetic improvement.

Materials and Methods

Introduction to study site

All materials were obtained in a plantation with Luotian and common Chinese fir, located in Luotian County in the northeast of Hubei province, with geographic site of E 115°06'~ 115°46' and N 30°35'~ 31°16', at the southern foot of Ta-pieh Mountains. The territory is mountainous, with terrain inclining from northeast to southwest, surrounded by mountains in the north. Seven mountains have an elevation of more than 1000 m, while the rest of the mountains are above 300 m. The terrain of the central region is relatively open, with widely distributed hills with elevations of more than 200 m. The climate is north subtropical monsoon, which is dry and cold in winter, humid in summer, warm in spring and cool in autumn. The average annual total sunshine duration is 2047 h, the average annual radiant energy is 109.25 kCal•m⁻². The average annual temperature in the county is 16.4 °C, and the maximum temperature reaches to 41.6 °C, the minimum temperature is as low as -14.6 °C. The average frost-free period is 240 days and the average annual precipitation is 1330 mm which mostly occurs in May, June and July, accounting for about 50% of the annual rainfall. The study area is in the junction of Hubei and Anhui province on the southwestern slope of Ta-pieh Mountains with the highest elevation at 1408.2 m. It is one of the main waters shed between the Yangtze and Huai River and contains more than 1600 species of plants, including more than 200 species that are nationally protected. Additionally, there are over 100 species rare animals and 20,000 acres of virgin forest in this area, with forest coverage as high as 90%.

Sampling

A plantation of preserved *C. lanceolata* var. *Luotian* was selected which was built in 1993 with clone seedlings, with the density of 2×3 m, mixed with *C. lanceolata* in the plantation. Three sample trees of Luotian and Lanceolata were random collected in the plantation. After the sample trees were felled, some parameters were measured, such as diameter at breast height (DBH), height (H), crown size (CZ), and height to the lowest live crown base (HCB) (Table 1). For all sample trees, the crown was separated into three layers, i.e., from the crown tip to the base, the first, the second, and the third layer. In each layer, 3-5 standard lateral branches were chosen as samples for analyses, and these branches were divided into three parts: base, middle, and tip part. Samples were obtained at the upper side and downside of each branch part mentioned above according to

gravity, i.e., upper side of base part (UB), down side of base part (DB), upper side of middle part (UM), down side of middle part (DM), upper side of tip (UT), downside of tip (DT). The barks of all samples were peeled and their secondary xylems were scraped into 3 mm of the samples. These secondary xylems were used for analyses of phytohormones and nutrients.

Table 1. Summary of data for *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata* sample trees

Tree	No. of sample trees	H (m)	DBH (cm)	HCB (m)	CZ (m)
Luotian	3	14.3	15.7	11.6	1.8
Lanceolata	3	12.8	17.4	4.2	5.3

Note: H: height, DBH: diameter at breast height, HCB: height to the lowest live crown base, CZ: crown size.

Determination of phytohormones and nutrition components

All endogenous hormones (IAA, GA₃, ABA, and ZR) were analyzed by Enzyme-linked Immunosorbent Assays (ELISA) at the lab of Prof. Wang (Engineering Research Center of Plant Growth Regulator, Ministry of Education, China Agricultural University, China), as previously described (Chen *et al.*, 2009).

The soluble protein (SP) content was estimated as described by Bradford (1976), with bovine serum albumin as the standard. Anthrone-sulphuric acid colorimetry was used to determine the content of total soluble sugars (SS) and starch (ST) (Riazi *et al.*, 1985).

Data analyses

The variables related to endogenous hormone, hormone ratios and nutritional ingredients were analyzed by using SPSS 22.0 and Microsoft excel 2010, significance levels between Luotian and Lanceolata samples, and the tendency of endogenous hormone levels, and nutritional ingredients concentrations at the same position of branches in different crown layers were also determined, and the significance level was set at $p = 0.05$.

Results

Hormone concentrations

Changes in ABA concentrations

In both the upper and down sides of the branches in the first crown layer of Luotian, ABA concentrations reduce gradually from the base to the tip parts of the branches, and ABA concentrations in the downsides of the tip part and base part of the branch was significantly lower than those in the corresponding upper sides ($p < 0.05$, Table 2). On the contrary, ABA concentrations in the upper and down sides of branches in the second and the third crown layers gradually increased from the base to tip part, and ABA concentrations in the tip parts of branches were significantly higher than those in the base and the middle parts of branches ($p < 0.05$, Table 2). For each part of the branches in the second and the third crown layer, ABA concentrations were not significantly different between the upper and the down side ($p > 0.05$, Table 2)).

In general, in the first crown layer of Lanceolata, ABA concentrations also reduced gradually from the base part to tip part, and at each part ABA concentrations were not significantly different between the upper and the down side ($p > 0.05$, Table 2). In the second and the third crown layer, ABA concentrations were the highest at the tip parts of the branches (Table 2)

In order to investigate the role of ABA in abscission of Luotian branches, ABA concentrations were compared between Luotian and Lanceolata. ABA concentrations at the tips of branches in the second crown layer of Luotian were significantly higher than those of Lanceolata trees ($p < 0.05$, Figure S1), and ABA

concentrations were not significantly different in other parts of the branches in all the three crown layers ($p>0.05$, Figure S1).

As for ABA concentration changes in the whole crown, Luotian and Lanceolata showed similar patterns (Figure S2). From the first to the third crown layer, ABA concentrations in the upper and the down sides at the base and the middle parts of the branches gradually reduced, and gradually increased at the tip parts of the branches (Figure S2).

