Research paper

Anatomical Characteristics of Artificially Induced Tension Wood in Seedlings of Honduras Mahogany

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【Summary】

Seedlings of Honduras mahogany (*Swietenia macrophylla* King) were laid horizontally for 4 and 10 mo to induce reaction wood. Gelatinous fibers occurred on the upper side of the trunks of the treated seedlings, and the longer the treatment, the higher the extent of the gelatinous fibers. More-abundant axial parenchymatous cells and starch grains were observed in the opposite wood than in the reaction wood. Such anatomical characteristics suggest that there are distinct patterns of regulation between the cambium zones of the reaction wood and opposite wood. Starch storage in axial parenchymatous cells may play a role in the active or passive processes involved in the biochemical regulation and source-to-sink transportation.

Key words: gelatinous fibers, Honduras mahogany, reaction wood, starch grains, *Swietenia macrophylla*.

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研究報告

大葉桃花心木苗木人為誘導的抗張材之解剖特徵

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摘　要

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本研究中，大葉桃花心木(*Swietenia macrophylla* King)苗木經4與10個月水平橫放生長，以誘導反應材形成。膠質纖維主要產生在主幹上側的反應材組織中，且水平橫放處理時間越久，膠質纖維形成量越多。相較於反應材，對應材組織中含較高比例的縱向薄壁細胞及澱粉粒。這些解剖特徵顯示反應材形成層與對應材形成層具有不同的調節機制，縱向薄壁細胞中所儲藏的澱粉可扮演著主動或被動的生化調節功能與養分供需運輸角色。

關鍵詞：膠質纖維、大葉桃花心木、反應材、澱粉粒。

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INTRODUCTION

The formation of reaction wood is related to stem reorientation in response to environmental factors. In order to maximize exposure to sunlight, the leaning trunks of most dicotyledons are able to recover to a vertical position by producing tension wood, a wood with high tensile stresses, on the upper side of the trunk, whether the cause is natural or artificial (Wardrop 1964, Fisher and Stevenson 1981). One of the characteristics of tension wood is gelatinous fibers which are named for their unusual inner wall layer called the gelatinous layer (G-layer). The G-layer is known to contain high cellulose, low lignin contents, and small microfibril angles (MFAs) (Scurfield 1973, Dickison 2000). These differences in chemical composition and structure give tension wood with particular properties in comparison to normal wood, notably high longitudinal shrinkage. The growth stress and G-layer of tension wood may cause log splitting and lumber distortion during the cutting and drying processes, respectively (Dinwoodie 1966, Washusen et al. 2003a).

Swietenia macrophylla King (Honduras mahogany or Caoba) is a member of the mahogany family (Meliaceae). It is an important commercial species with a desirable figure and good working properties. Many studies of the natural generation, dendroecology, genetic structure, and gene flow have been conducted on this species (Dünisch et al. 2003, Lemes et al. 2003). However no report has investigated reaction wood formation. In this work, artificially induced reaction wood of *S. macrophylla* seedlings was anatomically studied.

MATERIALS AND METHODS

Experiments were conducted at Hengchun in southern Taiwan. Several 1~2-yr-old seedlings of *S. macrophylla* were collected from a nursery of the Taiwan Forestry Research Institute. The seedlings were initially grown in an upright orientation to obtain normal wood as a control. Eight seedlings were then laid horizontally to induce different levels of reaction wood (Fig. 1A-C). Before cutting, the upper sides of the trunks were marked. The basal curved parts of the trunks of 4 seedlings treated for 4 months were dissected into 3 segments (Fig. 1B, C). Three additional segments were collected from the upper trunks of the 4 seedlings treated for 10 mo (Figs. 1C, 4A). The eccentricity (Ec) is defined as the eccentric distance (De) divided by the short diameter (Ds) (Japan Material Society 1982).

$$Ec = De/Ds = | Dg - Da | /Ds;$$

where Dg is the distance between the rim of the lower side and the geometric center, and Da is the distance between the rim of the lower side and the pith.

