Morphological and chemical analyses of the “chi-chi” of *Ginkgo biloba* L.

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Abstract

Outline of this thesis

This thesis is entitled “Morphological and chemical analyses of the “chi-chi” of *Ginkgo biloba* L.” and it consists of 4 chapters. This study was conducted with the following objectives.

(a) To clarify the morphological characteristics of the aerial “chi-chi".

(b) To clarify the lignin chemical characteristics of the aerial “chi-chi".

(c) To clarify the morphological characteristics of the basal “chi-chi" and the differences between the aerial “chi-chi" and the basal “chi-chi".

In chapter 1, Introduction is shown.

In chapter 2, (a) the morphological characteristic of “aerial chi-chi” and (b) the lignin chemical characteristics of “aerial chi-chi” were studied in relation to each other. (a) was performed with Scanning electron microscopy (SEM), field emission (FE)-SEM, X-ray microcomputed tomography (X-ray μCT), and optical microscopy. (b) was performed by the acetyl bromide method and gas chromatography-mass spectrometry (GC-MS) after thioacidolysis. The “aerial chi-chi” had many woody bulges and latent buds under the bark. The tracheids around the parenchymatous tissue of latent bud were curving and had compression-wood-like feature.

In chapter 3, (c) clarification of the morphological characteristics of the "basal chi-chi" and the differences between the "aerial chi-chi" and the "basal chi-chi" were performed optical microscopy. The “basal chi-chi” had root-like structure but had not root cap. The “basal chi-chi” grew on the main root underground when the trunk was tilted, but it did not grow when the trunk was tilted and shaded with aluminum foil.

In chapter 4, the results and discussion of this thesis are summarized.

Chapter 1. General Introduction

Leaves of the *G. biloba* tree have a distinctive shape that even those unfamiliar with the plant can recognize at first glance as ginkgo. Aside from the leaves, the wood of the *G. biloba* resembles that of conifers, and until 1896, when Sakugoro Hirase discovered swimming sperm cells in *G. biloba*, it was thought that *G. biloba* was a close relative of conifers. Seed plants deliver sperm cells to eggs through tubes extending from pollen grains, but Ginkgo, which is fertilized by swimming sperm, is clearly in an isolated position in the phylogenetic relationships of the present plant species, meaning that it is closer to ferns and mosses, which have swimming sperm, than to conifers. The oldest known ginkgo is *Ginkgo cordilobata*, found in 190 million-year-old Early Jurassic rocks in Afghanistan. The leaves are similar to those of the present ginkgo, divided into six segment and each segment is itself deeply bilobed. They look more like the leaves of ginkgo seedlings than like the leaves of mature trees, but there is no dispute about their identity; it does not take a specialist to immediately see the link to the familiar tree of our streets and gardens. Steinthorsdottir et al. give the photo of the leaves of *Ginkgo cordilobata* in their thesis. Zhou et al. described new *Ginkgo* fossil which was over 121 million-year-old. Their new finding extends the geological range of the modern form and is evidence of a roughly 120 million-year-old morphological stasis in ovulate organs of *Ginkgo*. For these reasons, *G. biloba* is considered a "living fossil.

The number of *Ginkgo* species was more than 20 in the early Cretaceous (145 million to 65 million years ago), but the species decline began in the mid-Cretaceous, about 100 million years ago, and now there is only one species in one genus, *G. biloba*. At the same time, the distribution range of *G. biloba* declined, probably due to a decrease in the number of animals responsible for seed dispersal. The final refuge for *G. biloba* was the mountains and valleys of southern and western China. Ginkgo was spared from extinction in part because human beings began to eat the endosperm (ginnan) inside the ginkgo seed and cultivate ginkgo; at the end of the 17th century, the physician and botanist Engelbert Kempel came to Nagasaki and introduced ginkgo to the West. From this time on, the ginkgo tree began to grow in Europe.

Older and larger ginkgo trees produce "aerial chi-chi," or downwards extending branches, from the branches near the trunk (**Fig. 1.1**). When the ginkgo tree is threatened by a leaning trunk, a lignotuber called a “basal chi-chi” is produced in the ground (**Fig. 1.2**). Tredici of the Arnold Botanical Garden at Harvard University has demonstrated the presence of embedded buds from freshly germinated seeds. He reasoned that vegetative regeneration by "basal chi-chi" would have played a major role not only in the longevity of *G. biloba*, but also in the survival of the species since the Cretaceous period. In addition, *G. biloba* has become revered by human beings, which has led to the survival of the species and the expansion of its distribution area around the world.

In the present age, when the importance of maintaining biodiversity and preserving the global environment is increasing, it is significant to study the *G. biloba*, which appeared on earth 200 million years ago, long before the appearance of present humans, as a reference for preserving other species, as a purely scientific research subject, as a research subject for medicinal ingredients and for other purposes. It is also significant to use it as a model plant that takes advantage of its characteristic of being a single species in one genus.

