



Research article

Water, starch, and nuclear behavior in ray parenchyma during heartwood formation of *Catalpa bungei* ‘Jinsi’

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ABSTRACT

Catalpa bungei ‘Jinsi’, a cultivar of *C. bungei* C. A. Mey., is valued for its heartwood with good overall mechanical properties, naturally durable and golden-yellow color. Little is known about heartwood formation in *C. bungei* ‘Jinsi’ trees. The behavior of starch, water, and nuclei was studied in the xylem tissue of *C. bungei* ‘Jinsi’ concerning aging in ray parenchyma cells. Blocks containing heartwood, golden zone, transition zone, and sapwood were collected from the stems of six *C. bungei* ‘Jinsi’ trees. The moisture content of the blocks was measured by oven drying. Changes in starch and nuclei in ray parenchyma were investigated in radial profiles from sapwood to heartwood blocks using microscopy and various staining techniques. The nuclear size and starch content gradually decreased to heartwood. While the horizontal distribution of moisture content of *C. bungei* ‘Jinsi’ was very varied, with the heartwood and golden zone being lower than sapwood but slightly higher than the transition zone. Starch grains were rare, but nuclei were still present in some ray parenchyma cells in the heartwood and golden zone. The nuclei showed irregular shape and elongation before disintegration. These results suggest that the most apparent change occurs in the transition zone, the critical location involved in forming *C. bungei* ‘Jinsi’ heartwood. Water and starch appear to be actively engaged in heartwood formation. The loss of function of ray parenchyma cells results from heartwood formation.

1. Introduction

Catalpa bungei C. A. Mey is one of China’s most valuable timber trees, with high natural durability and good overall mechanical properties [1,2]. In particular, *C. bungei* ‘Jinsi’, a cultivar of *C. bungei* C. A. Mey., forms a typical heartwood (HW) that is colored golden-yellow [3]. The color is pleasing and meets the needs of advanced high-grade furniture and interior decoration. This timber resource is scarce due to overcutting or illegal logging. *C. bungei* trees are self-sterile and have been cultivated mainly through asexual propagation [3]. In China, there has been an effort to cultivate fast-growing *C. bungei* trees to harvest many high-quality HW [4]. Despite HW importance to economics, HW formation in *C. bungei* ‘Jinsi’ remains poorly understood [5].

Much research has been devoted to HW formation since its inception. HW formation process was once thought to be a loss of physiological functions of parenchyma cells [6,7], whereas change in ray parenchyma cells is an important indicator of HW formation. Ray parenchyma is the horizontally growing tissue in trees, and plays various roles in material transport, nutrient absorption, support, and protection during tree growth and development (Morris, 2016). Some studies have reported that ray parenchyma cells can remain longevity for many years (Spicer and Holbrook, 2007[8,9]). In ray parenchyma cells, the behavior of water, starch, and nucleus serves

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as an important indicator of cellular metabolism and growth, which holds immense significance in elucidating the physiological state and biological functions of these cells. Firstly, water acts as a medium for all chemical reactions occurring within cells, thereby playing a pivotal role in various vital functions, including material transportation, ionic homeostasis, and signal transduction (Brown, 1978). Within ray parenchyma cells, the status and distribution of water provide valuable insights into the physiological state and functional activity of these cells [10]. The maximum flow velocity in the sapwood (SW) position varies among species, but a decrease in HW direction is similar [11,12]. Beauchamp et al. [12] suggest that sap flux reduction could trigger HW formation as a temporal signal. Secondly, starch is the main energy storage substance in ray parenchyma cells, which can be decomposed into glucose to provide energy for cells [13]. In ray parenchyma cells, the synthesis and decomposition of starch can help us understand the energy metabolism status of cells and their response to environmental conditions (Von Arx, 2017). As the xylem depth increases, the starch content also decreases; a small amount of starch is present at the SW/HW boundary, and no starch is present in HW [13,14]. The disappearance of starch is accompanied by the accumulation of phenolic compounds, which is related to HW color [15–18]. Finally, the nucleus is the storage location of genetic information within cells, controlling their genetic activities [19]. In ray parenchyma cells, morphological changes in the nucleus include nuclear pyknosis, nuclear fragmentation, and nuclear lysis (Nairand Chavan, 1983). These changes are part of the process of cell necrosis, leading to the loss of normal morphology of the nucleus [20]. In the xylem, due to the gradual depletion of oxygen, the living cells in the sapwood are suffocated by normal respiration, causing changes in the size and shape of the nuclei in the direction of the cambium towards the heartwood, and ultimately the disappearance of the nuclei in the ray parenchyma cells (Nakaba, 2013, Nakada, 2018). The above studies indicate no sudden transition from SW to HW. Therefore, scientists present a theme focusing on the transition zone (TZ) between HW and SW in trees [21–23].