Table 2. Comparison of hormone concentrations in different positions at the same crown layer in *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Tree	Position of branch	The first crown layer				The second crown layer				The third crown layer			
		ABA	GA3	IAA	ZR	ABA	GA3	IAA	ZR	ABA	GA3	IAA	ZR
Luotian	UB	152.9±2.5a	7.1±2.3a	41.7±5.0a	9.8±2.3a	53.4±14.8b	4.7±1.2a	29.1±9.0a	3.8±1.0c	26.3±4.8b	5.7±1.3a	27.5±1.8a	3.8±0.3a
	DB	137.9±24.7ab	7.5±2.9a	33.5±1.2a	8.0±1.9ab	59.1±12.7b	6.5±0.0a	27.5±5.4a	4.2±0.6bc	37.2±12.7b	5.6±0.6a	33.0±16.1a	4.4±1.4a
	UM	116.8±25.2ab	6.7±2.3a	28.8±1.7a	6.3±1.1ab	63.3±3.8b	6.5±3.2a	36.3±22.1a	4.9±1.6abc	50.5±5.3b	6.3±1.7a	33.7±13.6a	4.8±1.3a
	DM	122.8±5.9ab	7.9±4.0a	34.8±4.6a	7.3±2.9ab	68.0±21.7b	7.4±2.0a	26.4±6.1a	4.7±0.9abc	59.0±18.5b	5.9±1.3a	29.6±3.7a	4.6±0.5a
	UT	110.7±24.0ab	6.9±0.8a	41.6±10.9a	6.0±1.6b	123.1±4.4a	6.3±2.2a	38.9±8.5a	6.2±0.8a	141.2±40.4a	5.5±1.6a	33.2±7.6a	4.4±1.1a
	DT	96.2±50.2b	6.1±1.4a	43.4±18.9a	5.7±2.5b	122.4±13.1a	6.8±0.9a	42.2±6.4a	5.9±1.1ab	138.2±50.3a	5.5±1.6a	31.6±8.8a	4.1±1.0a
Lanceolata	UB	126.5±27.2a	5.9±1.6a	39.3±7.6a	7.0±1.8a	69.4±49.9a	4.4±0.7a	39.9±4.6a	6.2±2.1ab	30.8±2.0b	4.5±1.3a	34.8±3.5a	6.1±1.8a
	DB	115.3±27.6a	5.3±0.5a	45.0±1.2a	7.3±1.6a	67.4±49.1a	4.1±0.7a	42.5±5.7a	4.9±0.5b	42.8±30.3b	5.2±1.3a	36.7±4.6a	6.3±1.4a
	UM	113.5±37.6a	6.5±0.8a	42.6±12.8a	6.7±2.5a	58.3±25.1a	5.7±1.8a	37.1±3.4a	6.3±0.9ab	37.3±28.5b	4.0±1.49a	34.7±5.5a	5.6±2.2a
	DM	121.8±30.2a	6.5±1.0a	45.71±10.8a	8.4±2.3a	58.7±23.0a	5.5±1.2a	35.2±9.3a	5.2±1.1ab	33.3±25.7b	5.3±2.0a	36.3±7.5a	5.2±1.4a
	UT	110.1±37.4a	5.4±1.3a	45.5±3.0a	8.2±2.3a	112.0±1.5a	5.4±1.3a	42.3±10.5a	7.7±2.1a	121.6±13.1a	4.8±1.4a	40.7±7.0a	7.1±0.8a
	DT	107.1±41.1a	5.1±1.2a	49.3±8.0a	8.1±2.0a	103.7±4.9a	4.3±0.9a	40.4±15.8a	6.2±1.2ab	116.3±32.2a	4.9±1.5a	45.1±9.0a	7.2±3.1a

Note: Different letters in each column indicate that they are significantly difference (LSD test, $p \leq 0.05$) according to Least Significant Difference (LSD) (The same below)

Changes in GA3 concentrations

There was no significant difference in GA3 concentrations between different parts of the branches in the same crown layer of Luotian and Lanceolata (Table 2). For the first crown layer of Luotian, GA3 concentrations were higher at the upside of the base and middle parts of branches than those at the downsides. For changes in GA3 concentrations in Lanceolata, in general, GA3 concentrations were higher at the upper sides than those at the down sides of the branches in the first and the second crown layer, but in the third crown layer, the situation was just contrary (Table 2).

GA3 concentrations in the upper and down sides of branches in the first and the third crown layer were higher in Luotian than those in Lanceolata, but the differences were not significant ($p>0.05$, Figure S3). GA3 concentrations in the down sides at the base and the tip part were significantly higher in Luotian than those in Lanceolata ($p<0.05$, Figure S3).

In general, GA3 concentrations were higher in Luotian than those in Lanceolata in the upper and down sides of the same positions of the branches, but GA3 concentrations showed no significant differences between Luotian and Lanceolata ($p>0.05$, Figure S4), except few cases.

Changes in IAA concentrations

For Luotian, IAA concentrations in the upper sides at the base parts of branches in the first and the second crown layer were higher than those in the down sides at the same position, and the situation was just contrary at the base parts of the branches in the third crown layer (Table 2). But for Lanceolata, IAA concentrations in the upper sides at the base parts of branches in the first and the second crown layer were lower than those in the down sides at the same position (Table 2).

Between Luotian and Lanceolata, IAA concentrations in the upper and the down sides of at almost all parts of the branches in the three crown layers were higher in Luotian (Figure S5).

There were significant differences in IAA concentrations between Luotian and Lanceolata at the downside of the base parts of the branches in the second crown layer and at the upside of the base parts of the branches in the third crown layer ($p < 0.05$, Figure S5).

In the whole crown of Luotian, IAA concentrations in the upper sides at the base parts of the branches reduced gradually from the first to the third layer, IAA concentrations in the down sides at the base parts of the branches seemed to be consistent (Figure S6A, B). At the same time, changes in IAA concentrations in the upper and the down sides at the base parts of the branches in all the three crown layers were little (Figure S6A, B). IAA concentrations in the middle and the tip parts showed little changes in Luotian and Lanceolata (Figure S6, C-F).

