Concentration and Distribution of Nuclei and Plastids in Xylem Cells in *Cunninghamia lanceolata* and *Aquilaria sinensis*

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After programmed cell death (PCD), heartwood formation, storage, and processing, wood DNA degradation occurs to varying degrees. The concentration and distribution of nuclei and plastids in xylem cells of *Cunninghamia lanceolata* and *Aquilaria sinensis*, treated under different conditions of processing and storing, were studied by analyzing the distribution frequency, area, and signal intensity, in specimens that had been stained with aceto-carmine, DAPI, and I_2-KI. Most of the nuclei and plastids were present in the ray cells, and a small quantity of nuclei and plastids were present in the axial parenchyma cells. There was an indication that the concentration of the remaining nuclei and plastids in the xylem cells was mainly affected by the xylem heartwood formation, storage time, and temperature. The nuclei and plastids content of the sapwood was greater than that of the heartwood. However, the nuclei and plastids content of the fresh wood was greater than that of the processed and stored wood. An estimation of the quantity of nuclei and plastids using staining methods could provide a direct basis for the appropriate selection of a procedure for DNA extraction.

Keywords: Nuclei; Plastid; Xylem; Aceto-carmine; DAPI; I_2-KI

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INTRODUCTION

Nuclear ribosomal DNA and plastid DNA play an important role in the identification of timber species from the general to the individual level. In eukaryotic organisms, the nuclear ribosomal DNA (rDNA) has two internal transcribed spacers (ITS), *i.e.*, ITS1 and ITS2. The length and sites of ITS sequences are different, which can be used as a basis for the identification of wood species. The popularity of the rDNA-ITS region for molecular systematic analyses of closely related species can be attributed to both rapid evolution of the ITS spacers and PCR-amplification with conserved primers (Gonzalez *et al.* 2009; China Plant BOL Group 2011; Hanssen *et al.* 2011). Plastids are specific organelles of plants, and their forms include chloroplast, chromoplast, leucoplast, proplastids, etioplasts, gerontoplasts, and more specialized leucoplasts. These plastids can be transformed into each other. Compared with unique nuclei, there are a number of copies of plastid genomes in each cell (Deguilloux *et al.* 2002). The conserved gene order of chloroplast genome, the widespread availability of primers, and a general lack of heteroplasmy and recombination have made the chloroplast genome an attractive tool for phylogenetic studies of plants (Olmstead and Palmer 1994). DNA barcoding has been researched and applied in wood identification, using genes such as rpoC1, psbK–psbI, trnH–psbA, rbcL, matK (CBOL Plant Working Group 2009; Kress and Erickson 2007).

Wood is arborous, containing secondary xylem and many kinds of cells differentiated from cambium, such as vessel, ray, xylem fiber, and tracheid. DNA that remains in cells has provided a new approach to wood identification, *viz*. a DNA barcode and a DNA fingerprint. Secondary growth includes several consecutive processes, such as vascular tissue differentiation, secondary cell wall deposition, lignification, programmed cell death (PCD), and heartwood formation (Tian *et al.* 2007). In the process of PCD, nuclei are decreased and draped. At the same time, DNA is cut into fragments among the nucleosomes (Turner *et al.* 2005). Subsequently, DNA goes through further degradation during storage and processing.

DNA extraction is the front line for wood identification. Such identification is done by using an applied genetic method and is used for regulating the timber trade and fighting illegal logging. However, DNA in wood cells gradually breaks down, which makes DNA extraction and molecular identification very difficult, particularly from heartwood. So far, an all-round procedure for DNA extraction is still unavailable for application to different wood materials, although some successful cases have been published (Asif and Cannon 2005; Jiao *et al.* 2012; Tnah *et al.* 2012; Jiao *et al.* 2014). Furthermore, in some experiments, DNA cannot be amplified or sequenced even after successful DNA extraction (Asif and Cannon 2005; Zhang *et al.* 2014). Therefore, in order to choose the appropriate procedure, it is very important to estimate the concentration and quality of DNA before extraction occurs.

Nuclei can be observed effectually by using aceto-carmine, 4'-6-diamidino-2-phenylindole (DAPI), and aceto-carmine. Carmine is a natural dye extracted from a red pigment called cochineal. Apart from carmine, the aqueous solution of aceto-carmine contains acetic acid and ferrum. Acetic acid is a solvent for carmine, and it increases cell permeability and helps the ions enter the cells (Belling 1926; Rattenbury 1952). Chromosomes become crimson or red after an aceto-carmine reaction, and these appear visibly different from the other cell organelle and tissues. Consequently, it has been used in studies of the nuclei distribution of xylem (Islam and Begum 2011; Nakaba *et al.* 2012) and ray parenchyma cells in *Pinus dendiflora*, *Pinus rigida* (Nakaba *et al.* 2008), in *Populus sieboldii*, *Populis grandidentata* (Nakaba *et al.* 2012), and in *Tectona grandis* (Islam and Begum 2011). DAPI is a kind of DNA-specific probe, which forms a fluorescent complex by becoming attached in the minor groove of A-T rich sequences of DNA. In both sites, DAPI is bound with a long axis, approximately parallel to the grooves of the DNA helix (Kubista *et al.* 1987; Kapuscinski 1995). DAPI can penetrate a cell membrane and is applied in DNA locating and the quantitative analysis of clones (Nguyen *et al.* 1995). On the other hand, amyloplast, a kind of plastid, will show navy blue after being stained with Lugol's solution (I_2-KI). It can then be effectively distinguished from nuclei, cell walls, and so on (Abe *et al.* 2011).

