

Vascular bundles in *Manihot esculenta* Crantz (*Euphorbiaceae*)

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Abstract

This study describes the stem anatomy of *Manihot esculenta* Crantz and its hybrid with *M. oligantha* Pax. This is the first report of the presence of internal phloem in this genus. Cross-sections were made, fixed, conserved and colored by safranin-alcian blue. *M. esculenta* and its hybrid showed the same vascular structure in bicollateral bundles with internal phloem, which may be related to drought resistance and enables selection of individuals that are more adapted to arid regions.

Key words: bicollateral bundles, Euphorbiaceae, breeding improvement, internal phloem, *Manihot esculenta* Crantz, *Manihot oligantha* Pax.

Introduction

Presence of internal phloem in certain species of superior plants is related to physiological processes involved in the drought resistance. The organizational pattern of the bicollateral bundles, which is composed of internal and external phloem (Esau 1974; FloresVindas 1999; Ye 2002), has also been observed in Apocynaceae (Solereeder 1908), Convolvulaceae, Cucurbitaceae, Solanaceae and Asteraceae (Esau 1974; Mauseth 1988; Fahn 1990), and in Lythraceae (Mundo and Duarte 2007).

With regard to Euphorbiaceae, a report on collateral vascular traces in *Jatropha* was published by Miller and Webster (1962), who analysed basal regions of the petiole. Popham (1947) studied this genus by describing the *Jatropha cordata* anatomically and no mention was made of internal phloem, only referring to the differentiation of cell groups of primary phloem outside the procambium. However, Hayden and Hayden (1994) reported the occurrence of internal phloem in *Croton glandulosus* var. *septentrionalis*, a species belonging to the same subfamily of *Manihot*.

Metcalf and Chalk (1983) listed some genera of Euphorbiaceae that possess internal phloem; *Manihot* was absent of this list. Anatomical studies on the stem structure of this genus are rare with exception of Nassar *et al.* (2008b). On this view, this work aimed at clarifying the anatomical characterization of vascular bundles in *Manihot* and its further significance to the improvement of drought-resistant varieties.

Material and methods

Stem samples of *Manihot esculenta* Crantz (cassava cultivar) and its hybrid with *M. oligantha* Pax were collected from plants maintained in the living collection of the University of Brasilia Experimental Station (Brazil). The tetraploid type of the hybrid was also analyzed.

The stem samples were collected during primary (at the stem apex) and secondary growth (at the internodes of the apical portion) and then fixed with 70% FAA and conserved in 70% ethanol (Johansen 1940).

The transversal cross-sections were enumerated sequentially in the apex-base direction as follows: 1- cross-section near the apex; 2- cross-section up to 2 cm from the apex; 3- cross-section up to 5 cm from the apex; 4- cross-section of the middle portion of the second internode; 5 - cross-section of the median portion of the fifth internode (adapted from Hayden and Hayden 1994).

The free-hand cross-sections were clarified with 50% sodium hypochlorite solution (Kraus and Arduin 1997), stained with 1% safranin-alcian blue (Luque *et al.* 1996), dehydrated in ethanol series and butyl acetate, and subsequently mounted in a synthetic resin (Paiva *et al.* 2006). Photomicrographs were taken using a Zeiss Axioskop microscope and the images were captured with Motion Image Plus 2.0.

Results

The Region 1 shows the stem structure at the primary growth phase (Fig. 1A). Its epidermis is composed of common epidermal cells, stomata, and long unicellular tector trichomes. In the cortical region, one can note a three-stage differentiation: a) initially, 3-5 layers of irregular shape and slender cell walls, and laticifers, b) a segment of 4-6 layers of cells with slightly thickened walls followed by 1-3 layers of irregular cells limited by small intercellular spaces, and c) one last layer containing druses and crystals. Anticlinal and periclinal divisions could still be observed in such tissues.

Two regions could be seen in the tetraploid hybrid, with lesser number of layers and some druses (Fig. 3A). Stem cultivar differed at the advanced stages of differentiation by showing a greater number of layers, cell diameter, and presence of starch-rich cells in the last layer (Fig. 4A). The next region had 4-6 layers of small polygonal cells with slender walls and without intercellular spaces, but similar in content.

The vascular system consisted of external primary phloem, with the primary xylem spread in five bundles and followed by internal primary phloem. Between the primary phloem and the primary xylem, it was observed differentiation of procambial cells. The external primary phloem, which was in a cord distribution, showed approximated six layers of differentiating cells; in some places sieve elements, companion cells, parenchyma cells, and laticifers were observed. The external primary phloem was separated by small parenchyma cells (Fig. 1A).