Changes in ZR concentrations

For Luotian, ZR concentrations in both of the sides reduced gradually from the base parts to the tip parts of the branches in the first crown layer, and ZR concentrations were higher in the upper sides at the base part than those in the down sides, but the situations were just contrary in the second and the third crown layer (Table 2). ZR concentrations in the two sides of branches gradually increased in the second crown layer, and ZR concentrations at the base parts were significantly lower than those in the tip parts ($p < 0.05$, Table 2).

For Lanceolata, although ZR concentrations showed no significant changes from the base part to the tip part of branch in the first crown layer ($p > 0.05$, Table 2), ZR content in the upper side at the base part of the branch in the first crown layer was lower than that in the down side, but there was no significant difference ($p > 0.05$, Table 2).

In both the sides at the base part of the branch in the first crown layer, ZR concentrations were higher in Luotian than those in Lanceolata, but there were no significant differences ($p > 0.05$, Figure S7). But at the middle and the tip part of the branch in the first crown layer, ZR concentrations in the two sides were lower in Luotian than those in Lanceolata (Figure S7). The same situations occurred in the second and the third crown layer (Figure S7).

In general, in the whole crown of Luotian and Lanceolata, ZR concentrations gradually decreased from the first to the third crown layer (Figure S8)

Hormone ratios

Changes in ABA/GA3 ratios

In most cases, the ABA/GA3 ratios in Lanceolata were higher than those in Luotian, but in all the cases there were no significant differences in ABA/GA3 ratios all the positions ($p > 0.05$, Figure 1). In both Luotian and Lanceolata, the ABA/GA3 ratio was correlated with depth into crown base. As shown in Figure 1, for both Luotian and Lanceolata, higher ABA/GA3 ratios occurred in young branches and young parts of old branches. Furthermore, ABA/GA3 ratios were higher in Luotian than those in Lanceolata in the tip parts of old branches in the third crown layer.

For Luotian, the ABA/GA3 ratios in the tip parts of the branches in the third crown layer were significantly higher than those in the first crown layer in Luotian ($p < 0.05$, Table S1), and also were significantly higher than those in the base and middle parts of branches in the third crown layer ($p < 0.05$, Table S1).

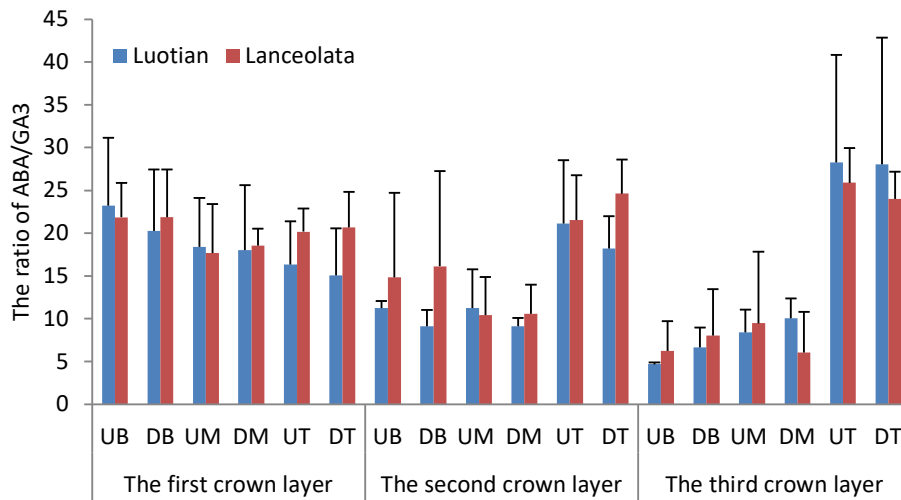


Figure 1. The ratios of ABA/GA3 at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Changes in ABA/IAA ratios

ABA/IAA ratio in Luotian was significantly higher than that in Lanceolata in the downside of the base part of the branch in the first crown layer and in the downside of the tip part of the branch in the third crown layer ($p < 0.05$, Figure 2). For Luotian, ABA/IAA ratios in both sides of the tip part of branch were significantly higher than those in the other parts of branch in the third crown layer ($p < 0.05$, Table S1), and ABA/IAA ratios gradually increased from the base to the tip part of branch in the third crown layer (Figure 2). Meanwhile, the ABA/IAA ratios in Luotian in all the three layers were higher than those in Lanceolata, except for the downside of tip part of branch at the first crown layer (Figure 2).

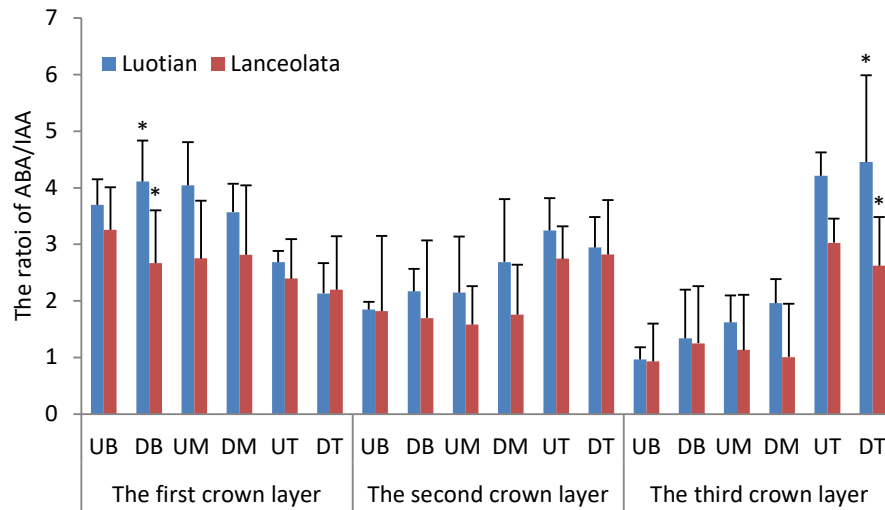


Figure 2. The ratios of ABA/IAA at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Note: * in each bar indicate that they are significantly difference (LSD test, $p \leq 0.05$) (The same below)

Changes in ZR/GA3 ratios

The differences in ZR/GA3 ratios were obvious between Luotian and Lanceolata (Figure 3). Compared to Lanceolata, in all the tip parts of Luotian branches, ZR/GA3 ratios were significantly lower ($p < 0.05$, Figure 3). Furthermore, in all parts of branches in the second and the third crown layers, ZR/GA3 ratios were significantly lower in Luotian, compared to Lanceolata ($p < 0.05$, Figure 3), and ZR/GA3 ratios were not significantly different in the base and the middle parts of branches in the first crown layer ($p > 0.05$, Figure 3).