The aim of this study was to understand the degree of degradation of wood nuclei and plastids under different sapwood, heartwood, storage, and processing conditions. The distribution frequency of nuclei and plastids, the area of nuclei, the signal intensity of nuclei in the ray cells, and the percentage of plastid area in rays of *Cunninghamia lanceolata* (Cuppressaceae) and *Aquilaria sinensis* (Thymelaeaceae) were investigated after being stained with aceto-carmine, DAPI, and I_2-KI in order to assist in choosing the appropriate DNA extraction procedure.

EXPERIMENTAL

Materials

C. lanceolata is widely distributed in China and is an important conifer species used in architecture and industry. Wood samples were collected from a 36 year old standing tree in the Chenshan Forestry Centre in Ji'an, in the Jiang Xi Province of China. Twelve wood discs (10 mm × 10 mm × 5 mm) were cut at a level of 2 m from the ground, with a diameter of 24.2 cm at breast height. Six wood samples were immediately placed into 2.5% glutaraldehyde in order to keep fresh. Other six wood samples were keeping in air temperature. On arrival at the laboratory, they were treated according to Table 1.

Table 1. Sample Information of *C. lanceolata*

Sample type	Location	Sample number	Storage temperature(℃)	Storage time(years)	Radial position (distance to pith)(cm)
Fresh wood	sapwood	3	4	2	18
	heartwood	3	4	2	3
Air-dried wood	sapwood	3	Room temperature	2	18
	heartwood	3	Room temperature	2	3

A. sinensis is a precious and unique resource for the production of "agarwood" in China and an endangered flora that has been listed since 2004 in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (http://www.cites.org/eng/app/appendices). Wood samples were collected from a standing tree of *A. sinensis* in the city of Guang Zhou in the Guang Dong Province of China.

The tree height was 2.3 m, the diameter at breast height was 7.48 cm, and the average diameter of the heartwood was 5.77 cm. The wood samples were immediately placed into an ice storage box after being collected from the stumpage. They were then immediately transported and stored in a refrigerator at -20 °C on return to the laboratory. Eighteen sticks (10 mm × 10 mm × 5 mm) were randomly chosen from the sapwood and heartwood respectively. They were treated according to Table 2.

Table 2. Sample Information for *A. sinensis*

Sample type	Location	Sample number	Storage or treated temperature °C	Processing time(hours)	Radial position (distance to pith) (cm)
Fresh wood	Sapwood	3	4	24	6.5
	Heartwood	3	4	24	2
Dried wood	Sapwood	3	80	3	6.5
	Heartwood	3	80	3	2
Dried wood	Sapwood	3	120	240	6.5
	Heartwood	3	120	240	2

Methods

Section preparation

First, the surfaces of the wood sticks were washed with 70% ethanol and then with purified water to reduce the influence of microbes on the DNA. Second, samples were fixed in a 2.5% solution of glutaraldehyde (pH=7.4) at room temperature for one day and thereafter washed four times with 0.2 mol/L phosphate buffer (PB) (0.2 mol/L monosodium orthophosphate and disodium hydrogen phosphate) (pH=7.4). Radial sections with a thickness of 10 μm were cut using a freeze sliding microtome (Leica CM3050S, Germany).

Control sample

Sections washed with PB or PBS without any staining, as control samples, were observed with a light microscope (Olympus BX61, Japan) and a Leica TCS SPE confocal microscope (Leica Microsystems, Germany)

Aceto-carmine staining

The sections were washed twice with 0.2 mol/L PB (pH=7.4) and then immersed in a 2% aqueous solution of aceto-carmine for 20 min. After washing twice with 0.2 mol/L PB (pH=7.4) to remove any unreacted aceto-carmine, the sections were placed under a light microscope (Olympus BX61, Japan) for observation.

DAPI staining

The sections were washed three times with a phosphate buffer solution (PBS) (pH=7.4) and then immersed in a 2.9 mM/L solution of DAPI for 20 min. After washing with PBS twice, the sections were then placed on a glass slide with glycerol. A Leica TCS SPE confocal microscope (Leica Microsystems, Germany) was adapted to observe at a 405 nm wavelength (PMT Gain value=900; PMT Offest=0; Laser output power=15%).

I_2-KI staining

The sections were washed twice with 0.2 mol/L PB (pH=7.4) and then immersed in an I_2-KI solution for 20 min. After washing twice with 0.2 mol/L PB (pH=7.4) to remove any unreacted I_2-KI, the sections were placed under a light microscope (Olympus BX61, Japan) for observation.