As early as 1950, Chattaway [6] found that some trees have a band of intermediate wood, which is sapwood, but approaches heartwood in some features. Since transformation processes occur in the intermediate wood band, it was referred to as TZ by Frey-Wyssling and Bosshard [7]. TZ is different from HW and SW in color, usually pale white. Thus, TZ has also been called the white zone by some scientists (Shigo 1973 [24–26]). Although a TZ cannot be recognized by the naked eye, it can be identified by the difference to SW or HW in properties such as moisture content (MC) [27]. The MC of green wood in the TZ is lower than that of SW (often HW), so the TZ is sometimes called dry TZ (Shain, 1973). However, some believe that TZ and intermediate wood cannot be equated. For example, Nobuchi et al. [63] considered the pale-colored narrow zone to be a transitional zone between intermediate wood and heartwood. The International Association of Wood Anatomists [28] defined intermediate wood as inner layers of sapwood that are transitional between sapwood and heartwood in color and general character. TZ is believed by many scientists to be part of SW, not only because it is on the outer edge of sapwood, but also because it usually has some living cells. Some ray parenchyma cells in the TZ still have nuclei and “survive” [29]. Still active parenchyma cells are responsible for converting primary metabolites (e.g., starch) into secondary metabolites, which are later accumulated in HW [30–32]. Given the many similarities between the two, TZ is used as a synonym for intermediate wood in some studies [26,27]. There is also TZ that is different from HW and SW colors in *C. bungei* ‘Jinsi’ trees. However, there are few reports on wood properties of TZ in *C. bungei* ‘Jinsi’ trees, with the exception of Deng et al. [5] briefly mentioning TZ’s width. Intriguingly, the outer layer of HW in *C. bungei* ‘Jinsi’ displays a golden-colored concentric zone, which is the trees’ name derived from and can be distinguished from other cultivars of *C. Bungei* [1,3]. We would temporarily call it “Golden Zone” (GZ). However, the GZ does not appear to be called “intermediate wood” as defined by International Association of Wood Anatomists [28], although it is located between SW and HW like the TZ. Unfortunately, none of the studies developed for GZ have been found.

We aim to study the TZ and GZ of *C. bungei* ‘Jinsi’, regarding their significance in HW formation. We monitored starch grains and nuclei in the ray parenchyma to identify a relationship between starch loss and nuclei autolysis. In addition, we monitored radial MC changes to assess water functions during the loss of physiological functions of ray parenchyma cells.

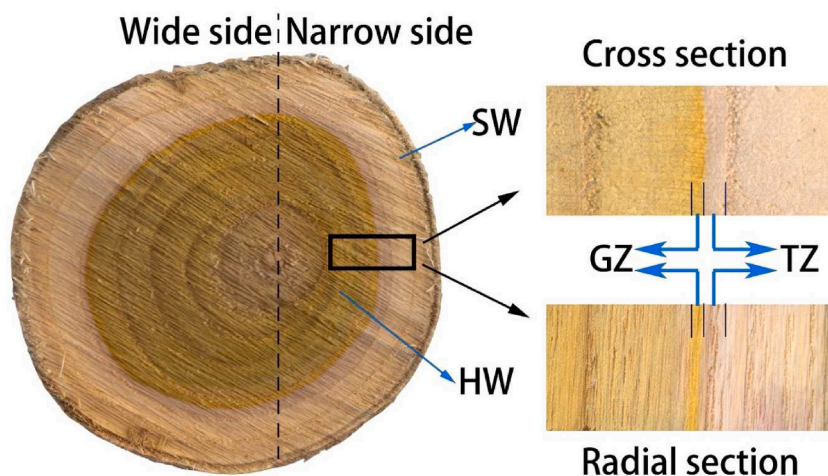


Fig. 1. Schematic diagram for wood disc and section from *Catalpa bungei* ‘Jinsi’ trees. SW, sapwood; TZ, transition zone; GZ, golden zone; HW, heartwood. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Material and methods

Six 8-year-old *C. bungei* ‘Jinsi’ trees with straight stems were randomly selected from an even-aged pure plantation in Yanshi District (36°41’N, 140°41’W, elevation 60 m), Luoyang City, Henan Province, China. The regional climate is continental, with an average annual temperature of 13.7 °C and an average total annual precipitation of 550 mm. Sample trees were harvested early in the morning on April 25, 2022, to minimize sap flow in the xylem. Logs of 2 m in length were cut from each tree at the height of 2 m. The log ends were covered with polythene to reduce dehydration. These logs were immediately transported to the laboratory (45 km from the site).

