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Original Research Article

Microscopic Observation of Different Tissues from *Ginkgo biloba*

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ABSTRACT

In the present study, we observed the microscopic structure of root, stem, leaf, embryo, callus, anther and ovule from *Ginkgo biloba* using paraffin embedding method. The results showed that the root vascular tissue of ginkgo root was developed, and contained cambium and secondary structure. The primary xylem edges of ginkgo root had two xylem bundle. The cross section of ginkgo stem had 30 pith rays, which helpful for lateral transporting and storage of nutritional components. There was no obvious different in size and thickness between epidermal and hypodermal cells. The mesophyll consisted spongy parenchyma and palisade tissues. The structure of embryo included radicle, hypocotyl, germ and cotyledon, shaped as a torpedo. The microstructure of callus indicated that dedifferentiation degree of callus was relatively high. Our results of observation revealed that the number of pollen in anther was not great, while endothelial cells of anther were relative large. However, cells at the wall gaps were thinner and smaller than other place. Ovule integument was varied in thickness, and embryo has appeared in embryo sac. The observation of ginkgo microscopic structure in this study was consistent with the characterization of gymnosperm physiological development with developed sporophyte, degraded gametophytes and naked ovule.

Keywords: *Ginkgo biloba*; paraffin section; microscopic structure

INTRODUCTION

Ginkgo biloba L. is the one of oldest existing seed plants, also known as "living fossils." *G. biloba* originated from China, with a high economic, ecological, ornamental and scientific value, especially medicinal value attributed to flavonoids and lactones terpenes [1]. *G. biloba* has been paid more and more attention from worldwide researchers. At present, there were a large mount of reports on *G. biloba*, especially on structure and pharmacology of ginkgo flavonoids and terpene lactones [2]. In the past decade, the molecular mechanism of the biosynthesis of flavonoids and terpene lactones was intensively studied at genetic

level [3-5], while however, there were few literature related to observational studies of *G. biloba*. Therefore, this study observed and analyzed the microscopic structure of different tissues from *G. biloba* in order to explore its structural features.

MATERIALS AND METHODS

Experimental materials

15-year-old grafts of *G. biloba* were grown in a greenhouse at Yangtze University, China. The roots, stems, leaves, microspores and macrospores were collected from 15-year-old grafts of *G. biloba* in the sunny morning. The ginkgo embryo was isolated from one-year stratification seeds. The gingko callus was obtained by embryo tissue culture [6].

Methods

The microscopic observation of the cross section of root, stem, embryo, ovule and anther, longitudinal section of leaf and embryo, and callus was performed by paraffin embedding method [7]. Different sample was sectioned by handy microtome (Leica RM2235, German), and images were captured and analyzed by the micrography system (LEICA M165FC, German).

RESULTS

Microscopic structure of *G. biloba* root

As shown in Fig. 1A, the primary structure of root cross section was composed of three parts, epidermis cells, cortex and vascular cylinder. Among these, the epidermal cells were larger and closer packed than others. The cortex parenchyma cells were relatively large and neatly arranged with six laps. Endodermis cells of cortex were narrow and slender, and its shape was irregular. The vascular cylinder of primary xylem is consists with small oval cells, which had two xylem bundle. The cells of primary phloem were smaller than other tissues and most of which were the irregular rectangular.

Microscopic structure of *G. biloba* stem

In this study, we observed secondary structure of *G. biloba* stem through cross section. As shown in Fig. 1B, the diameter of the pith is about half of the diameter of stem cross section. The number of pith ray was 30. The above structure feature was helpful for lateral transporting and storage of nutritional components. The secondary xylem cells arranged closely and neatly into 8 lines. The cambium cells closely arranged in a ring. The secondary phloem cells are relatively large and loosely arranged, while the epithelial cells are relative small and closely arranged.

Microscopic structure of *G. biloba* leaf

From the cross section of *G. biloba* leaf, the epidermis and hypodermis were composed of monolayer cells, which shape in rectangular or square. There was no obvious different in size and thickness between epidermal and hypodermal cells. The mesophyll tissue differentiated into spongy parenchyma and palisade tissues. The palisade tissue was composed of one or two layer cells with relative large intercellular gap. Furthermore, chloroplasts were obviously seen in spongy parenchyma and palisade tissues and arranged loosely.

Anatomical observation of *G. biloba* embryo

In our present study, we observed the cross and longitudinal section of *G. biloba* embryo. As shown in Fig. 1E, the radicle, hypocotyl, cotyledon, and embryo were obviously seen in longitudinal section and shaped in torpedo. From the cross section of embryo, cells inside was relative small and arranged closely. The cells become larger and looser from inside to outside.