Statistical analysis

Three specimens were selected for each treatment and five fields of each specimen were used for analysis. For each of the detected fields, the nuclei frequency (the number of signals of DAPI and aceto-carmine of nuclei of rays (mm^{-2})), the nuclei area (the single nuclei area of rays, μm^{-2}), the signal intensity (0-255) of nuclei, and the percentage of plastid area (the percentage of plastid area in rays) were recorded. A double factor analysis of variance (ANOVA) was carried out using the SAS program, version 9.0, to evaluate quantitative nuclei and plastids differences among the samples.

Nuclei and plastids in axial parenchyma cells were ignored, because they could only be observed occasionally and the amount was so small compared with the nuclei and plastids in ray cells. In the evaluation of nuclei and plastids area and signal intensity, 50 signals of nuclei were selected at random in every sapwood sample. Fifteen signals of heartwood were measured because the number of signals of heartwood was small.

RESULTS AND DISCUSSION

Nuclei Distribution

In the control samples, the nuclei could not be observed under the light microscope (Fig. 1a, 1b, 1f, 2a, 2b, and 2f); however, they appeared black without auto fluorescence (Fig. 3a, 3b, 4a, 4b, and 4e), as detected by the confocal microscope. The nuclei stained with aceto-carmine appeared crimson in the ray cells and the axial parenchyma cells. These were effectively separated from the lipids, amyloplast, and cell walls (Fig. 1c, 1d, 1e, 1g, 2c, 2d, and 2g). Meanwhile, the nuclei stained with DAPI was detected by fluorescence under a confocal microsystem (Fig. 3c, 3d, 3g, and 4d).

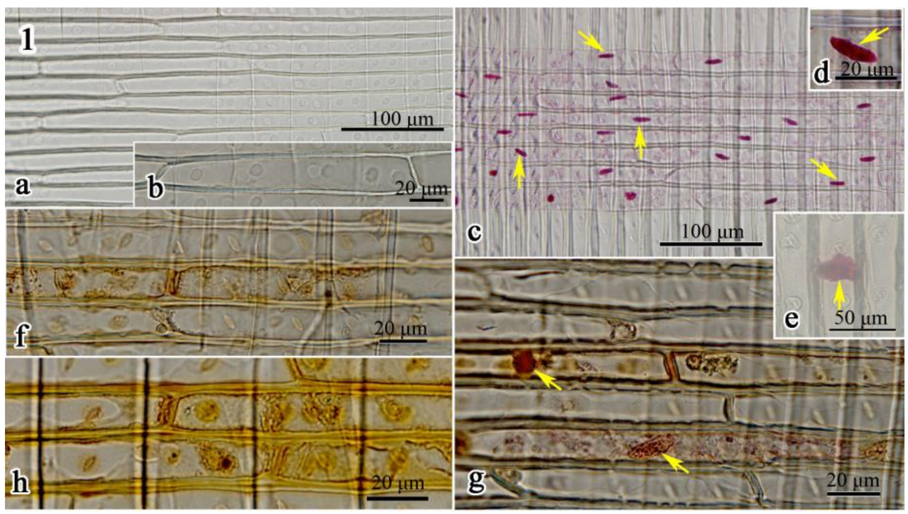

Fig. 1. Radial sections of *C. lanceolata*. a, b and h: Control samples. c, d, e, f and g: Samples stained with aceto-carmine. a: Rays of sapwood of fresh wood. b: Magnification of a. c: Rays of sapwood of fresh wood. d: Magnification of d. e: Axial parenchyma cells of fresh wood. f and g: Rays of heartwood of fresh wood. h: Rays of heart of air-dried wood. Parts indicated by arrows are nuclei in figure c, d, e, and g