From each log, cross-sections (two discs) at breast height (1.3 m) were cut with 20 mm thickness. One disc was used for MC measurement, and the other for wood anatomy. With the naked eye, it was easy to distinguish the boundaries of the four zones (HW, TZ, GZ, and SW, see Fig. 1). Although the stems of the sample trees were straight, they were more or less eccentric. HW radius, TZ width, GZ width, and SW width were measured from wide and narrow sides, respectively. Samples for analysis were taken from the narrow side of each disc to avoid the possible influence of stress wood and to ensure similar growth conditions for the studied wood. A 20 mm wide, pith-centered strip was cut along a narrow radius from each disc. Four thick chips were cut from the four zones along the strip, which was used for MC measurement. The radial dimension of the chips varied from 2 to 4 mm due to variations in TZ and GZ width. After cutting, the chips were weighed immediately and then oven-dried at 103 ± 2 °C to reach 0% moisture content. After cooling in desiccators, the oven-dry weight of the chips was measured. The MC of the chips was then calculated from the dry and fresh weights by equation (1)

$$MC = [(G1 - G2) / G2] \times 100\% \quad (1)$$

where G1 and G2 are the chip’s fresh and oven-dry weights, respectively.

A small block with radial dimensions of about 30 mm was cut from the strip and used for sectioning (Fig. 2). The block covered TZ, GZ, and adjacent portions HW and SW. All the blocks were then immediately fixed in an equal-part solution of formaldehyde, glacial acetic acid and 70% ethanol and kept for a few days at room temperature [9]. Before slicing, the blocks were rinsed twice in 70% ethanol and embedded in paraffin. Radial and transverse sections of 15 µm were made from each block using a Leica slicing machine. The transverse sections were stained with 1% safranin in water to determine the cell wall. The transverse section is mainly used to help identify the position of the four wood zones on the radial section. The radial section was stained with iodine solution in potassium iodide to identify starch grains and 0.5% Evans blue in water to identify nuclei [33]. The sections’ two-dimensional photographs (resolution of 300dpi, 84.667 µm/pixel) were obtained using a Mshot-MD50 (Micro-shot Technology, Guangzhou, China). The photographs were taken at × 200 magnification, with a high light intensity background illumination to maximize the contrast between the stained starch grains or nuclei and the surrounding tissue.

Nuclei morphology and disappearance were observed. Rate of the number of nucleated ray parenchyma cells to total ray parenchyma cells (RSN) were calculated for each wood zone by equation (2)

$$RSN = (N1 / N0) \times 100\% \quad (2)$$

where N0 is the total number of ray parenchyma cells in the measured area, and N1 is the number of ray parenchyma cells containing nuclei. Each rate was determined by the percentage of nucleated ray parenchyma cells among at least 100 ray parenchyma cells located

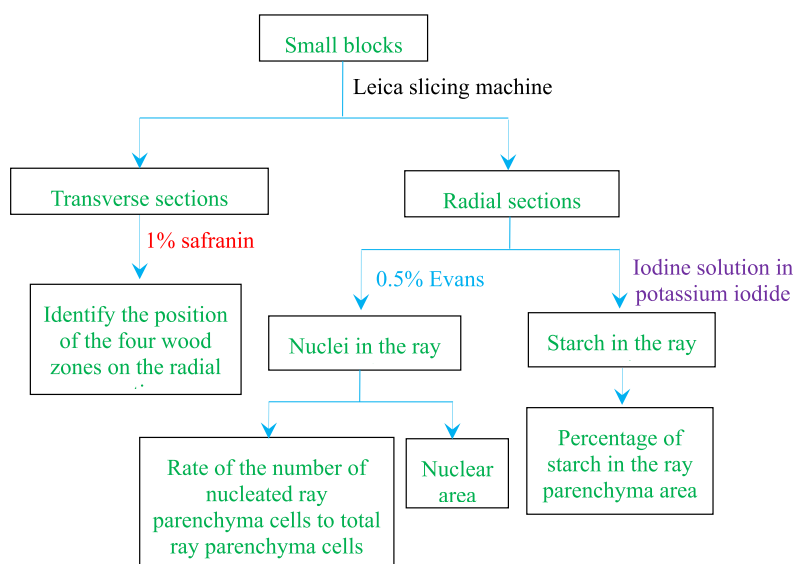


Fig. 2. Flow chart of wood anatomy for nuclei and starch in ray parenchyma from *Catalpa bungei* ‘Jinsi’ trees.

in the zone. The nuclear area (NA) was determined using Adobe Photoshop software (Version 21.0, Adobe Systems Software Ireland Ltd, 4–6 Riverwalk, City West Business Campus, Saggart D24, Dublin, Ireland). At least 60 nuclei were measured per wood zone.

