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# Root Anatomical Structure of *Jatropha curcas* Seedlings—A Short Report

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## Abstract

*Jatropha curcas* has been expected as a biodiesel plant which can be grown in degraded lands. The structure of roots at the seedling stage, in particular cell wall modification in exodermis and endodermis, was microscopically observed. In addition, it was discussed if the first four peripheral roots that emerge from the base of the primary root (taproot) are lateral roots or adventitious roots. The primary root and the first-order lateral roots formed diarchy stele, in which two protoxylem poles present in primary xylem of root. Consequently, the first four peripheral roots cannot be lateral roots, but should be adventitious roots formed at the base of hypocotyl. In both the primary and first-order lateral roots, exodermis and endodermis formed highly lignified cell walls. Moreover, the exodermal and endodermal cell walls formed Casparian strips, which could be observed without special staining by fluorescent dye under ultraviolet microscopy. Such cell-wall modification in root exodermis and endodermis may play an important role for *J. curcas* under soil stresses in degraded lands.

## Keywords

Adventitious Root, Casparian Strip, *Jatropha curcas*, Root Endodermis, Root Exodermis

## 1. Introduction

*Jatropha curcas* is a tropical Euphorbiaceae shrub and its seeds contain rich fatty acids such as linoleic acid and oleic acid [1]. As *J. curcas* can be grown in degraded lands, it has been expected as a promising biodiesel plant grown with small competition against food production [2] [3] [4].

In degraded lands, soil stresses to plants such as soil drought, low soil fertility, inapposite soil pH, and soil salinity can be occurred. It is suggested that the roots

of *J. curcas* should play some roles for the high tolerance of this species to such soil stresses. In the roots of many of higher plants, the most outer and inner layers of root cortex, hypodermis (exodermis) and endodermis, respectively, represent apoplastic barriers that control the uptake and radial transport of water and solutes by the root. These cell layers also have several additional functions such as mechanically protecting the stele and protection against pathogens and parasites [5] [6]. Thus, it should be meaningful to clarify the feature of cell-wall modification of root hypodermis (exodermis) and endodermis in *J. curcas* plants.

Besides, Reubens *et al.* (2011) focused on the taproot and "four perpendicularly oriented laterals" (or "four main second order roots") and pointed out their potential to control of soil erosion [7]. In the very first stage of the root system development, the taproot emerges at the edge of seed, and soon four peripheral roots appears at the junction of hypocotyl and taproots (**Figure 1**). The taproot and four peripheral roots thereafter compose the framework of the *J. curcas* root system. However, morphological origin of the four peripheral roots (whether they are adventitious roots emerged from hypocotyl or lateral roots of taproot) is unclear.

In this study, the anatomical root structure of *J. curcas* seedling was observed under microscopy to demonstrate the hypodermis (exodermis) and endodermal cell-wall modification and identify the origin of the four peripheral roots.

## 2. Materials and Methods

Seeds of *J. curcas* (Cultivar QVP3014, Quinvita India Private Limited) were germinated in wet vermiculite kept 30˚C in a laboratory of School of Agriculture, Tokai University (Kumamoto, Japan).

After 4 - 6 days incubation, when the length of primary root (taproot) reached 1 - 4 cm, fresh hand sections of the basal part of hypocotyl, the primary root, the first four peripheral roots, and first-order lateral roots of the taproot were made with razor.

The sections were stained by 0.05% toluidine blue solution, and observed with light and ultraviolet (UV) fluorescent microscope (BX60, Olympus Corporation) equipped with a CCD color camera system (VB 7010, Keyence Corporation). Under UV fluorescent microscopy, the toluidine blue suppressed autofluorescence of cell wall cellulose in order to emphasize autofluorescence of cell wall lignin [8].


**Figure 1.** Hypocotyl, taproot and the first four peripheral roots at the germination stage of *Jatropha curcas*. (a) (b) After 2-day incubation in wet vermiculite at 30˚C. Root tips of the four peripheral roots emerged at the junction of hypocotyl and taproot. (c) After 3-day incubation in wet vermiculite at 30˚C.

## 3. Results

As shown in Figure 2, all of primary root, the first four peripheral roots, and first-order lateral roots formed diarch stele with two primary xylem strands. With UV illumination, autofluorescence was observed in exodermis, endodermis and xylems. Moreover, the Casparian strips (Casparian bands) in radial walls of exodermis and endodermis appeared clearly with autofluorescence (Figure 2(e), Figure 2(f)).

In the hypocotyl, there were four large longitudinal vascular bundles (Figure 3). The positions of the four peripheral roots were regularly related to the positions of the four large vascular bundles of the hypocotyl.