Fig. 1.1 Aerial "chi-chi" of the Hida Kokubunji temple in Takaya city, Gifu prefecture, Japan.
a) Bell tower gate and *Ginkgo* tree. b) Many aerial "chi-chis" near the trunk. c) Bulletin board
showing that the tree is designated national monument and a folk belief on lactation in Japan.

Fig. 1.2 Basal "chi-chi" surrounded by light green oval. It grew on the main root underground when the trunk was tilted.

Chapter 2. The "chi-chi" of Ginkgo biloba L. grows downward with horizontally curving tracheids having compression-wood-like features

The elongation of the chi-chi can be classified into two stages. Near the tip growing part of the chi-chi, multiple aggregates of tracheids curving in the horizontal plane and intersecting ray cells, i.e., swirls, have a parenchymatous tissue of latent bud in the center and form wood. This results in the growth of woody bulges, and the chi-chi grows downward.

Tracheids in the swirl have no S_3 layer in their cell wall structure and have H-units in the lignin chemical structure. However, the cell walls of the curved tracheids were not thickened, not rounded, and had no intercellular spaces; therefore, they have only a part of gymnosperm compression-wood like features. With time, the growing axes of tracheids and ray cells in chi-chi become similar to those in normal wood of the stem, and the lignin is composed only of G-units.

Chapter 3. Morphological characteristics of the "basal chi-chi" of Ginkgo biloba L. and the differences between the "aerial chi-chi" and the "basal chi-chi"

Del Tredici concluded, based on a study of native Ginkgo trees in Tian Mu Shan (TMS), that the formation of "basal chi-chi" in TMS is critical for ecological conservation of young trees in disturbed native habitats. The "basal chi-chi" is formed in the early stages of tree growth and is distinct from the "aerial chi-chi". The paper includes a photo of a "basal chi-chi" produced by a ginkgo sapling tilted at a 45-degree angle and discusses the process known as "basal chi-chi." When the axis of the seedling experiences a traumatic event, one of the cotyledonary shoots embedded within it grows normally downward from the trunk to form a woody, rhizome-like structure. This study explores the impact of vegetative regeneration by "basal chi-chi" on the long-term survival of Ginkgo biloba in Chinese forests, as well as its potential role in the genus' survival since the Cretaceous period (146-65 million years ago) [1, 2]. The study involved tilting Ginkgo seedlings at a 30-degree angle (Figure 1a, b) to create basal chichi structures in the ground (**Figure 1c**), which were later examined using optical microscopy.

Furthermore, L. Griffing states in "Plant Physiology and Development, Sixth Edition" by Sinauer Associates, Inc. (2015) that plants possess a robust internal structure to withstand their weight as they grow in the direction of light against gravity. This concept can alternatively be expressed as plants having the ability to react to light and gravity. The formation of basal chi-chi on the subterranean roots near the surface of the ground when the trunk is tilted may be due to a mechanism that senses the trunk's inclination towards gravity and transmits signals to the subterranean roots. The objective of this study was to investigate how basal chi-chi formation varies when the trunks are covered with black paper and aluminum foil in plastic bags and shaded from the ground surface up to 20cm, given the assumption that the trunks have the ability to detect light.

Experiments were conducted to induce basal chi-chi, and morphological characteristics of the obtained basal chi-chi were observed. The basal chi-chi occurred on the sides of the underground main roots when the trunk was tilted, but not when the trunk was tilted and shaded with aluminum foil. Recently, a mechanism for detecting the direction of gravity in cells has been reported. However, the stimulus response that triggers basal chi-chi formation may involve not only tilting but also being aboveground, i.e., light stimulation. When ginkgo plants were grown on an incline, the basal chi-chi had a root-like morphology in the soil against the direction of gravity, but did not have a root crown. This basal chi-chi itself does not have a root function, but it has been reported that both shoots and roots occur, suggesting that this may be some kind of response related to postural control. The lignin in the basal chi-chi did not contain H unit, suggesting that the lignin in the basal chi-chi was not the same with that in the aerial chi-chi. This suggests that some functional expression related to the formation of Ginkgo wood is affected under shading conditions, which may act on both the above-ground xylem and basal chi-chi.

Chapter 4. Conclusions

The elongation of the chi-chi can be classified into two stages. Near the tip growing part of the chi-chi, multiple aggregates of tracheids curving in the horizontal plane and intersecting ray cells, i.e., swirls, have a parenchymatous tissue of latent bud in the center and form wood. This results in the growth of woody bulges, and the chi-chi grows downward.

Tracheids in the swirl have no S_3 layer in their cell wall structure and have H-units in the lignin chemical structure. However, the cell walls of the curved tracheids were not thickened, not rounded, and had no intercellular spaces; therefore, they have only a part of gymnosperm compression-wood like features. With time, the growing axes of tracheids and ray cells in chi-chi become similar to those in normal wood of the stem, and the lignin is composed only of G-units.

Ginkgo trees grown on a slant were able to induce basal chi-chi, which do not function as roots and may express some function related to posture control. When *G. biloba* was shade-grown at a sloping angle, the shaded area did not form the basal chi-chi. Light may be one of the external stimuli necessary for the formation of the compression wood.