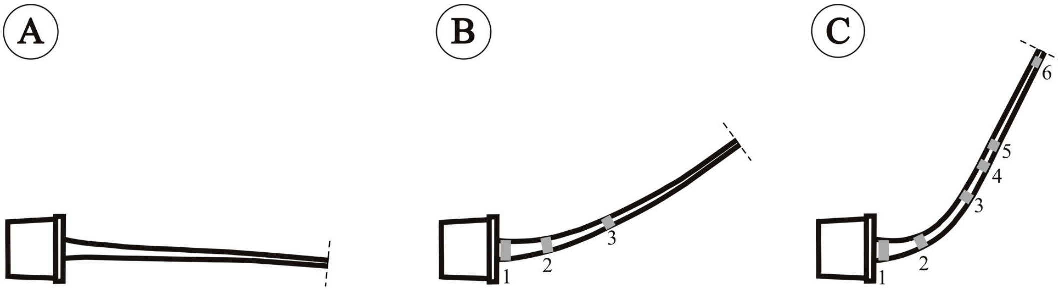

Fig. 1. One to 2-yr-old seedlings of *Swietenia macrophylla* laid horizontally to induce tension wood. (A) At 0 mo; (B) after 4 mo, the trunk had bent upwards; (C) after 10 mo, the trunk had almost bent back to the vertical position. Cross-sections cut from segments 1~3 in both (B) and (C) were used for calculating the area percentages shown in Fig. 5. Segments 1~6 in (C) were the sites cut for measuring eccentricity.

Small wood blocks ($1 \times 1 \times 2$ mm^3) containing tension wood or opposite wood were processed through fixation (in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer), postfixation (in 1% OsO_4 in 0.1 M sodium phosphate buffer), and dehydration (in an acetone series), and were then embedded in Spurr's resin (Spurr 1969). Semi-thin sections (1 μm) were made using an ultramicrotome (Ultracut E) and were stained with 1% toluidine blue in borax buffer. Free-hand or microtome sections (20~30 μm in thickness) were made from the wood blocks, and were stained with safranin O and Herzburg reagent. Typical G-layers of the gelatinous fibers in the tension wood were recognizable by their morphology and their reaction with different stains. Sections were stained with iodine-potassium iodine to identify starch grains (Gahan 1984). Sections stained with zinc-chlor-iodide (Krishnamurthy 1999) with a few drops of hydrochloric acid were used for morphometric analysis. The lignified secondary wall stained light yellow, the G-layer orange, and the starch grains blackish-brown. All sections were examined using a Leica Diaplan Microscope. Images (n = 50) were captured with a Nikon Coolpix 995 digital camera. The percent areas of lignified cell walls, G-layers, cell lumens, and starch grains were calculated using the image analysis software ImagePro Plus.

RESULTS AND DISCUSSION

Swietenia macrophylla forms a diffuse porous wood (Fig. 2A). Axial parenchymatous cells appeared regularly in the vicinity of vessel elements or were sometimes grouped along the initial or terminal part of the annual rings that usually refer to apotracheal banded (or marginal) parenchyma (Carlquist 2001). Starch grains were found in the ray and axial parenchymatous cells (Fig. 2B), however they often spread out during the free-hand sectioning procedure.

All trunks of seedlings laid horizontally for 4 or 10 mo exhibited radial growth promotion on the upper side. The discs cut from the curved parts of these trunks were more or less oval in shape (Figs. 3A, 4A, B), and the eccentricity decreased from the base to the apex of the seedling (Fig. 4C). To maintain normal angles with the vertical trunk, it is known that sufficient amounts of compression wood are continuously formed on the lower