The number of pixels at 1 mm² square was quantified throughout the photographs [34]. Each nuclear area (NA) was outlined manually to determine its number of pixels. Number of pixels converted to NA. Using the same method, we quantified the area percentage of starch coverage in the outlined ray parenchyma area. The percentage of starch in the ray parenchyma area (PS) was calculated for each wood zone by equation (3)

$$PS = (A1 / A0) \times 100\%$$

(3)

where A0 and A1 are the pixel area of starch coverage and the total ray parenchyma area, respectively.

Results were statistically processed with IBM SPSS Statistics software (Version 24.0, International Business Machines Corporation, Armonk, New York, United States). Before starting the statistical analysis, the raw data were tested for normality using Shapiro–Wilk and Levene tests. Variance analysis (ANOVA) was used to assess differences in traits in the sample zone, with an alpha value < 0.05. The relationship between starch, water distribution, and availability of nuclei was characterized using the Pearson correlation and determination coefficient.

3. Results

In this paper, recognition of SW, TZ, GZ and HW was based on visual color observed (Fig. 1). The colors on the rough surface obtained by chain saw cutting differed between the four zones. The boundaries between the four zones were easy to distinguish and separate. HW was yellowish-brown. SW was yellowish-white and much paler than HW. TZ and GZ were always present between HW and SW in all discs investigated. TZ was grey-white, located in the latewood part, and continued tangentially generally along the growth ring boundary. The GZ was golden-yellow and continued tangentially along the TZ. GZ width was less than TZ (Table 1). In addition, the widths of TZ and GZ are not uniform in the tangential direction. The mean width of TZ and GZ on the wide side of the tree discs was significantly lower than on the narrow side. The SW width difference between the narrow and wide sides was not significant.

A large number of purple and round starch grains were observed in SW ray parenchyma cells stained with KI + I₂ (Fig. 3A). In TZ, the shape of starch grains was similar to SW (Fig. 3B), but the number was smaller. The number of starch grains became much smaller, and the shape of existing starch grains became irregular or fragmented within a ray parenchyma cell from GZ and HW (Fig. 3C and D). Nuclei were present in all samples taken from *C. bungei* ‘Jinsi’ trees. The radial pattern of morphological variation in starch grains also occurs in nuclei in ray parenchyma cells. The nuclei were blackish-green, round or oval-shaped granules in specimens from SW stained with Evans blue (Fig. 3E and 4A). In TZ (Fig. 3F and 4B), several nuclei were observed similar to SW. Changes in nuclei morphology in ray parenchyma cells, relative to SW and TZ, GZ (Fig. 3G and 4C) and HW (Fig. 3H and 4D) showed a small, primarily triangular shape with tapering ends.

Fig. 5 shows distribution of MC, PS, RSN, and NA in SW, HW, TZ, and GZ. First, the mean MC of HW was 80.2 ± 2.4% and was not significantly different from TZ (75.1 ± 2.5%) and GZ (80.0 ± 2.0%). However, the mean MC of SW was 103.6 ± 2.9%, which was more than 20% higher than the MC of HW and GZ (Fig. 5A). Second, the mean PS values was highest in SW (93.0 ± 13.0%), followed by TZ (75.1 ± 6.4 %). In comparison, HW and GZ showed similar but lower PS, significantly different from the other zones (Fig. 5B). Finally, examining all SW, GZ, and HW in radial sections showed that the nuclei of ray parenchyma cells varied in shape and size (Fig. 5C and D). From SW to HW, RSN and NA decreased gradually. There is a significant difference in RSN among the four wood zones. The maximum RSN was 88.2% in SW, and the minimum was 32.0 % in HW. The most significant difference in NA was found between SW and HW. NA varied from 60.6% to 34.3% in specimens.

Pearson correlation analysis showed there was an extremely significant positive correlation between PS, RSN and NA based on data for secondary xylem (Table 2). The highest correlation coefficient was observed between PS and RSN (r = 0.83), followed by NA and RSN (r = 0.68), and NA and PS (r = 0.68). A moderate correlation was found between MC and other parameters (r = 0.42~0.50).

4. Discussion

The 8-year-old *C. bungei* ‘Jinsi’ trees we collected were juvenile [1] but produced HW with five rings (see Fig. 1). The initiation of HW formation appears to agree with earlier research findings that *C. bungei* ‘Jinsi’ is a tree species with early HW formation [5]. Early HW production can reduce operating costs. The HW distribution in the cross section is not centrosymmetric, which appears to be related to stem eccentricity. Stem eccentricity is most often the case in practical forestry and is a reaction to the influence of natural factors [35]. The GZ around HW is very narrow and may play a vital role in HW formation. However, as mentioned in the introduction,

Table 1
Difference in HW radius, SW width, TZ width and GZ width between the wide and narrow sides of *Catalpa bungei* ‘Jinsi’ trees.