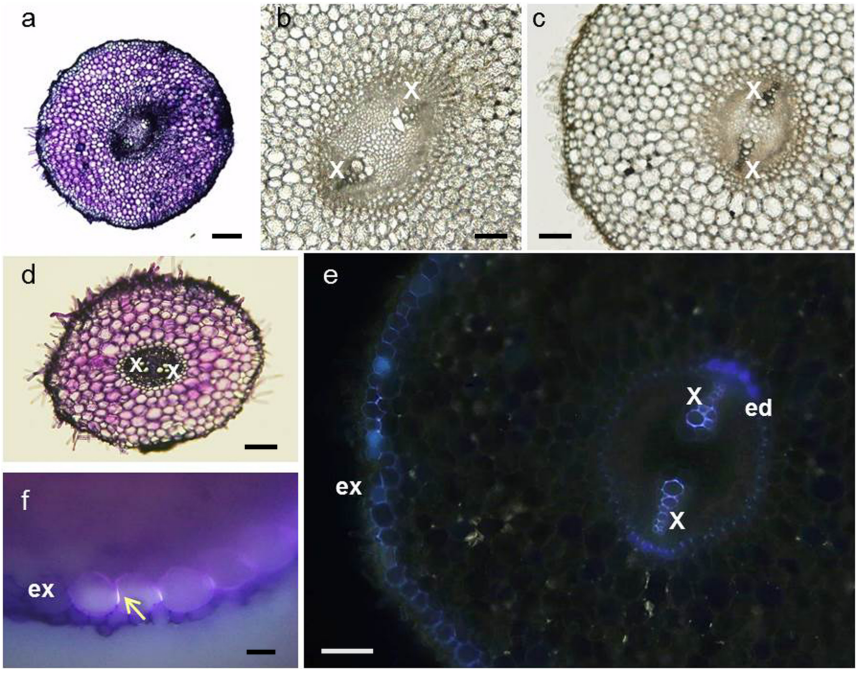


Figure 2. Cross sections of *Jatropha curcas* roots. (a) primary root (2 cm from the root tip), (b) magnified view of a (central part of cross section of primary root), (c) the first four peripheral root (2 cm from the root tip), (d) first-order lateral root (e) UV view of the first four peripheral root (2 cm from the root tip), (f) marginal UV view of first-order lateral root. **ed**: endodermis, **ex**: exodermis, **x**: primary xylem, **yellow arrow in f**: autofluorescence of Casparian strip; Bar in **a** = 200 μm, Bars in **b-e** = 100 μm, Bar in **f** = 20 μm.

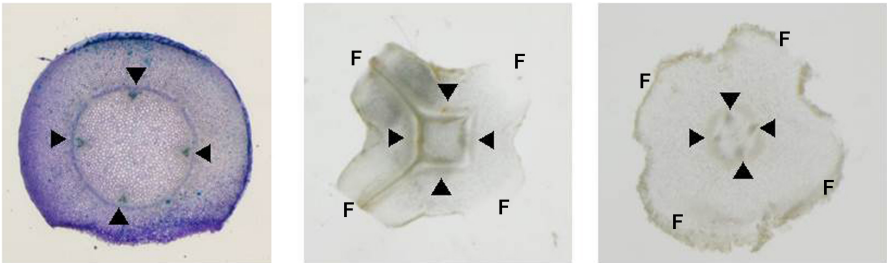


Figure 3. Cross sections of basal part of *Jatropha curcas* hypocotyl. (a)-(c) apposed in downward sequence. **black arrowheads:** large vascular bundles, **F:** base of the first four peripheral roots.

## 4. Discussion

The exodermis is defined as a special type of hypodermis that develops Casparian strips [9]. As well as the Casparian strips of endodermal cells, the Casparian strips in exodermis functions as the root apoplastic barrier to the water and solutes. The roots of *J. curcas*, either primary or first-order lateral roots, formed exodermis with Casparian strips. In many species of plants, Casparian strips can be observed with staining by fluorescent dye such as berberine and fluorol yellow that labels suberin deposition to cell walls [8]. An exception is rice root, in which Casparian strips can be visualized by autofluorescence without staining under UV illumination [10]. In the roots of *J. curcas*, as well as rice roots, the Casparian strips could clearly appeared by UV illumination without staining by fluorescent dye. It is suggested that the root exodermis and endodermis of *J. curcas* roots form highly modified secondary cell walls. It has been thought that the modification of cell walls in root exodermis and endodermis, such as lignification and Casparian strip formation is related with tolerance against soil drought, excessive water, heavy metals and so on [11] [12] [13]. The modification of cell walls of root exodermis and endodermis may be a key characteristic of *J. curcas* for its high tolerance to soil stresses. Khattab *et al.* (2005) reported the presence of Casparian strips in endodermis of *J. curcas* roots [14], but as for exodermis this is the first report as far as the author knows.

Although Reubens *et al.* (2011) called the first four peripheral roots as "four perpendicularly oriented laterals" or "four main second order roots" [7], they cannot be "lateral roots" of taproot. Khattab *et al.* (2005) also pointed out the possibility that the four peripheral roots are adventitious roots initiated in hypocotyl, but they called them as "four weak perpendicular lateral roots" [14]. "Lateral roots" are branching roots that are initiated in pericycle of the parent root, and the sites of initiation are closely related with the position of protoxylems of the parent root in dicot plants. As the primary root (taproot) was diarch root in *J. curcas*, there should be two lines of lateral roots formed—not four. Moreover, the emerging positions of the four peripheral roots were regularly related to the position of the four longitudinal large vascular bundles of hypocotyl. Thus, the four peripheral roots should be adventitious roots formed at the base of hypocotyl.

## 5. Conclusion

In the primary and first-order lateral roots of both taproot and four peripheral adventitious roots, exodermal and endodermal cells are highly lignified and clearly form Casparian strips in *J. curcas.* The contribution of such remarkable cell-wall modification in root exodermis and endodermis of *J. curcas* to the tolerance of this species to soil stresses should be investigated in further studies.

## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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