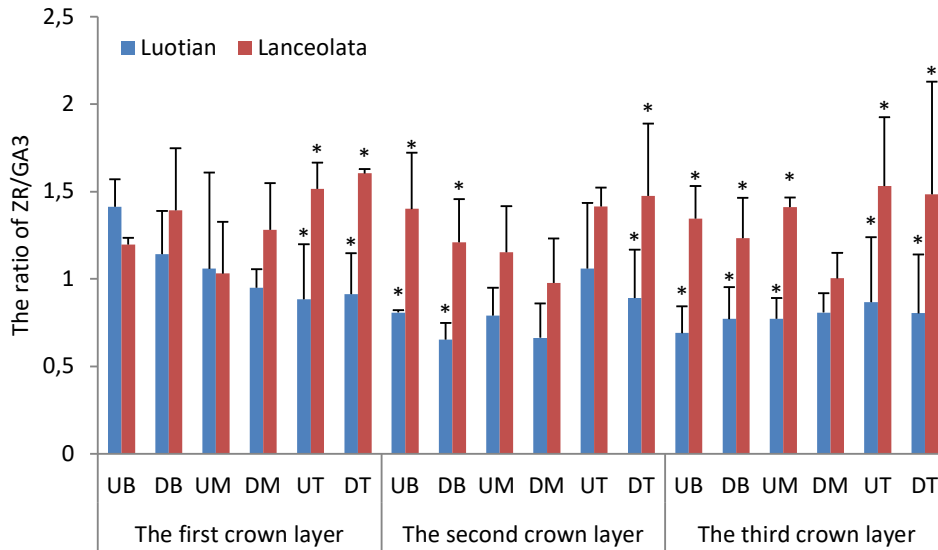


Figure 3. The ratios of ZR/GA3 at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Changes in IAA/ZR ratios

There were no significant differences in IAA/ZR ratios at all the positions of the branches between Luotian and Lanceolata ($p > 0.05$, Figure 4). In the first crown layer of Luotian, IAA/ZR ratios in the tip parts of branches were higher than those in the other parts, especially in the downside of tip part, whose IAA/ZR ratio was significantly greater than that in the downside at the base and middle part ($p < 0.01$, Figure 4). There were no significant differences in IAA/ZR ratios among the positions in the third crown layer of Luotian ($p > 0.05$, Table S1). In the tip of Lanceolata branch, IAA/ZR ratio at the downside was higher than those at the upside among all crown layers. At the middle of Lanceolata branch, the value of IAA/ZR ratio at the upside of the first crown layer was greater than those at the downside, but IAA/ZR ratio at the upside of the second and third crown layer was less than that at the downside. While the upside of the first and second crown layer had higher IAA/ZR ratio than that at the downside, however, which was opposite at the third crown layer (Table S1).

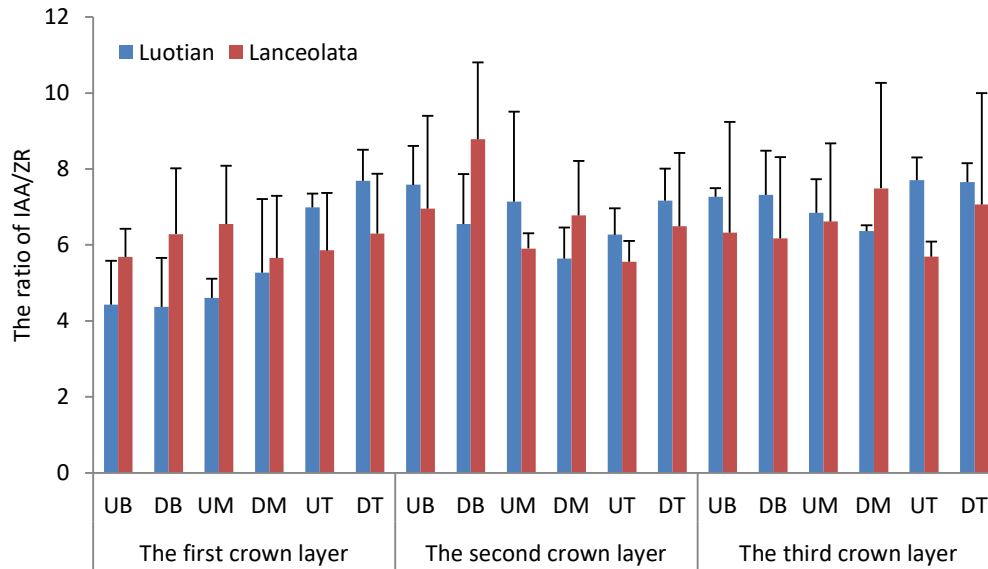


Figure 4. The ratios of IAA/ZR at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Changes in IAA/GA3 ratios

IAA/GA3 ratios showed that in *Lanceolata* which were higher than those in *Luotian* (Figure 5). There were significant differences in IAA/GA3 ratio at the downside of the base part at the second crown layer ($p < 0.01$) and the upside of the middle part at the third crown layer ($p < 0.05$) among all the positions of sample trees (Figure 5). Although the IAA/GA3 ratio has no significant difference among different positions in every crown layer both in *Luotian* and *Lanceolata* ($p > 0.05$, Table S1), IAA/GA3 at downside of the tip of the first crown layer was notably greater than that at downside of middle part at the second crown layer in *Luotian* ($p < 0.05$, Table S1).