	SW (mm)	TZ (mm)	GZ (mm)	HW (mm)
Wide side	17.61 ± 5.63	2.13 ± 0.56	1.32 ± 0.26	52.42 ± 8.46
Narrow side	16.35 ± 5.82	4.5 ± 1.33	2.1 ± 0.13	20.22 ± 5.34
Significance	n.s.	*	*	*

The values are mean ± standard deviation.
*Difference is significant at the p < 0.01 level. n.s. Difference is not significant. SW, sapwood; TZ, transition zone; GZ, golden zone; HW, heartwood.

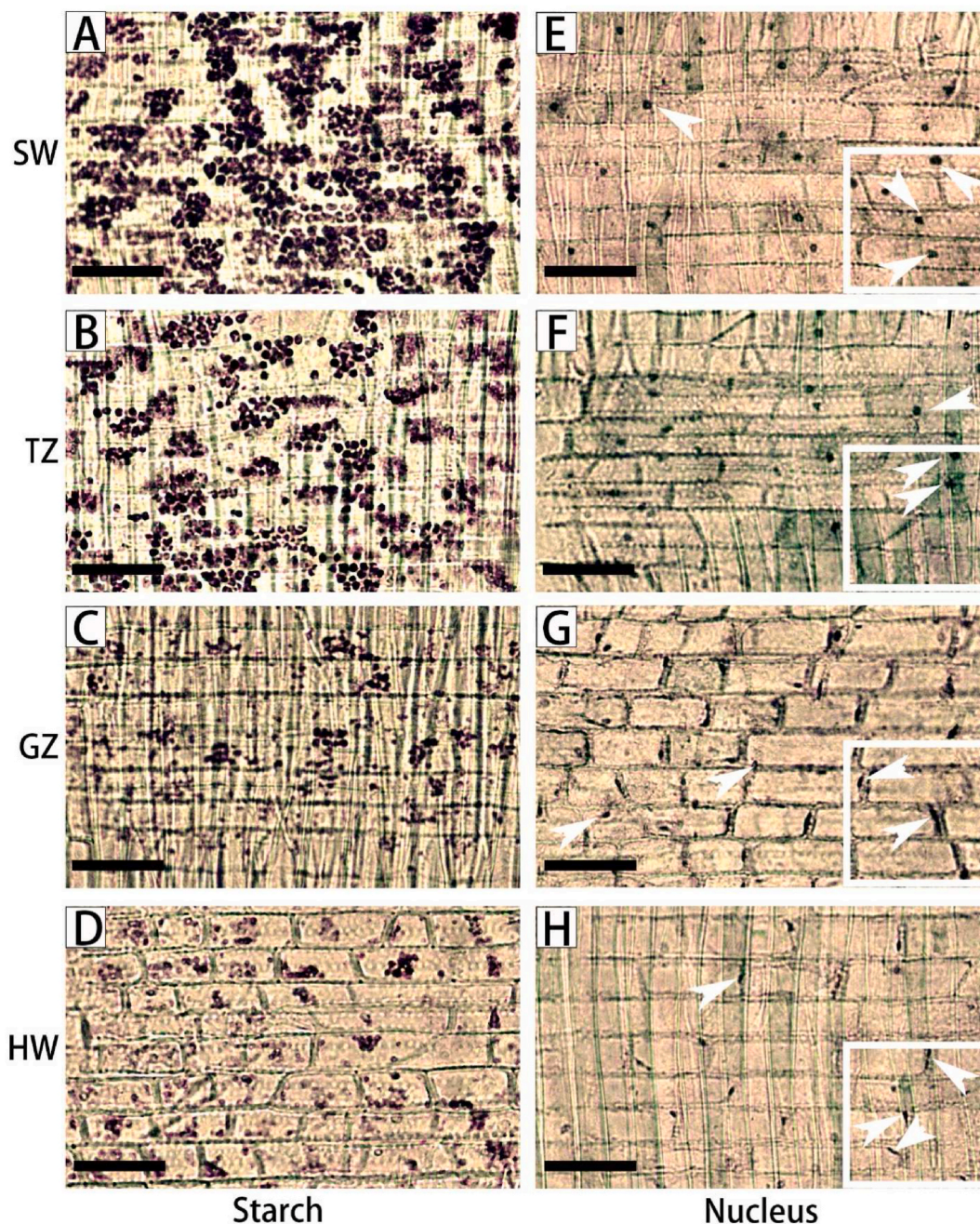


Fig. 3. Micrographs of radial sections, stained with Evans blue and KI + I₂, showing starch grains (A–D) and nuclei (E–H) in ray parenchyma cells sampled at stem height of 1.3 m from *Catalpa bungei* ‘Jinsi’ trees. The white arrow refers to the nucleus. The area enclosed by a white rectangle is magnified in Fig. 4. SW, sapwood; TZ, transition zone; GZ, golden zone; HW, heartwood. Scale bars = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