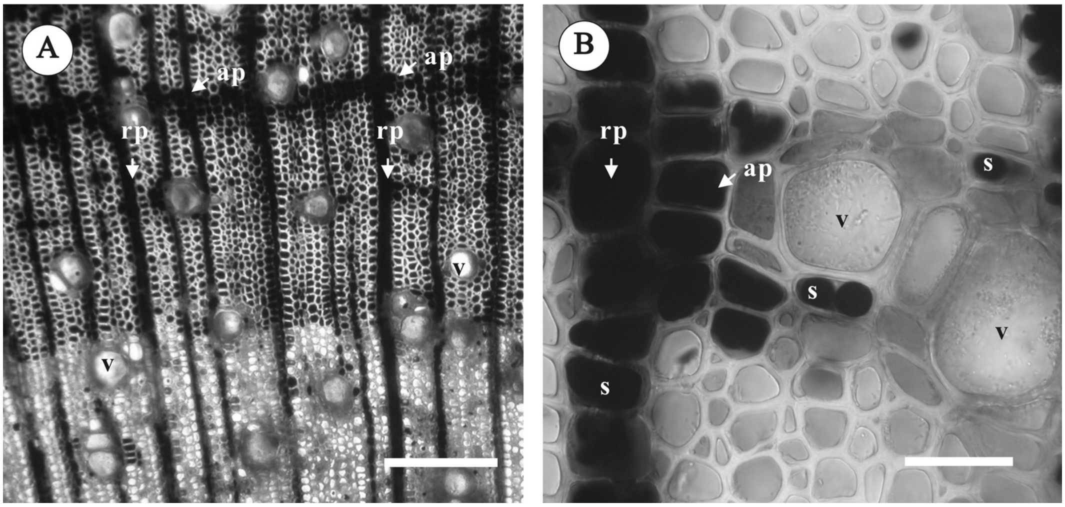

Fig. 2. Normal wood structure of the basal part of seedlings. (A) Transverse section of the trunk showing the diffuse porous wood with vesicentric scanty and apotracheal banded parenchyma. (B) Starch grains stained with iodine solution occurring in the ray and axial parenchymatous cells. ap, axial parenchymatous cells; rp, ray parenchyma; s, starch grain; v, vessel. Bars = 200 µm in (A) and 30 µm in (B).

sides of branches of gymnosperms (Westing 1965). The adaxial promotion of the growth of tension wood in the horizontally laid trunks of *S. macrophylla* seemed to follow a similar pattern.

Eccentricity of the stem can be used to show the significance of growth strain from a macroscopic view, while the anatomical structure, i.e. the distribution of gelatinous fibers, can represent these facts from a more-microscopic field of view (Washusen et al. 2003b). In the trunks of seedlings laid horizontally for 4 mo, the axial fibers in the tension wood were mostly G-fibers (Figs. 3B, 5A), while those in the opposite wood were mainly normal fibers with a few G-fiber in thin layers (Figs. 3D, 5A). In the trunks of those treated for 10 mo, the area percentage of the G-layers was closely related to the eccentricity of the trunks (Figs. 4C, 5B). Almost all of the G-fibers were found in the sections of the upper side of the eccentric or basal part of the trunks (Figs. 4D, 5B), but not in those sections of the lower side (Figs. 4E, 5B), while equal amounts of G-fibers were found in all sections from the almost upright parts of the trunks (Fig. 5B).

Gelatinous fibers shorten at maturation and induce longitudinal tension strains in tension wood areas. When the seedlings receive the gravitational signal, the xylem mother cells follow different pathways of differentiation. That is, more xylem mother cells develop into gelatinous fibers in the reaction cambium, but into axial parenchymatous cells in the opposite cambium. The process of cell differentiation during wood formation is a complicated research topic involving many biochemical pathways and genetic regulators.