Changes in ABA/ZR ratios

ABA/ZR ratio in *Luotian* was higher than that in *Lanceolata* at all the positions of branches, except the upside of the base at the first crown layer (Figure 6). The significant differences of ABA/ZR ratios between *Luotian* and *Lanceolata* were showed at the tip of the third crown layer ($p < 0.01$, Figure 6).

The maximum ABA/ZR ratio in *Luotian* was 33.62, which was at the downside of the tip in the third crown layer, while the minimum value was 6.98, at the upside of the base in the same crown layer. While in *Lanceolata*, the highest value of ABA/ZR was 18.21, which was at the upside of the base at the first crown layer, while the lowest ratio of ABA/ZR (4.62) was at the upside of the base at the third crown layer. For *Luotian*, ABA/ZR ratio at the tip of the third crown layer significantly differed from the all-other positions ($p < 0.05$, Table S1), especially the other parts at the third crown layer ($p < 0.01$, Table S1). With regard to *Lanceolata*, the ratio of the tip was also significantly greater than those of the other parts in the third crown layer ($p < 0.05$, Table S1). At the same time, ABA/ZR ratio at the base and middle parts in the first crown layer was significantly higher than those in the third crown layer ($p < 0.05$, Table S1), respectively.

Compared to the two sides of the branches, the ratio at the upside was greater than that at the downside in the first crown layer, while for the second and third crown layer, the ratio at the upside was less than that at the downside in *Lanceolata*, almost as the same as in *Luotian* (Table S1).

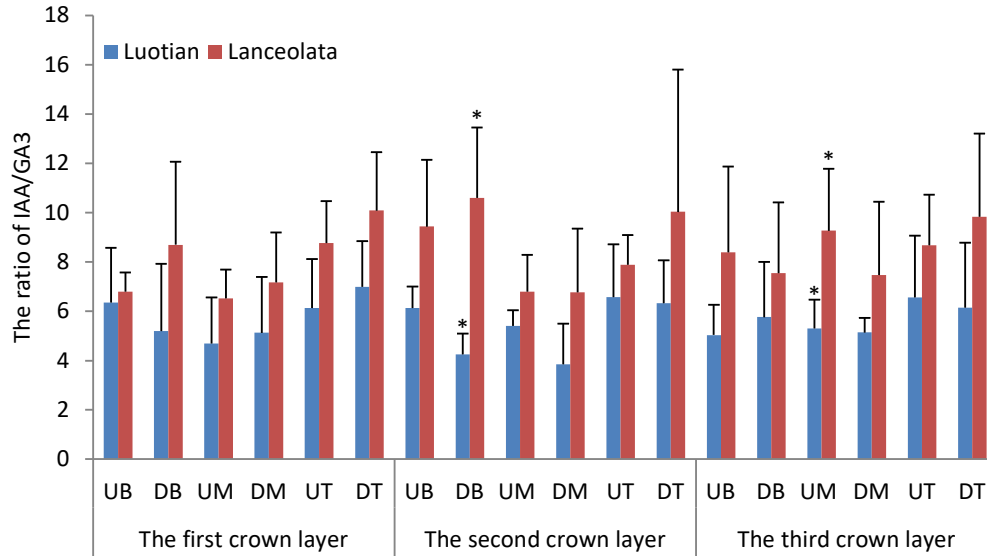


Figure 5. The ratios of IAA/GA3 at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

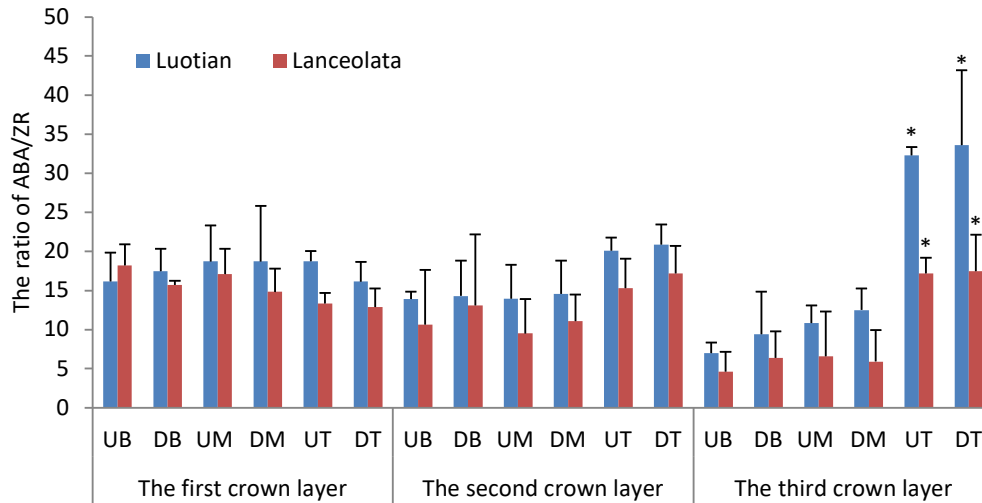


Figure 6. The ratios of ABA/ZR at different positions of *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Nutrient concentrations

Changes in soluble proteins

Soluble protein concentrations were measured both in *Cunninghamia lanceolata* and *Cunninghamia lanceolata* var. *Luotian* branches showed in Figure S9. Compared to *Lanceolata*, the soluble protein concentrations of the first and the second crown layers in *Luotian* were lower, except that at the upside of the tip of the second crown layer. However, in the third crown layer, except the downside of the middle part, the soluble protein concentrations in *Luotian* were higher than that in *Lanceolata*. There were significant differences in soluble proteins at the upside of the base and middle part ($p < 0.05$), and the downside of tip

($p < 0.01$) at the first crown layer, as well as those at the upside of the base ($p < 0.01$) and middle part ($p < 0.05$), the downside of the base part ($p < 0.05$) at the second crown layer (Figure S9).