there is no research in this aspect. *C. bungei* ‘Jinsi’ has a discernible TZ. The TZ is wider than the GZ. The width is not uniform in the tangential direction and is associated with stem eccentricity. The pith is further away from the side containing narrow TZ, which means that the variability of the TZ in the cross-section of the xylem should also have obvious directionality. The directional variation of the TZ was also found in *Robinia Pseudoacacia* [36], *Pinus sylvestris* [32], *Tectona grandis* [37], and *Dalbergia odorifera* [22]. However, these authors have no comment on this topic. Interestingly, our observations on the asymmetric distribution of HW in the cross section of the xylem support the eccentricity assumption suggested above. At the same time, the variation of TZ width in the tangential direction is against the hypothesis. Given the functional significance of the TZ, there may be other still unknown mechanisms that lead to its width

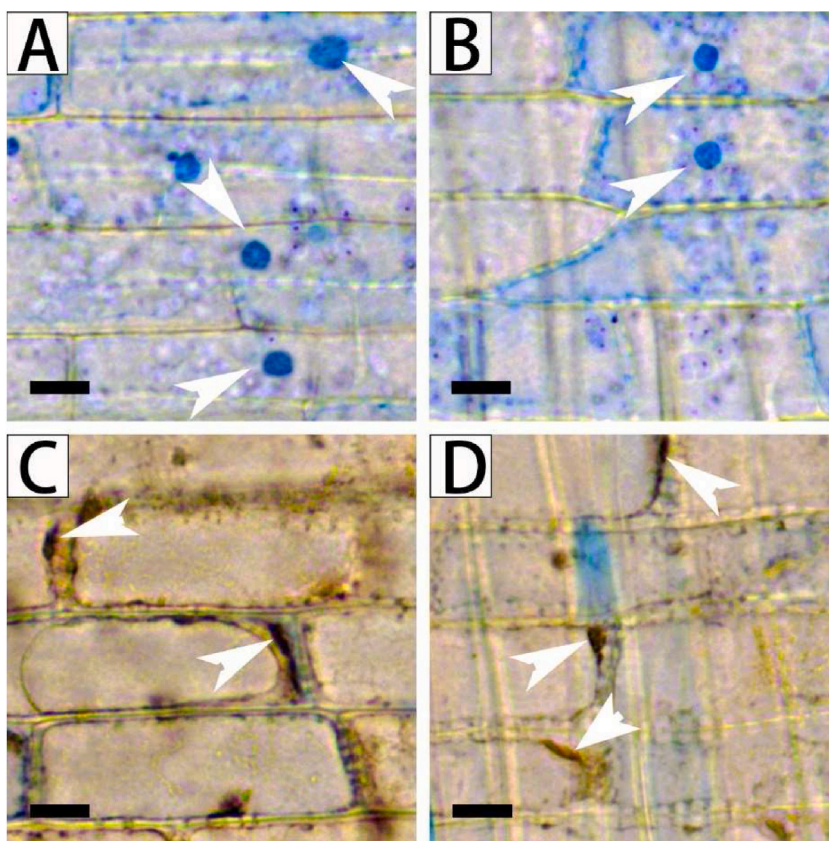


Fig. 4. Magnified micrographs of radial sections, stained with Evans blue, showing nuclei (A–D) in ray parenchyma cells sampled at sapwood (A), transition zone (B), golden zone (C), and heartwood (D). The white arrow refers to the nucleus. Scale bars = 10 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