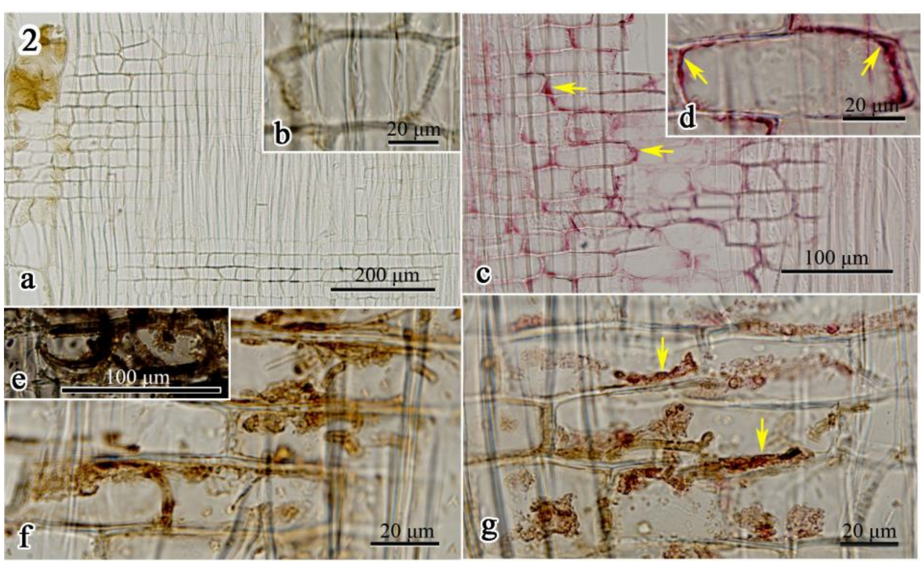

Fig. 2. Radial sections of *A. sinensis*. a, b and f: Control samples. c, d, e and g: Samples stained with aceto-carmine. a: Rays of sapwood. b: Magnification of a.c: Rays of sapwood. d: Magnification of c. e: Rays of heartwood of air-dried wood. f and g: Rays of heartwood of fresh wood. Parts indicated by arrows are nuclear chromatin in figure c, d and g

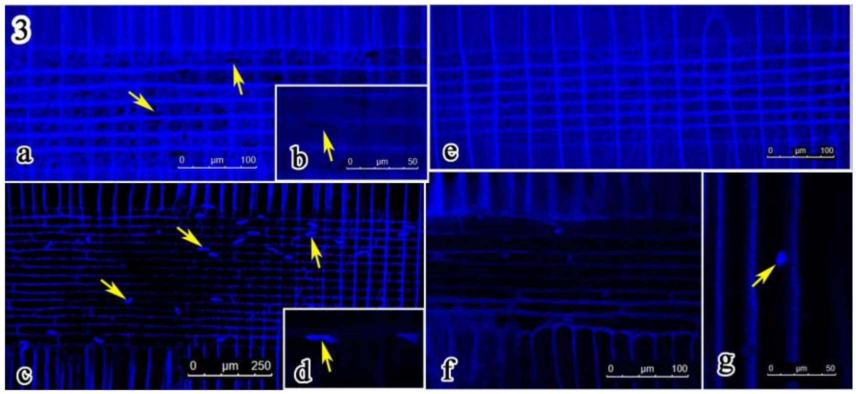

Fig. 3. Radial sections of *C. lanceolata*. a, b and e: Control samples. c, d, f and g: Samples stained with DAPI. a: Rays of sapwood. b: Magnification of a. c: Rays of sapwood. d: Magnification of c. e and f: Rays of heartwood. g: Axial parenchyma cells of fresh wood. Parts in figure a, b c, d and g indicated by arrows are nuclei

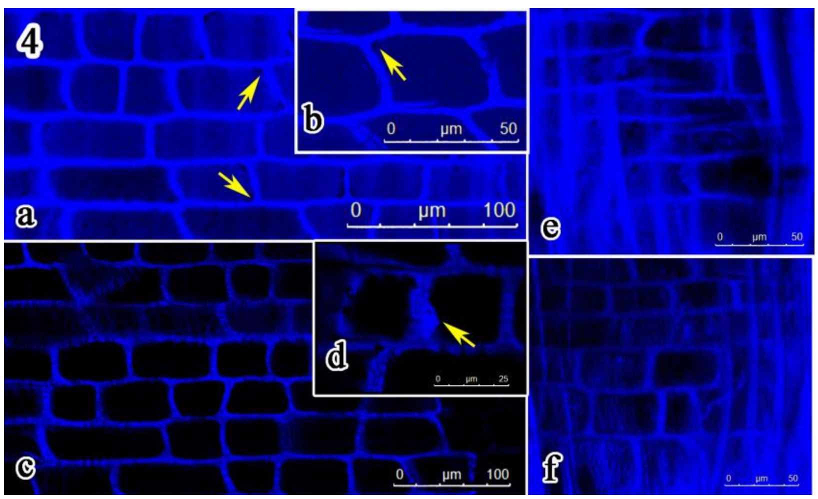

Fig. 4. Radial sections of *A. sinensis*. a, b and e: Control samples. c, d and f: Samples stained with DAPI. a: Rays of sapwood. b: Magnification of a. c: Rays of sapwood. d: Magnification of c. e and f: Heartwood. Parts in figure a, b and d indicated by arrows are nuclear chromatin

Generally, the chromatins of *C. lanceolata* were spindly in the cell lumen of ray parenchyma cells and axial parenchyma cells (Figs. 1 and 3). Most of the nuclear chromatin was localized in the upper middle and lower lines of the ray cells. However, when the nuclear chromatins of *A. sinensis* were attached to a cell wall, it was difficult to separate the chromatin from the cell wall (Figs. 2 and 4). When the nuclei of heartwood had been dispersed, there was not enough fluorescence emitted (Figs. 2g and 4f).

In the case of *C. lanceolata* sapwood samples, signals of DAPI and aceto-carmine were equally distributed in rays of both early wood and late wood. However, in heartwood samples, these signals were mainly distributed in rays of late wood near the growth ring boundary. For *A. sinensis*, crimson signals of nuclei were distributed in almost all ray parenchyma cells of fresh sapwood. Numbers of these signals in heartwood and dried sapwood were less than fresh sapwood, but they too were evenly distributed. It is possible that because *A. sinensis* trees grow in tropical areas, wood formation was not influenced by seasons.