The soluble protein concentration was increased with the growth of the branches among all crown layers in Luotian, as well as in Lanceolata (Table 3). In both Luotian and Lanceolata, the concentrations of soluble proteins at the downside of the tip among three crown layers varied significantly ($p < 0.05$), with similar variations in trend (Figure S10).

Table 3. Comparison of nutritional ingredient concentrations in different positions at the same crown layer in *Cunninghamia lanceolata* var. *Luotian* and *C. lanceolata*

Tree	Positions of branch	The first crown layer			The second crown layer			The third crown layer		
		SP	SS	ST	SP	SS	ST	SP	SS	ST
Luotian	UB	1.1± 0.4a	153.0± 21.0a	144.4± 54.9a	1.8± 0.8a	268.3± 77.6a	138.2± 79.6a	14.1± 19.7a	172.6± 38.6a	116.2± 55.4a
	DB	2.4± 1.5a	200.2± 95.1a	150.4± 14.4a	1.2± 0.7a	185.6± 82.6a	101.6± 59.2a	5.3± 5.5a	207.6± 77.9a	146.6± 70.3a
	UM	1.7± 1.4a	325.4± 258.9a	159.4± 58.5a	1.1± 0.3a	225.3± 64.4a	123.7± 78.9a	2.6± 2.0a	266.2± 155.9a	127.5± 21.2a
	DM	1.6± 1.5a	235.4± 115.2a	161.3± 36.9a	2.6± 1.8a	232.1± 80.2a	84.6± 52.3a	7.1± 6.5a	242.1± 123.6a	128.8± 40.6a
	UT	3.4± 1.7a	256.6± 107.5a	199.9± 78.1a	6.9± 8.6a	245.1± 79.3a	113.4± 60.4a	19.9± 11.2a	158.8± 69.9a	84.4± 50.0a
	DT	2.2± 0.3a	184.4± 58.0a	179.0± 33.6a	6.9± 5.6a	251.3± 13.4a	116.9± 66.2a	14.5± 8.9a	117.5± 91.2a	86.6± 18.2a
Lanceolata	UB	5.3 ± 1.8a	409.3± 74.4a	151.5± 53.6a	5.0± 0.4a	139.8± 101.7a	205.6± 158.4a	3.7± 3.7b	231.0± 169.2a	80.4± 27.6a
	DB	6.4 ± 2.2a	447.4± 114.2a	157.6± 70.7a	6.0± 2.0a	83.9± 56.8a	209.9± 136.2a	4.7± 0.3ab	242.0± 199.1a	102.3± 28.2a
	UM	5.7 ± 0.5a	481.8± 242.1a	206.6± 168.9a	5.34± 2.1a	233.7± 176.8a	200.0± 143.9a	8.5± 4.6a	163.2± 36.7a	172.3± 118.3a
	DM	6.0 ± 2.5a	449.7± 116.8a	269.9± 250.0a	7.3± 4.2a	162.5± 110.9a	208.5± 152.0a	6.5± 1.8ab	145.3± 46.6a	125.4± 75.7a
	UT	5.4 ± 0.9a	391.7± 97.9a	102.9± 79.4a	6.2± 2.8a	237.2± 140.8a	239.0± 140.9a	7.6± 1.2ab	144.4± 33.8a	90.0± 62.9a
	DT	3.7 ± 0.3a	392.2± 167.0a	202.3± 139.1a	8.3± 3.2a	254.2± 208.7a	224.5± 70.2a	8.4± 0.9a	140.4± 38.5a	126.7± 66.2a

Note: SP: soluble protein, SS: soluble sugars, ST: starch

Changes in concentrations of soluble sugars

The ranges of soluble sugar concentrations in Luotian and Lanceolata were 117.51-325.38 mg•g⁻¹ and 83.90-481.76 mg•g⁻¹, respectively (Table 3, Figure S11). The soluble sugar concentrations distributed in the first crown layer in Luotian were lower than those in Lanceolata, especially at the base of the branch ($p < 0.05$, Figure S11). And the highest soluble sugar concentration occurred at the upside of middle part of the first crown layer both in Luotian and Lanceolata. The soluble sugar concentrations varied greatly at the upside of the base part and the downside of the tip part among three crown layers in Luotian. Whereas in Lanceolata, except the upside of the middle, the downside of the tip showed no significant difference in soluble sugar concentrations ($p > 0.05$, Table 3), and soluble sugar concentrations in the all-other positions had significant difference at all crown layers ($p < 0.05$, Figure S12).

Changes in starch concentrations

The starch concentrations in Luotian sample trees were lower than those in Lanceolata sample trees, except those at the upside of the tip at the first crown layer, the base and the downside of the middle part at the third crown layer (Figure S13). All of the differences among all positions were not obvious between Luotian and Lanceolata ($p > 0.05$). There was no significant difference at the different positions at the same crown layer in Luotian, which as the same as in Lanceolata (Table 3).

Among three crown layers, higher starch concentrations occurred in the first crown layer in Luotian, while in Lanceolata, the starch concentrations in the first and second crown layers were presented higher than those in the third crown layer. However, there were no significant differences at the same positions among crown layers in starch concentrations in Lanceolata. Compared to Lanceolata, the starch concentrations varied significantly at the downside of the tip in Luotian sample trees ($p < 0.05$, Figure S14).