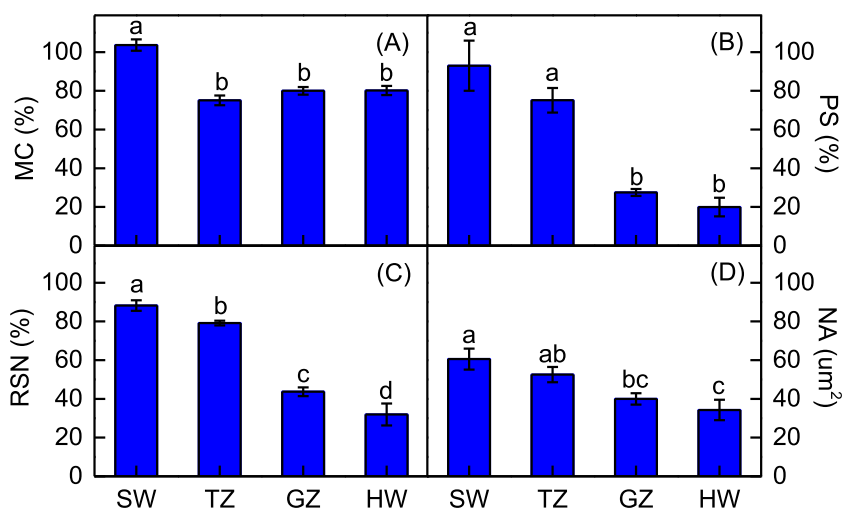


Fig. 5. Distribution of MC (A), PS (B), RSN (C), and NA (D) in different types of wood tissues. The mean and standard deviation are based on six independent measurements. MC, moisture content; PS, Percentage of starch in ray parenchyma; RSN, Rate of the number of nucleated ray parenchyma cells to total ray parenchyma; NA, Nuclear area; SW, sapwood; TZ, transition zone; GZ, golden zone; HW, heartwood. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2Pearson correlation coefficient between MC, NP, NA, and SP in wood tissues of *Catalpa bungei* 'Jinsi' trees.

	SP	RSN	NA
MC	0.423*	0.504*	0.462*
RSN		0.825**	0.679**
NA			0.681**

** Correlation is highly significant at $p < 0.01$. * Correlation is significant at $p = 0.05$. MC, moisture content; PS, Percentage of starch in ray parenchyma area; RSN, Rate of the number of nucleated ray parenchyma cells to total ray parenchyma; NA, Nuclear area.

variation. The SW of the sample tree is narrow with only two rings, which could partly be related to the little crown of *C. Bungei* 'Jinsi' [38]. The pipe model theory generally accepted today shows that the relationship between the crown's foliage biomass and the stem's SW area is linear without an intercept [39,40]. We did not measure the SW amount of *C. bungei* 'Jinsi' in different growth stages, but Deng et al. [5] revealed that the SW amount changes little with the growth of trees.

SW is located at the xylem periphery, has energetic parenchyma cells, and actively participates in water transport. SW sampled here near the SW/HW boundary, with a mean MC of $103.6 \pm 2.9\%$. The MC may be higher in the outer SW near the cambium [41]. GZ's MC was close to HW's MC. Strikingly, a 25% reduction in TZ's MC relative to SW was observed. TZ' MC was even lower than HW. Several studies have proposed that the low MC of this zone was due to the withdrawal of water by the energetic parenchyma cells for the hydrolysis of starch in them, which initiated HW formation [42]. However, in this study, the MC of TZ was high, and whether the water deficiency is sufficient to support the transformation process of SW/HW remains to be further studied.

Our study found that the PS of ray parenchyma in *C. bungei* 'Jinsi' SW was up to $93.0 \pm 12.0\%$ (Fig. 5B). Much starch is regular in the SW of ray parenchyma because starch is the carbohydrate reserves to be mobilized in SW and then resorbed when needed to supply energy for tree growth [43]. Starch in trees varies seasonally [44,45]. Guan et al. [46] showed that starch in SW is more present in late growing season and winter than in spring (when sample trees were collected in this study). PS of ray parenchyma dropped significantly from SW to HW. However, the reasons for starch reduction in HW are different from SW. Under normal environmental conditions, starch stored in HW is not used to support tree growth and metabolic demand as in the SW of some temperate trees [45], but is usually converted into extractives, especially phenolics, for example, in the HW of *Juglans* sp [30]. There are studies that wood color is mainly due to the presence of extractives (Shigo1973 [22,42]). We did not study the transformation of starch into extractives in *C. bungei* 'Jinsi' HW. However, the most abrupt change in PS of ray parenchyma occurred between the TZ and the GZ, the starting point for wood color change, which seemed to support the idea that starch transformation is the cause of HW formation [13,47].

Interestingly, some starch grains of ray cells in the GZ are broken to pieces, and some have been degraded, although it is adjacent to the TZ and in the same growth ring. A small amount of starch grains was found in the HW. Speculating that the GZ is part of HW is tempting. During HW formation, starch storage material such as starch gradually disappears and newly formed phenolics accumulate at the boundary between sapwood and heartwood. The reason for the presence of starch in HW is unknown, but may be due to the rapid growth and HW formation in the sample trees in this study. Because of the need for tree growth, the change of reserved starch in the xylem parenchyma has a strong seasonality (As already stated). Meanwhile, trees prioritize storage over growth independent of growth conditions, likely as a mechanism to ensure long-term survival (Von Arx, 2017). The rich starch grains may not yet be fully transformed into various organic substances. This assumption opens an exciting avenue for future investigations into seasonal changes in starch grain presence in ray parenchyma cells.