Plastid Distribution

In the control samples, the plastid could not be observed under the light microscope (Fig. 1a, 1b, 1f, 2a, 2b, and 2f). In the stained samples, the plastids exhibited ianthine (Fig. 5).

In *C. lanceolata* sapwood samples, numerous amyloplasts were distributed in almost every ray parenchyma cell and axial parenchyma cells contained brown substance in fresh sapwood (Fig. 5a and 5b). The air-dried sapwood contained less amyloplast than the fresh one (Fig. 5a and 5c). About 3% of the rays of fresh heartwood contained amyloplast, and the content of amyloplast was rarely evident (Fig. 5d). There was amyloplast in the other rays of fresh heartwood and in almost all the air-dried heartwood (Fig. 5e). Signals of I_2-KI were equally distributed in rays of both early wood and late wood. However, in heartwood samples, these signals were mainly distributed in rays of early wood near the growth ring boundary. In the stained *A. sinensis* samples which had undergone a different processing method, there was no amyloplast (Fig. 6).

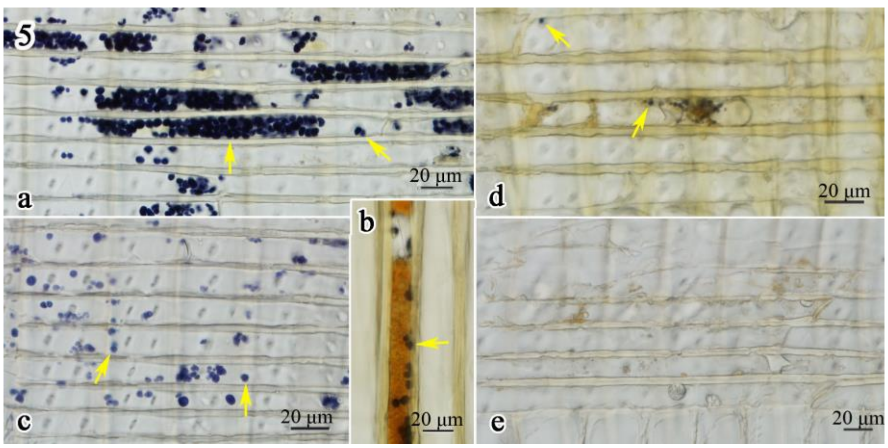

Fig. 5. Radial sections of *C. lanceolata* stained with I_2-KI. a: Rays of fresh sapwood. b: Longitudinal parenchyma of fresh sapwood. c: Rays of air-dried sapwood. d and e: Heartwood. Parts indicated by arrows are plastids in Parts a, b, c, and d.

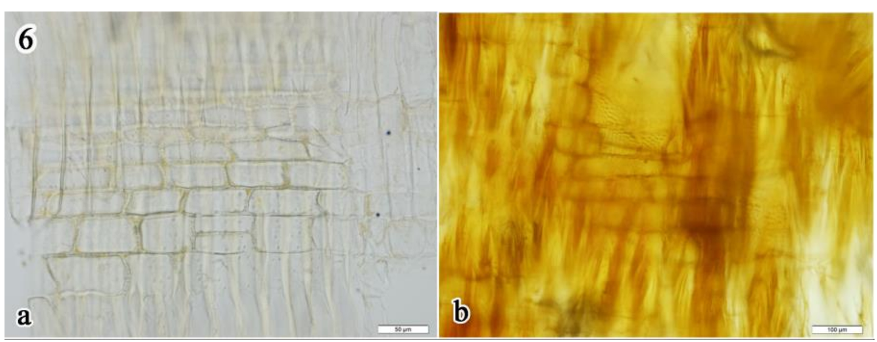

Fig. 6. Radial sections of *A. sinensis* stained with I_2-KI. a: Rays of sapwood. b: Rays of heartwood

Because nuclei and plastids in the trunk of *A. sinensis* could not be observed, the fresh branches of *A. sinensis* were stained in order to verify the correctness of the results. Results from signals of branch xylem stained with aceto-carmine and I_2-KI were the same as those of trunk xylem（Fig. 7b and 7c). However, some small particles, which should have been nuclei, degraded deeply, appeared in the rays of branch xylem stained with

DAPI, and could not be observed from xylem with longer formation time (Fig. 7e and 7f).