Discussion

Crown structure and tree morphology have powerful effects on the productivity of plantations. Research on plant architecture helps manipulate branch numbers, branch orientations, and tree sizes. Plant architecture influences the growth, carbon sequestration, shading, filtering of fine air particulates, and risk of windbreak for other trees (Pretzsch *et al.*, 2015). An ideal plant architecture could minimize the need for pruning and maximize light use efficiency (Li *et al.*, 2020). In this study, the crown architecture of Luotian is narrow, pointed, and tower-like, and the older drooping branches die naturally at the age of 6-7 years old, thus the forest species is an ideal one for plantations with high densities and farmland protection. Based on our previous study, the branching angles of Luotian were remarkably larger than those of Lanceolata, 71.8% of the branching angles in Luotian were more than 90°, resulting the branch drooping of Luotian (Xu *et al.*, 2020). To reveal the underlying physiological mechanisms involved in drooping and natural death of the branches of Luotian, analyses in phytohormones and nutritional ingredients were performed in Luotian and Lanceolata trees. The results indicated that the drooping branches were influenced by the phytohormone levels and nutritional ingredient distribution.

Phytohormones play critical roles in plant morphogenesis and organ longevity. ABA, as a plant growth inhibitor, has regulatory roles during plant growth and development (Leng *et al.*, 2014). In this study, ABA concentration in Luotian was higher than that in Lanceolata, particularly at the tip part in the second and third crown layers (Figure S1), which was in accordance with the research in weeping Mei (*Prunus mume*) (Zhang, 2016). Previous studies have suggested that ABA may be an inhibitor of bud development (Chatfield, 2000), suggesting that ABA may inhibit the development of the axillary buds of Luotian branches, whose branches grow faster than those of Lanceolata, thus leading to the formation of the crown structure of Luotian and naturally drooping branches. These results also explained the hypothesis that the thicker crowns of Luotian is related to the strong sprouting ability of the axillary buds of the main trunk in Luotian or to the simultaneous inhibition of the apical buds on the lateral branches (Xiong, 1984; Xu *et al.*, 2020). However, the direct relationship between ABA and early branch death of Luotian remains to be further verified by subsequent experiments.

Gibberellic acid (GA3) is also significantly associated with plant architecture. In this study, there were unremarkably difference in GA3 content among different positions both in Luotian and Lanceolata (Figure 2), which was in a line with the results of the studies on Mei (Wang, 2014). However, GA3 concentration in Luotian was higher than those in Lanceolata among all positions (Figure S4). In the third crown layer, the upsides of all parts had a higher GA3 concentration in Luotian than those at the downsides, in accordance with the reports in Japanese cherry (Nakamura *et al.*, 1994; Kei-ichi *et al.*, 1995, Yoshida *et al.*, 1999). It is speculated that higher concentration of GA3 in one side of a branch cause faster growth than the other side, resulting in the bending of branches and the formation of pendulous and straight branches.

Indole-3-acetic acid (IAA), one of the best-known phytohormones, has an extremely wide spectrum of activity, and is particularly important in plant growth and developmental processes. ZR, a plant cytokinin, can promote plant cell division, prevent chlorophyll and protein degradation, slow down respiration, maintain cell vitality, and delay plant senescence. Both in Luotian and Lanceolata, the concentrations of IAA and ZR had a similar tendency, which in Luotian was lower than those in Lanceolata, in particular at the tip of the branches (Figures S3 and S4). IAA and ZR promote branch growth in Luotian and Lanceolata. The IAA concentrations

at the upsides were lower than those at the downsides in Lanceolata, especially in the third crown layer (Figure S6). The branches may curve upward because the downside grows faster than the upper side. Nakamura (Nakamura *et al.*, 1994) applied exogenous IAA on weeping peach (*Prunus persica*) and weeping cherry (*Prunus spachiana*). Their results showed that neither of IAA and ZR changed the shape of the vertical branches of cherry and peach, and it was believed that IAA and ZR might synergistically regulate the formation of xylem during the bending of the vertical branches to gravity with GA3. Furthermore, higher concentrations of IAA and ZR caused the greater base diameters and lengths of branches in Lanceolata, which was explained in the investigation carried out by Xu (2020).

Based on the overall analysis of four hormone concentrations in different crown layers both in Luotian and lanceolata, it was found that the deeper the depth into the crown base became, the less the concentrations of the four hormones were almost, these concentrations appearing a tendency of decline (Figures S2, S4, S6, S8). However, ABA concentration increased in the tip of branches both in Luotian and Lanceolata from the first to the third crown layer (Figure S2), while GA3 concentrations had differed at the downside of the second crown layer between Luotian and Lanceolata (Figure S4). The differences in IAA concentrations appeared at the base and the downside (Figure S6), as well as the ZR concentrations between Luotian and Lanceolata (Figure S8). The results indicated that IAA and ZR played a critical function on the initial of the branch growth and GA3 related to the bending of branch. Meanwhile, ABA may improve the drooping of the branches in Luotian.