RSN was significantly different among SW, TZ, GZ and HW of *C. bungei* 'Jinsi' ($p = 0.05$, Fig. 5C). RSN was the highest in SW ray parenchyma ($88.2 \pm 6.1\%$). Ray parenchyma cells in SW occasionally lose their nuclei during section slicing, but such cells are rare. The RSN in TZ ($79.2 \pm 3.0\%$) was significantly lower than that in SW, while the rapid decline in RSN occurred in GZ ($43.7 \pm 3.7\%$). It is suggested that the rapid loss of function of most parenchyma cells occurred at the SW/HW boundary. The physiological function of wood ray parenchyma cells may be maintained for a long time. For example, 16–42 years in *Pinus sylvestris* (Tulik, 2019), 20 years in *Pinus strobus* and 48 years in *Fraxinus americana* (Spicer and Holbrook, 2007). Our studies found that a few ray parenchyma cells still with nuclei in the HW of *C. bungei* 'Jinsi' are in sharp contrast to the many starch grains exhausted in parenchyma cells. This result indicated that the loss of function of ray parenchyma cells may occur after starch loss and HW formation. However, the presence of nuclei in GZ and HW gives no idea of the amount of material being physiologically active, especially in the case of the nuclei degrading (Fig. 4C and D).

Based on microscopic observations of ray parenchyma cells in *C. bungei* 'Jinsi' SW, the nucleus was spherical in shape. Spherical nuclei were also observed in *Bridelia retusa* [48] and *Carya ovata* [49]. It has been reported that the nuclear shape of SW ray cells in *Populus sieboldii* \times *P. grandidentata* trees was fusiform or spindle [50]. These reports indicated that nuclear shapes varied with species. Of course, perhaps it was also because of our choice of sampling locations within SW (near TZ). Regardless of the initial shape change between nuclei, the nuclei became rounded and then irregular from the SW/HW boundary [50,51]. Similar to SW, TZ ray parenchyma cells have circular nuclei, more or less intermediate in the cells (Fig. 4A and B). Our results support the hypothesis that TZ may be part of sapwood. From TZ to GZ, the nuclear shape of the ray parenchyma cells in *C. bungei* 'Jinsi' became irregular, but the change was not as noticeable from GZ to HW, and no typical pattern of nuclear shape change was found. The nuclei in GZ and HW are located at the edge of cells with a disordered shape and are thought to be nuclei remnants, or dispersed chromatin (Fig. 4C and D). This finding suggests that GZ may be part of HW. Combining the fact that the colors of GZ and other parts of HW are different, heartwood formation may still be in progress at GZ (e.g., biosynthesis of heartwood substance).

As Bosshard [52] suggested, the nuclear shape should be considered in conjunction with its size. The nuclear size in SW ray cells was the largest of the four radial positions in the xylem we observed (Fig. 4). The nuclear size in the outer SW may be larger than in the inner SW. These cells have large nuclei, which means they have high functional activity [52]. From SW to HW, a progressively decreasing NA pattern helps study HW formation. HW and GZ still have a few ray parenchyma cells with nuclei (as previously mentioned). However, these nuclei have shown irregular shapes and elongating before disintegration. The results indicate that the functional activity of nuclei-bearing parenchyma cells depends on the age of the cambium. The nuclei are three-dimensional. Using data from 2D images, we cannot accurately estimate their actual size. We should recognize some limitations in the measurement method.

Our study found that PS, WC, RSN, and NA decreased significantly from SW to HW (Fig. 5). We attempt to correlate structural observations on different xylem tissues with starch grains, water distribution, and nuclei availability (Table 2). However, correlation did not explain the causes of HW formation involving many interconnected anatomical, chemical and physiological processes [18,23,53]. Future studies should track extractives across the xylem to better understand where extractives are formed.

5. Conclusion

The radial distribution of MC is very different in *C. bungei* 'Jinsi' wood. HW and GZ had lower MC than SW but slightly higher than TZ. The TZ water shortage is insufficient to support the SW/HW transformation process. PS and RSN declined from SW to HW, the most abrupt change occurred between the TZ and GZ, and it was implied that the TZ is the crucial location involved in HW formation in *C. bungei* 'Jinsi'. In HW and GZ, starch grain deficiency is severe, but some ray parenchyma cells still have irregular nuclei. Our observations support that loss of parenchyma cell function is the result, not the cause of HW formation.

Our present study cannot answer why *C. bungei* 'Jinsi' trees formed the GZ at the outer edge of HW. Further studies on HW formation in *C. bungei* 'Jinsi' should be focused on the extractive, as abrupt extractive increase may cause loss of parenchyma cell function, and may also be the main reason for HW and gold color development.

Declarations

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Additional information

No additional information is available for this paper.

Data availability statement

Data included in article.

CRediT authorship contribution statement

Pingping Guo: Data curation. **Xiping Zhao:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **Zifei Yang:** Project administration, Methodology, Investigation, Funding acquisition. **Yingxin Wang:** Software, Methodology, Investigation. **Hongying Li:** Project administration, Investigation, Funding acquisition. **Lepei Zhang:** Software, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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