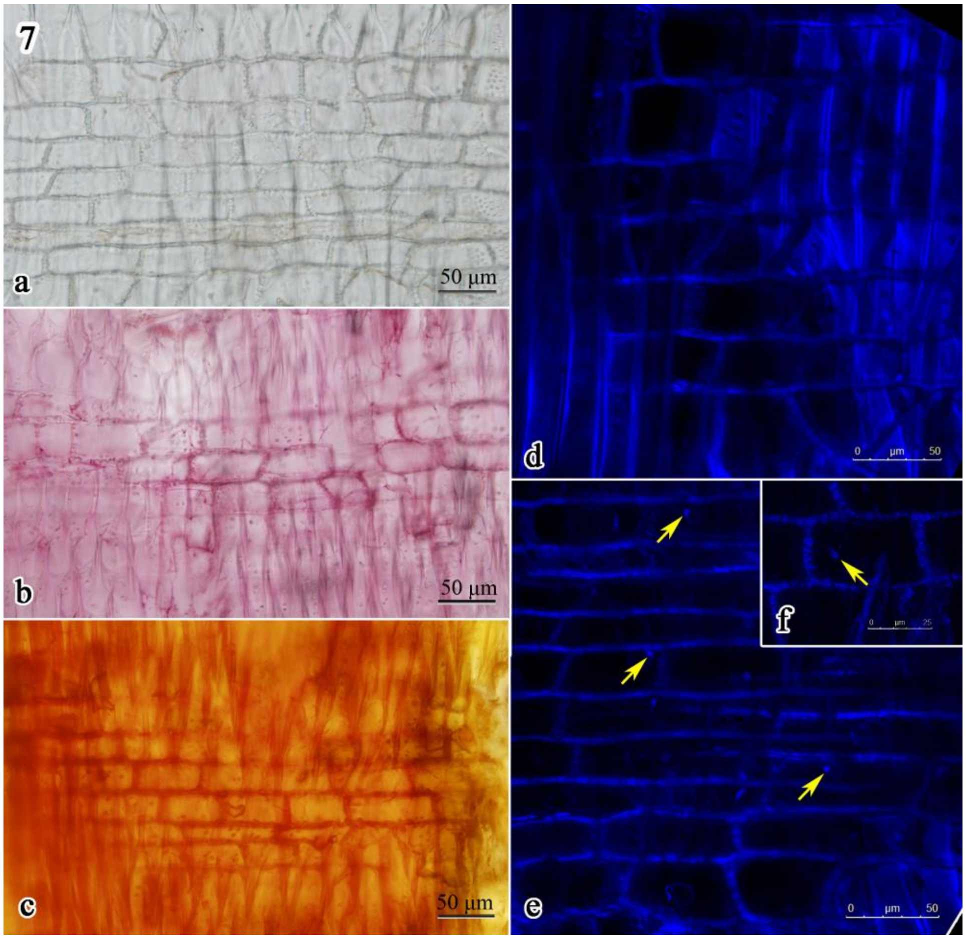

Fig. 7. Radial sections of the fresh branches of *A. sinensis*. a: Control sample of b and c. b: Sample stained with aceto-carmine. c: Sample stained with I_2-KI. d: Control sample of e. e: Sample stained with DAPI. f: Magnification of e. Parts e and f indicated by arrows are nuclear chromatin.

The nuclei and plastids in the ray parenchyma cells of *C. lanceolata* and *A. sinensis* varied considerably in different radical positions. Some of ray parenchyma cells and axial parenchyma cells contained nuclei and plastids, because these cells survived longer when compared to other cells, such as fiber, tracheary elements, and vessels. (Abe *et al.* 2011; Nakaba *et al.* 2006; Nakaba *et al.* 2008). Xylem was formed after PCD. In this process, various cells showed their own characteristics (Turner *et al.* 2005). Organelles containing DNA had already started to decrease during cellular senescence. Chromatins degraded soon after the nucleoli degraded in cells of fiber, tracheary elements, and vessels. Subsequently, DNA underwent a further degradation during heartwood formation for some species with heartwood, as well as during storage and processing. The size and shape of nuclear chromatin varied (Mittler and Lam 1995).

The nuclei between *C. lanceolata* and *A. sinensis* varied greatly, as did plastids. It is probably because the degree of evolution of *A. sinensis* is higher than that of *C. lanceolata*. Firstly, there are many anatomic construction features with a higher degree of evolution in wood of *A. sinensis*, such as rounded or oval vessels, vessels in tangential bands, vessel clusters common, simple perforation plates, and alternating intervessel pits (Bailey and Tupper 1918; Bailey 1944; Frost 1930a, 1930b and 1931). Secondly, the taxonomy of this *Aquilaria* genus is as follows—angiosperm: dicotyledoneae: rose subclass: myrtles: Thymelaeaceae. On the one hand, the degree of evolution of angiosperms is higher than that of gymnosperms. On the other hand, myrtle were located at the highest evolution level in four evolutionary classification systems of angiosperms, including A. Engler’s system, J. Hutchinson’s system, A. Takhtajan’s system, and A. Cronquist’s system (Qiang 2006).

Nuclear Quantity Analysis

Large quantities of nuclei remained in the fresh sapwood of *C. lanceolata*, while the amount of nuclei that remained in the fresh heartwood, air-dried sapwood, and air-dried heartwood was less obvious. Comparing the sapwood to the heartwood, the variation was significant (F-probability <0.0001) and greater than the value of the variation between the fresh wood and the air-dried wood (0.0006). Furthermore, there was more nuclei in the sapwood that had been stored for two years than there was in the fresh heartwood. Therefore, heartwood formation degraded the nuclei more than the storage time did (Table 3).