The distribution of carbon sources in the plant tissue is functionally allocated. It is interesting to note that in the trunks of seedlings laid horizontally for 4 mo, starch grains were found in most ray and axial parenchymatous cells of opposite wood (Fig. 3D, E), while in tension wood (upper side), starch grains were

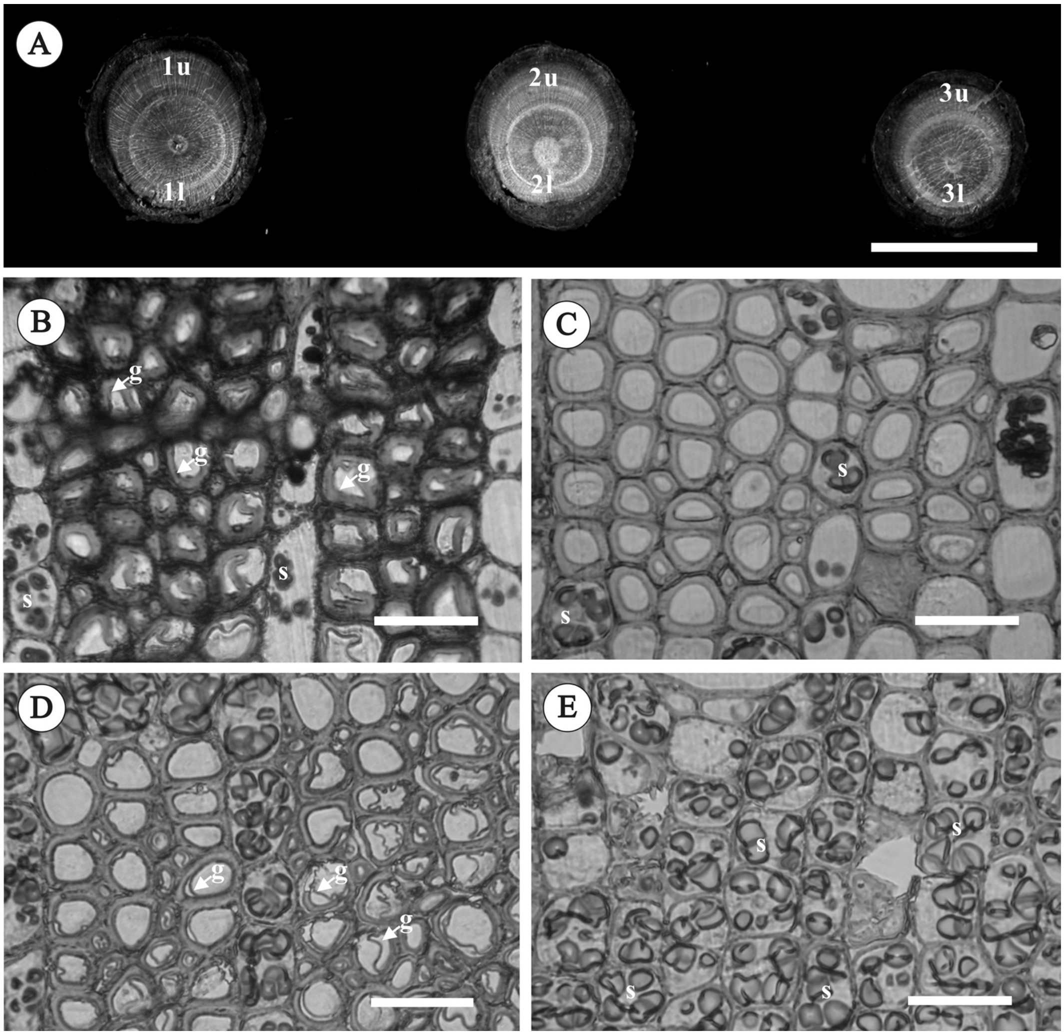

Fig. 3. Tension and opposite wood structures of seelings laid horizontally for 4 mo. (A) Discs cut from the curved parts of a seedling. Transverse sections of the upper (B, C) and lower sides (D, E) of the trunk representing the distributions of G-fibers and starch grains. g, gelatinous fiber; l, lower side; s, starch grain; u, upper side. Bars = 1 cm in (A) and 30 µm in (B-E).

only found in ray parenchymatous cells (Fig. 3B, C). Based on the morphometric analysis, we found that on average, about 60~70% of the starch grains were found in wood of the lower side (Fig. 5A). However, in the basal trunks of seedlings laid horizontally for 10 mo where severe tension wood was formed, most starch grains (about 90%) were found in wood on the lower side, while in the almost upright parts of the trunk, starch grains were found equally in both the lower and upper sides (Figs. 4D-G, 5B).