Plant growth is not only affected by the respective concentrations of endogenous hormones, but also related to the balance between various hormones, especially the ratios and balance between promoting growth hormones and inhibiting growth hormones. In the present study, both in Luotian and Lanceolata, the ABA/GA3 ratios were correlated with the depth into crown base, with the similar variations in trend (Figure 1). However, ABA/GA3 of Luotian showed more rapid changes than those of Lanceolata (Figure 1). In particular, the ratios of ABA/GA3 in the tips of branches increased with the increased depth of the crown layers (Figure 1). The ratios of ABA/IAA and ABA/ZR had the same trend with the ratios of ABA/GA3 both in Luotian and Lanceolata (Figures 2 and 6). However, ABA/IAA and ABA/ZR in Luotian were higher than those in Lanceolata, which was almost opposite with ABA/GA3 ratios (Figures 1, 2 and 6). Moreover, the ratios of ABA/GA3, ABA/IAA, and ABA/ZR in the tips of branches in the second and third crown layer were higher than those in the base and middle parts of branches localized in the same crown layer both in Luotian and Lanceolata, especially in the third crown layer of Luotian, suggesting that the ratios of ABA/GA3, ABA/IAA, and ABA/ZR in the abaxial parts were greater than those in the adaxial parts of branches. Based on these results, ABA could play an important role on the branch death in Luotian. With regard of IAA/GA3 and ZR/GA3, Lanceolata had both greater ratios in all positions than those of Luotian (Figures 3 and 5). The ratios of IAA/ZR in the base and middle parts of branches in the first crown layer, the downsides of the base and middle parts of branches in the second crown layer, and the downsides of the middle parts of branches in the third crown layer in Lanceolata were greater than those in Luotian (Figure 4). Therefore, a higher ratio of IAA/ZR could induce faster branch growth, resulting in upward branches. As a result, the distribution and proportion of phytohormones in Luotian and Lanceolata has influenced the branch morphogenesis. The interdependence and influence of these phytohormones on pendulous branch growth need to be further studied. Their biosynthetic pathways, signal transduction pathways, and genetic control mechanisms of these hormones in Luotian have not yet to be studied.

The differences between Luotian and Lanceolata on the physiological processes were also reflected in concentrations of carbohydrates, proteins and starch (Figures S9-S14). The accumulation of nutrition components in branches of Luotian and Lanceolata were consistent with the morphology of these branches. Plants produce proteins in response to biotic and abiotic stresses, and some of these proteins are induced by phytohormones (Hussein *et al.*, 2007). In this study, soluble protein concentrations in Luotian were significantly greater than those in Lanceolata in the upsides of the base parts of branches in the third crown layer (Figure S9), suggesting more reactive physiological processes in the upsides of the base parts of the Luotian

branches, i.e., more rapid growth of the upsides of the base parts, and finally resulting in drooping of these branches. It is possible that the base parts of Luotian branches in the third crown layer were pulled down by the branch weight. Moreover, Xu (2020) reported that the leaf biomass in each layer of the Luotian was higher than that of Lanceolata, and leaf biomass increased with crown thickness. Similarly, the branch length in Luotian increased with increase in the crown layers. Both affected by higher leaf biomass and longer branch, the tension on the base of Luotian branches increased with the increase in crown layers. Thus, the branches in the third crown layer in Luotian are easier to drooping and falling. Meanwhile, in order to response to the tension, Luotian trees produced more soluble proteins at the base of the branch.

Sugars act as energy sources and structural materials in plant growth. The total soluble sugar concentrations in Lanceolata branches were higher than those in Luotian branches in the first crown layer (Figure S11), indicating that Lanceolata branches grew faster than those of Luotian branches in the first crown layer. Recent studies found that sugars, especially sucrose, can act as a molecular signal in a manner similar to phytohormones and play a regulatory role in many processes, such as plant growth, development, maturation and senescence (Graham *et al.*, 1994; Jang and Sheenh, 1997; Morcuende *et al.*, 1998; Koch *et al.*, 2000; Loreti *et al.*, 2001). But it is still unclear about crosstalk between sugar and phytohormones in regulating plant growth and development.

Starch is the accumulation of organic matters in plants. For Lanceolata, starch concentrations in branches in the first and second crown layer were higher than those in the third crown layer, while there were no significant differences among the three crown layers in Luotian (Table 3). Moreover, starch concentrations in Lanceolata were greater in all same positions than those in Luotian (Figure S14). It was similar with the study of Xu (Xu *et al.*, 2020), indicating that branch growth differed significantly between Luotian and Lanceolata, but the lateral branches grew at a similar rate among Luotian trees of different ages.

From the point of overall nutrition distribution, the starch accumulation is directly related to the diameter and length of branches. In this study, the starch concentrations in branches in the first and second crown layer in Luotian were higher than those in Lanceolata (Figure S14), therefore, the diameters and length of the branches in the first and second crown layer were higher than those of Lanceolata, which was consistent with the previous results of tree crown analysis of Luotian and Lanceolata (Xu *et al.*, 2020). Compared with the branches of Lanceolata in the same crown layer, it was found that the differences in nutrient accumulation was smaller and smaller from the first to the third crown layer, and the concentrations of soluble sugars and proteins in the branches in the third crown layer of Luotian were even higher than those of Lanceolata (Figures S12 and S14), which could not be separated from its influence on tree crown structure. As the branch angles of Luotian branches are bigger and bigger, the material exchange between the base of branches and the main axis of tree is less and less. Therefore, the branches in the third crown layer appear physiological stress response, and the concentrations of soluble sugars and soluble proteins are increased.

Conclusions

The experimental results of this study suggested that IAA and ZR played a critical function on the initial of the branch growth of Luotian and Lanceolata and GA3 related to the bending of branches in Luotian. To further assess the ratios of hormones in Luotian and Lanceolata, the distribution and proportion of phytohormones has influenced the branch morphogenesis and ABA is pivotal in the branch death in Luotian. Meanwhile, nutritional ingredients analysis indicated that the accumulation of nutritive components in Luotian and Lanceolata were consistent with the morphology of their branches. An integrated analysis implied that phytohormones had a strong influence on pendulous characteristics by interacting with other factors. Our results provide an important foundation for improving branch orientations and branch death in *Cunninghamia lanceolata*. In addition, elucidating the mechanism governing the pendulous trait may

contribute to provide theoretical basis and technical support for its cultivation, introduction, tending management and genetic improvement.

Authors' Contributions

All authors directly participated in planning this paper. Yujie YANG and Chu WU conceived and drafted the manuscript. Yujie YANG, Chu WU and Yezhou XU conceived and designed the experiments; Chaoqun DU and Hui YUAN performed the experiments. Yujie YANG and Chaoqun DU analyzed the data; Shaoliang YAN and Hui YUAN took part in field investigation. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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