Anatomical observation of *G. biloba* callus

The section of *G. biloba* callus displayed that intercellular gap of callus was relatively loose and

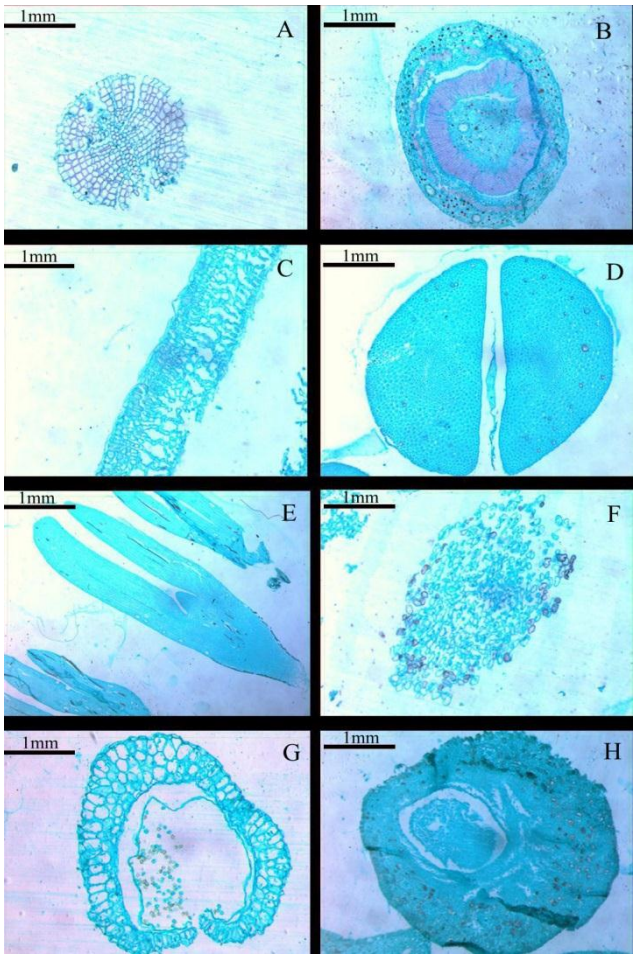

Fig. 1 Microscopic feature of different tissues of *Ginkgo biloba*

***A**, Cross section of root: **B**, Cross section of stem; **C**, Cross section of leaf; **D**, Cross section of embryo; **E**, Longitudinal section of embryo; **F**, Section of callus; **G**, Cross section of anther; **H**, Cross section of ovule*

the size of cells was consistent (Fig. 1F). Most cell shape is elliptic. The above results indicated that dedifferentiation degree of callus was relatively high and helpful for cell redifferentiation in the future.

Anatomical observation of *G. biloba* anther

The number of anther was about 60 and the cells were bigger (Fig. 1G). The cells at the wall gaps was thinner and smaller than other place. This structure was beneficial for Anther dehiscence. As shown in Fig. 1G, the tested anther in this study appeared to be dehiscent, indicating that the pollen grain has reached maturity status.

Anatomical observation of *G. biloba* ovule.

Because *G. biloba* is gymnosperms, its macrosporophyll was simplified as ovule collar and one or two ovules. Therefore, our present study selected *G. biloba* ovule as materials. As shown Fig. 1H, Ovule integument was varied in thickness, and embryo has appeared in embryo sac.

DISCUSSION

In this study, we preliminary understanded the microscopic structure of *G. biloba*. The results indicated that the root and stem of *G. biloba* had cambium and secondary structure, consistent with the reports from Baunch et al. [8], who demonstrated that the sporophyte was predominant, the vascular system was developed, the stele was reticular, and cambium and secondary appeared in gymnosperms.

Leaf tissue is sensitive and flexible during plant development and environmental change process. Under different stresses, leaf develops into different forms to adapt the environmental change. The anatomical structure of leaf can reflect the features of acclimatization [9,10]. We could find many vascular bundle from the cross section of leaf, indicating vascular system was developed in the leaf. This structure was helpful for leaf providing the water and inorganic and exporting photosynthetic product. Furthermore, vigorous secondary tissue in leaf was beneficial for supporting the leaf skeleton and providing the essential physiological function.

The pollen of *G. biloba* will spread by wind to ovule micropyle and germinate to form a pollen tube after maturation. The germ cells inside pollen tube divided into two sperm, and one of mature sperm fertilize a ootid to develop into the contact embryo possessing germ, radicle, hypocotyl, and cotyledon [11]. A part of original female gametophyte develops into endosperm. Endosperm and single-

layer integument develop into episperm to form a mature seed. Our study analyzed the microscopic structure of reproductive tissues, such as ovule, embryo, and anther. The results were similar to the findings of Wang et al. [12]. Based on previous work, this study carried out on the preliminary exploration of microscopic structure of reproductive tissues from *G. biloba* to provide the theory base for discovering the reproductive biological characteristics and phylogeny.

Our group have gotten embryo-derived callus of *G. biloba* through tissue culture. The aim of this research is to study the mechanism of growth, development and differentiation of *G. biloba*, as well as provide the technology accumulation for producing the medicinal ingredients using tissue culture. However, lack of perfect cell culture technology until now, the plant regeneration system of *G. biloba* is different, and we only got ginkgo callus after dedifferentiation [6]. This paper aims to analyze the effects of different culture condition on growth and development of callus at the microscopic level and provide the theory basis for screening *G. biloba* regeneration system through the microscopic observation of the callus structure.

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