The primary xylem had entered the phase of differentiation: the protoxylem was at advanced stage, exhibiting smaller elements and thickened walls (the farthest ones were collapsed); the metaxylem exhibited bigger elements, and the xylem was involved by parenchyma tissue consisting of small thin-walled cells with no apparent content. There were elements of primary phloem (sieve elements, companion cells, and laticifers) in front of the primary xylem spread into the parenchyma tissue.

With regard to the tetraploid hybrid, it was observed the same pattern, however the primary xylem exhibited thicker-walled cells and the internal phloem was at advanced stage presenting more cells (Fig. 3A). The cultivar differed from its diploid hybrid by the greater quantity of vascular tissue in the cell diameter and by the presence of druses (except in vascular tissue) (Fig. 4A).

The medulla showed polyhedral cells varying from 5-8 sides with no apparent cellular content (Fig. 1A). The cultivar, on the other hand, showed no intercellular space, cell divisions or druses (Fig. 4A).

In the Region 2, the same regions were observed in the external cortex (Fig. 1B), but at advanced stage of differentiation. There were parenchyma tissue and laticifers, angular collenchymas, parenchyma with starch-filled internal layer in all cells, which includes the starch sheath. No modifications were observed under these structures (Fig. 1D).

In the primary phloem, cell divisions were noted, mostly periclinal and some anticlinal ones. Sieve plates were also observed (Fig. 1C). Between the external primary phloem and primary xylem some cells were dividing, probably components of the fascicular cambium, characterizing the secondary growth. Secondary wall deposition was observed in primary xylem elements. Internal primary phloem was totally differentiated in the medullar region with polygonal-shaped cells. Internal primary phloem had no modifications (Fig. 1E).

Tetraploid hybrid showed different components of starch in the starch sheath and druses in medulla, revealing xylem and phloem with greater cell diameter. Collenchyma tissue, the external phloem and the starch sheath could be seen in development (Fig. 3B). Initial development of the fascicular cambium was also observed (Fig. 3C). In the cultivar, several tissues showed many divisions and initial development of the fascicular cambium (Figs. 4B and 4C).

In Region 3, the layers next to the vascular tissue showed secondary wall deposition, making them different from the fibers (Fig. 1F). In the external primary, phloem showed collapse of some cell types, mostly sieve elements and companion cells. Formation of both secondary phloem and secondary xylem was observed in the fascicular cambium. Cell divisions were noted in some regions, which were related to the inter-fascicular cambium formation. In the medulla, parenchyma also started to divide to form polygonal cells wider and narrower than the previous ones (Fig. 1F). The same events were noted in the tetraploid hybrid (Fig. 3D). Druses and starch grains were seen in cortex and medulla (Fig. 4C). In the cultivar, fascicular cambium was completely established only in this region (Fig. 4D).

The Region 4 shows cambium forming a complete cylinder and intense cell divisions (Fig. 2A). Laticifers of secondary origin were formed from these cells. It was observed intense dissolution of external primary phloem elements and addition of new peripheral parenchyma cells to medulla. The cultivar had an increased number of druses and starch grains in all tissues, except in the vascular one (Fig. 4E). The medullar region was in division (Fig. 4F).

In the Region 5, it was observed secondary growth with phellogen and active vascular cambium (Figs.2B). The stem showed, from outside to inside, the following tissues: epidermis (sometimes interrupted by periderm), variable number of phellogenic layers, sporadic lenticels, cortical parenchyma with idioblasts (druses and prismatic crystals) and isolated laticifers, and angular collenchyma with up to five cellular layers. One layer of parenchyma cells had crystals, druses and starch, forming a starch sheath. Pericyclic fibers were forming an interrupted cylinder with thickened wall. Dissolutions of the conductive cells occurred in the external primary phloem, remaining laticifers and parenchyma.

In the secondary phloem, with 12 layers, were observed sieve elements, companion cells and laticifers in rows of small clusters, including other cells (Fig. 2D). Phloem parenchyma rays were continuous with the secondary xylem. In the secondary xylem (Fig. 2C), it was observed vessel elements, xylematic fibers, tracheids, radial parenchyma, and vasicentric axial or scanty paratracheal parenchyma.

The vessel elements were solitary or clustered, often showing tyloses, and radial parenchyma cells were rectangular, distributed in rows (Fig. 2C). Xylematic fibers showed few lignified walls, wide lumen, and rectangular shape (Fig. 2E). In addition to these structures, it was observed in the secondary xylem tissue some layers that seemed to be secondary growth rings. Protoxylem and metaxylem elements were evident; moreover, phloematic elements were involved by parenchyma cells (Fig. 2B).