Table 3. Distribution Frequency of Nuclei Remaining in the Ray Cells (mm^{-2}) and the Quantity of DNA (ng/mg) Extracted from Freshwood using CTAB and Kit Methods of *C. lanceolata*

	Fresh wood	Air-dried wood (2 years)	F-probability	DNA quantity of freshwood *	
				CTAB	Kit
Sapwood	212.20 A	79.09 B		69.95	107.02
	(96.61)	(52.91)		(8.40)	(7.63)
Heartwood	12.57C	2.96 C		12.70	13.83
	(23.33)	(5.49)		(3.06)	(4.43)
<0.0001	0.0006		0.0097		

The values indicate the amount of nuclei remaining in wood per square mm^2. The values in parentheses represent standard deviations. A, B, C, and D: the same letter indicates that there was no significant difference among them. The significance level is 0.05.
* This parameter was normalized according to the procedure of Jiao (2012). Its treatment conditions and sample source of wood were same to this paper.

For *A. sinensis*, the variation in the distribution frequency of the cells containing nuclei from sapwood that were treated by keeping them fresh, dried at 80 °C, and dried at 120 °C were significantly different, which was the same result as with the *C. lanceolata* samples. The variation was significant (F-probability<0.0001) between the sapwood and heartwood. Additionally, the nuclei distribution frequency of the sapwood dried at 120 °C was greater than that of all heartwood samples, *i.e.*, the variation among the heartwood treated by keeping it fresh, dried at 80 °C, and dried at 120 °C was insignificant. Therefore, heartwood formation degraded the nuclei more than the high temperature did (Table 4).

Table 4. Distribution Frequency of the Ray Cells Containing Nuclei Remaining (mm^{-2}) and the Quantity of DNA (ng/mg) Extracted using Kit Method of *A. sinensis*

	Distribution frequency				DNA quantity *		
	Fresh wood	Dried at 80 °C	Dried at 120 °C	F-probability	Fresh wood	Dried at 80 °C	Dried at 120°C
Sapwood	411.79 A	98.98B	38.27 B		8.01	4.67	4.44
	(106.33)	(89.37)	(30.47)		(0.80)	(0.64)	(0.66)
Heartwood	8.73 C	6.11 C	3.90 C		4.39	4.73	4.24
	(12.5)	(17.27)	(11.04)		(0.49)	(0.53)	(0.60)
<0.0001	<0.0001			<0.0001			

The values indicate the amount of nuclei remaining in wood per mm^2. The values in parentheses represent standard deviations. A, B and C: the same letter indicates that there was no significant difference among them. The significance level is 0.05.
* This parameter was normalized according to the procedure of Jiao (2014). Its treatment conditions and sample source were similar to this paper.

The area of nuclei that remained in the fresh sapwood of *C. lanceolata* was significantly greater when compared to other fresh heartwood, dried sapwood, and dried heartwood samples. However, among fresh heartwood, air-dried sapwood, and air-dried heartwood, these values were not significant (Table 5). These results were similar to the distribution frequency results for *C. lanceolata*. This means that the heartwood nuclei degraded almost completely. The nuclei area of *A. sinensis* could not be computed, because it was difficult to separate the nuclear chromatin from the cell walls.

Table 5. Area of Nuclei Remaining in the Ray Cells of *C. lanceolata* (μm^2)

	Fresh wood	Air-dried wood (2 years)	F-probability
Sapwood	173.14 A	81.47 B	
	(99.36)	(68.79)	
Heartwood	54.51 B	38.76 B	
	(24.62)	(24.95)	
<0.0001	<0.0001		<0.0001

The values indicate the area of nuclei remaining in wood per mm^2. The values in parentheses represent standard deviations. A and B: the same letter indicates that there was no significant difference among them. The significance level is 0.05.

Table 6. Signal Intensity of the Ray Cells in *C. lanceolata*

	Fresh wood	Air-dried wood	F-probability
Sapwood	103.38A	41.49 B	
	(24.42)	(9.41)	
Heartwood	50.01 B	35.78B	
	(14.30)	(10.33)	
0.0321	<0.0001		0.0012

The values indicate the signal intensity (0-255) of nuclei remaining in the wood. The values in parentheses represent standard deviations. A and B: the same letter indicates that there was no significant difference among them. The significance level is 0.05.

A greater signal intensity of nuclei means a greater concentration of DNA. The value for fresh sapwood was significantly different compared to the values from fresh heartwood, air-dried sapwood and air-dried heartwood (Table 6). This means that the best DNA came from the fresh sapwood. However, the qualities of the nuclei that remained in the fresh heartwood, air-dried sapwood, and air-dried heartwood were similar to each other.

Plastid Quantity Analysis

The percentage of plastid area in rays was determined according to the ianthine signal of amyloplast in *C. lanceolata* stained with I_2-KI. The amyloplast area of 14.56% in rays of fresh sapwood was higher than 1.01% in air-dried sapwood, 0.05% in fresh heartwood, and 0.0023% in air-dried heartwood. Comparing the sapwood to the heartwood, the variation was significant (F-probability <0.0001), and the same as that between fresh and air-dried wood (F-probability <0.0001). Furthermore, there was more plastids in the sapwood that had been stored for two years than there was in the fresh heartwood. Therefore, PCD degraded the plastids more than the storage time did (Table 7).