Formation of gelatinous fibers is believed to involve additional energy costs and carbon sources compared with those of opposite wood. Physiologically, starch grains can

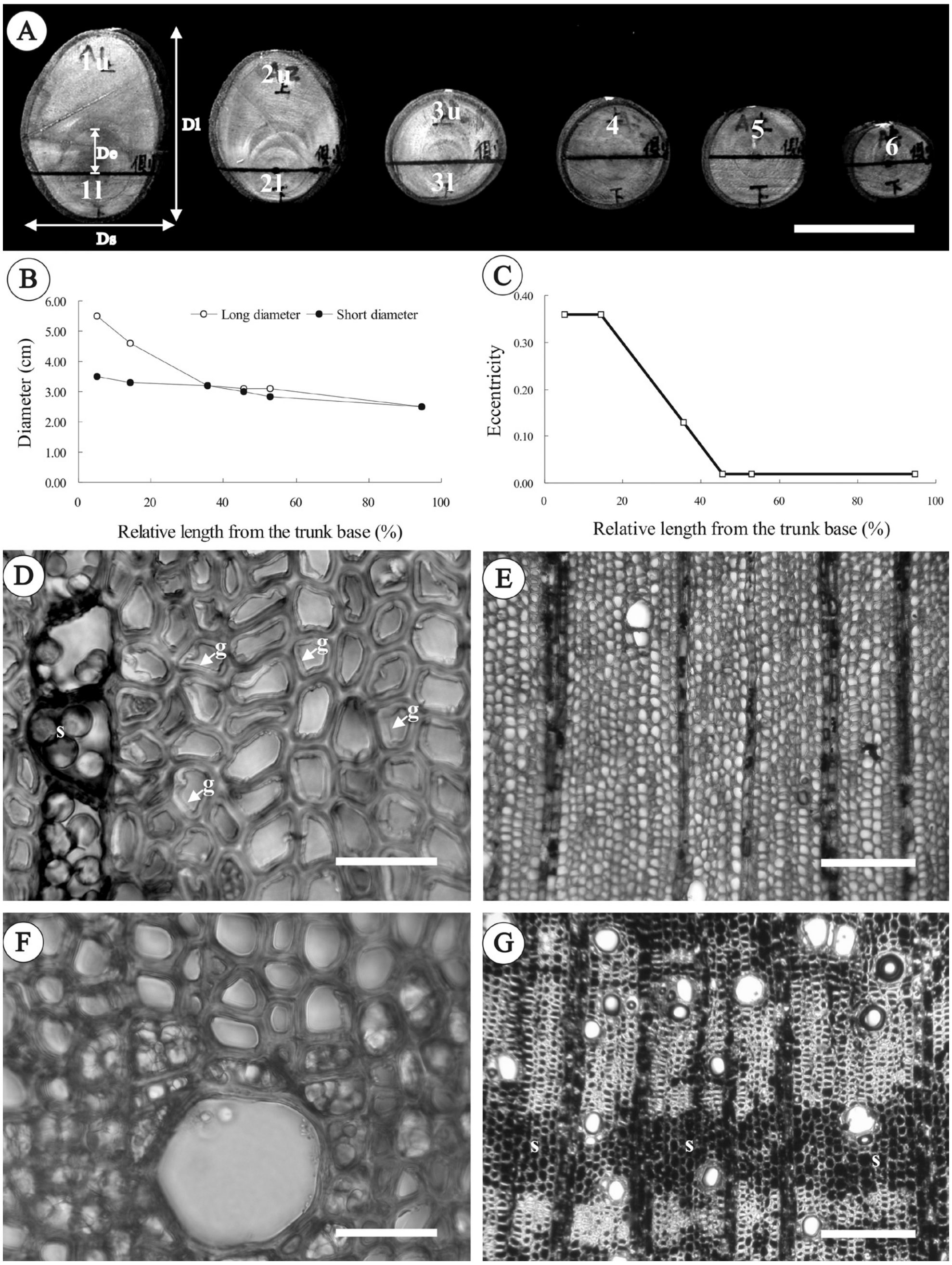

Fig. 4. Tension and opposite wood structures of seedling laid horizontally for 10 mo. (A) Discs cut from the base to the apex of the seedling. (B) Diameters and (C) eccentricity of each disc decreased from the base to the apex of the seedling. Transverse sections of the upper (D, E) and lower sides (F, G) of the trunk representing the distributions of G-fibers and starch grains. De, eccentric distance; Dl, long diameter; Ds, short diameter; g, gelatinous fiber; l, lower side; s, starch grain; u, upper side. Bars = 3 cm in (A), 30 µm in (D, F), and 200 µm in (E, G).

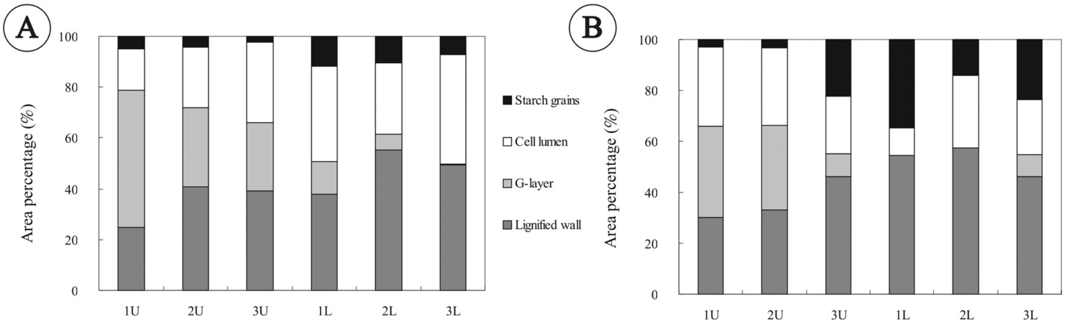

Fig. 5. Area percentages of the distributions of starch grains, cell lumens, G-layers, and lignified cell walls. (A) Seedling laid horizontally for 4 mo exhibited a high content of G-layers (83%) and a low content of starch grains (37%) in upper-side wood. (B) In the basal part after 10 mo of treatment, a few starch grains (16%) and all G-layers occurred in the fibers on the upper side of tree trunks, while in the almost upright parts of the seedling, equal areas of starch grains and G-layers occurred on either side of the tree trunk.

be converted into energy for cell metabolism or components for cell division in the cambium zone and for cell wall construction. These processes might be regulated by alterations in nutrition transportation to form gelatinous fibers combined with a source-to-sink movement of nutrients across the gradient. Oribe et al. (2001, 2003) described how the localization of starch grains in *Albies sachalinensis* is closely related to cambial reactivation, because they occur during dormancy of the cambium and disappear during activation. Carbohydrates across cambial region tissues, i.e., fructose, glucose (monosaccharides), and sucrose (disaccharide), and their metabolizing enzymes can be studied using tissue-specific microanalysis. It was found that sucrose was abundant in the phloem where it is translocated to and assumed to be decomposed in the cambium zone and in the developing xylem to supply the energy for cell development, while simultaneously being converted to monosaccharides for cell wall components in Scots pine (*Pinus sylvestris*) (Uggla et al. 2001). Although evidence of starch hydrolyzing enzymes was not pursued in our study, the importance of carbohydrate during wood formation is obvious.

Anatomical research of tension wood formation is enriched by our studies as well as those of many others. To reveal the nature of growth regulation by tree species, tensile measurements should be combined with information from anatomical research. At the same time, using biochemical and genomic methods to investigate the regulation of wood formation is also necessary to understand the processes of reaction wood formation. The combination of the data from tension strain measurements, anatomical ultrastructures, and biochemical and genetic regulations will shed new light on the research of tension wood formation.

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