Medulla was distinguishable into two regions according to cellular shape, content, and number of layers. The first one, peripheral medulla, was near to the primary xylem and had isodiametric cells; the second one, central medulla, had polygonal cells with 5-6 faces. The cells are bigger in this region (Fig. 2B).

In the tetraploid hybrids (Fig. 3E), fibers had slender walls, phloematic tissue had greater cell diameter and more laticifers, and xylem had more solitary vessel elements, greater diameter, and more starch deposition in fibers and radial parenchyma (Fig. 3F). The cultivar (Fig. 4G) had fewer fiber layers and slender walls (Fig. 4H), greater number of laticifers

(Figs. 5A and 5B), which were articulated and branched when seen in longitudinal cross-sections, bigger cells in the vascular tissue, more number of layers of xylem (Fig. 4G), prevailing solitary elements with greater diameter and starch amount in all kinds of cells (Fig. 4H). In the medulla, cells were bigger and narrower with slender walls. These data confirm that size, quantity and thickening of vascular elements differed according to the ploidy level.

Discussion

Bicollateral bundles occur in stems of several Dicotyledons, and, in some cases, it is characteristic of whole families. The most known examples include the Cucurbitaceae and Solanaceae (Esau 1974). Metcalfe and Chalk (1983) reported nine genera of Euphorbiaceae with presence of internal phloem; however, *Manihot* was not cited.

Diploid and tetraploid hybrids as well as the cultivar were found to have internal phloem in bicollateral bundles. In this type of organization, xylem stays in parallel with both external and internal phloem.

The external phloem is arranged in a continuous ring and the internal one arranged in cords next to the medullar region. Internal phloem can be found either in a continuous ring or in isolated cords (Metcalfe 1967; Cronquist 1981), the latter being observed in *Manihot* in the present study.

Internal phloem is very efficient for conduction, and consequently, is of great advantage for desert plants, which are under shorter but more intense photosynthetic periods (Fahn 1990; Appezzato da Gloria 1993). However, *Manihot* is not a desert plant despite its native origin possibly related to water deficit, nutrient insufficiency, and light intensity. According to Esau (1974), plants developing under greater light intensity show increased xeromorphy compared to light-protected plants.

Studies on the possible physiological functions of internal phloem address a series of hypothesis; one of them establishes that the internal phloem may exhibit directional and temporal differences in translocation if compared to the external phloem of the same plant (Bonnemain 1969; Fredon and Bonnemain 1970; Couillault and Bonnemain 1973). Another explanation suggests that there are differences in the cellular composition of the internal and external phloem (Botha *et al.* 1975; Botha and Evert 1978). Hayden and Hayden (1994) affirm the apparent storage function represented by the internal phloem in *Croton glandulosus*.

All these hypotheses converge towards the same point; the internal phloem of the bicollateral bundles is efficiently located to favour transport from or to medulla. It is the most significant hypothesis for internal phloem function.

In the *Manihot*, the characterization of vascular system with presence of internal phloem becomes a relevant issue when its improvement is taken into account. Even widely distributed in South America (Nassar 1978; Nassar *et al.* 2008a), the genus has few cultivated drought-resistant varieties. The anatomical study is a tool used by breeders for detecting drought-resistant species that can be further used in both selection and improvement of the crop.