Table 7. Percentage of Plastid Area in Rays in *C. lanceolata* (%)

	Fresh wood	Air-dried wood (2 years)	F-probability
Sapwood	14.56A	1.01B	
	(3.60)	(0.33)	
Heartwood	0.05C	0.0023C	
	(0.108)	(0.0063)	
<0.0001	<0.0001		<0.0001
The values indicate the percentage of plastid area in rays. The values in parentheses represent standard deviations. A, B, and C. the same letter indicates that there was no significant difference among them. The significance level is 0.05.			

To sum up, sapwood contained more plastids than heartwood, while fresh wood contained more plastids than processed wood. The variation tendency of the plastids quality was synchronous with the quantity (Rachmayanti *et al.* 2009; Tnah *et al.* 2012). Taking all the factors into consideration, there was an indication that the concentration of the remaining plastids in xylem cells was mainly affected by the xylem's PCD, heartwood formation, the storage time, and the drying temperature. The PCD made the strongest impact on the plastids degradation, heartwood formation is next, when compared to the effect of storage time and high temperature.

Content of Nuclei and Plastids and DNA Isolation

It was reported that hexadecyl trimethyl ammonium bromide (CTAB), the DNeasy Plant Mini Kit (Qiagen, Germany), and N-phenacylthiazolium bromide (PTB) had been successfully used in wood DNA extraction after modification (Asif and Cannon 2005; Jiao *et al.* 2012; Jiao *et al.* 2014). However, two or three extraction methods were used on the same wood sample in most of the existing research, because it is difficult to select the right wood DNA extraction procedure for wood that has unknown degrees of DNA degradation.

The differences among the distribution frequency of the nuclei and plastids remaining in ray cells of *A. sinensis* were similar to the quantity of DNA extraction from wood under the same treatment conditions. A DNeasy Plant Mini Kit (QIAGEN, Germany) succeeded in extracting the DNA in fresh sapwood, fresh heartwood, and dried heartwood of *A. sinensis*, but failed with heartwood dried at a high-temperature (Jiao *et al.* 2014). The same was true for both the Kit and CTAB when it came to DNA extraction from *C. lanceolata* (Jiao *et al.* 2012). This indicates that the extraction quantity varies according to the content of nuclei and plastids. The quantity of DNA extracted from the same samples using the DNeasy Plant Mini Kit was 1.5 to 2 times more than what was extracted using CTAB (Jiao *et al.* 2012). However, the kit did not succeed with the heartwood dried at a high-temperature (Jiao *et al.* 2014). CTAB and the kit yielded low-qualities and low quantities of DNA; these samples could not be amplified even though they were detectable. However, PTB succeeded in extracting DNA from the dried heartwood of Ramin (*Gonystylus bancanus*) (Asif and Cannon 2005), due to having greater DNA quantity and quality than what was obtained from CTAB and the kit. In another report, CTAB and the kit responded well when used in extracting DNA from the sapwood and heartwood of Penak (*Neobalanocarpus heimii*), but the result was not as good as that which was optimized using PTB. In general, fresh wood that contains a mass of DNA could be extracted using the CTAB or the kit without PTB. For aged wood that contains less nuclei and plastids than fresh wood, the kit or PTB ought to be chosen. When there is no sign of nuclei and plastids observable, a modified PTB procedure needs to be developed. However, PTB does not always work for all wood with substantially degraded nuclei and plastids.

In practice, the freshness and processing stage of wood is hard to evaluate visually. However, staining methods can be used for a fast estimation of remaining nuclei and plastids in wood materials. The optimized extraction procedures can be chosen according to the observation of the nuclei and plastids amount and quality by staining.

CONCLUSIONS

1. Nuclei and plastids were degraded by PCD first in xylem formation. Fiber, tracheary elements, and vessels lost nuclei and plastids. Subsequently, nuclei and plastids in ray cells and axial parenchyma cells underwent a further degradation during heartwood formation for some species with heartwood. After the tree was cut, nuclei and plastids were degraded continually by storage and temperature.
2. Staining methods, such as aceto-carmine, DAPI, and I_2-KI, can be adopted in the evaluation of nuclei and plastids quantity. It is advised that the optimized extraction procedures for wood DNA extracting be chosen.

ACKNOWLEDGMENTS

The authors would like to thank Professor Yin Yafang of the Chinese Research Institute of Wood Industry, the Chinese Academy of Forestry for his valuable comments on experiment and data analysis, Dr. Liu Bo for the guide of confocal microscope, and Mr. Jiao Lichao and Mr. Song Kunlin for collecting the samples. This work was financially supported by a project at the Chinese State Forestry Administration (No. 201304508) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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Article submitted: September 12, 2014; Peer review completed: October 15, 2014; Revised version received and accepted: December 19, 2014; Published: January 8, 2015.