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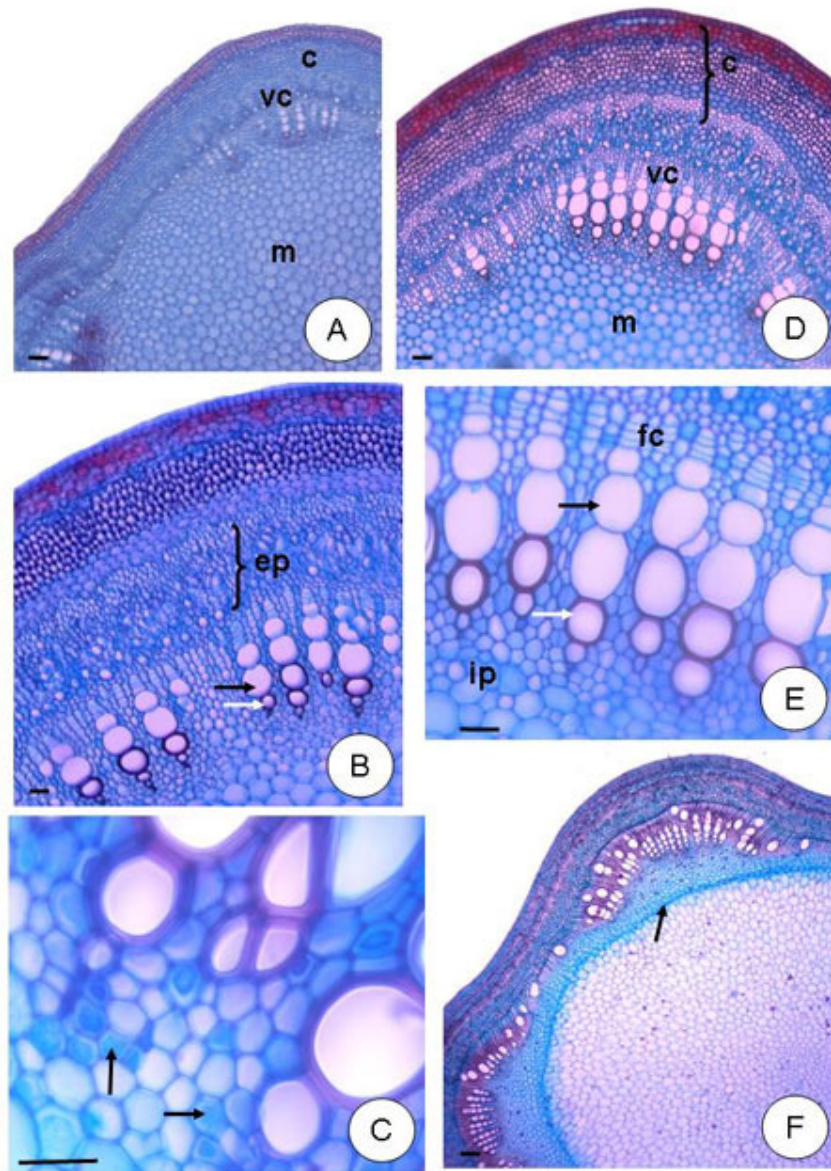


Figure 1. Transversal cross-sections of the diploid hybrid between *M. esculenta* x *M. oligantha*. **A.** Region 1 in general view with primary structures; cortical region (c), vascular cylinder (vc) and medulla (m). **B.** Region 2. Cortex with three distinguishable parts; vascular tissue in formation, external phloem (}), protoxylem (white arrow), metaxylem (black arrow) and internal phloem. **C.** Sieve plates (black arrow) in the sieve elements of the internal primary phloem. **D.** Region 2. Cortical tissues under differentiation (}): parenchyma with antocianin, angular collenchyma, parenchyma and starch sheath; beginning of the secondary growth with vascular cambium. **E.** Fascicular cambium, protoxylem (white arrow), metaxylem (black arrow) and internal primary phloem. **F.** Region 3. Fascicular cambium in activity with formation of secondary xylem and phloem, beginning of the interfascicular cambium, medulla cellular divisions (black arrow). Bar = 100 μ m.

Figure 2. Transversal and longitudinal cross-sections of the diploid hybrid between *M. esculenta* x *M. oligantha*. **A.** Region 4,

establishment of vascular cambium (vc) in intense divisions and new cells added in medulla (m). **B.** Region 5 with accentuated secondary growth: secondary phloem (sp) and secondary xylem (sx). **C.** Secondary xylem in detail: vessel elements solitary (ve) or clustered (*). **D.** Longitudinal cross-section showing laticifers (black arrow), sieve elements and companion cells (white arrow). pr: radial parenchyma, se: sieve element. **E.** Secondary xylem with parenchyma cells and fibers (fb) with slender walls. Bar = 100 μm .

Figure 3. Transversal cross-sections of the tetraploid hybrid between *M. esculenta* x *M. oligantha*. **A.** Region 1. Beginning of the primary structure, only two distinct parts are noted; great amount of elements and thickened walls in the vascular tissue (vc), divisions in medulla (m). **B.** Region 2. Formation of collenchyma cells (cc), and starch sheath (ss). **C.** Region 2. Development of extern phloem (ep) and primary xylem, by the presence of protoxylem (white arrow) and metaxylem (black arrow). **D.** Region 3. Secondary growth in detail, formation of secondary xylem and phloem from the vascular cambium (vc); medullar parenchyma (mp) in division to form narrower and elongated polygonal cells. **E.** Region 5 with accentuated secondary growth, great amount of vascular tissue, as bigger diameter of conductive elements and lesser quantity of tyloses than the diploid type. **F.** Secondary xylem in detail: vessel elements solitary (ve) or clustered (*). Bar = 100 μm .

Figure 4. Transversal cross-sections of *M. esculenta* cultivar. **A.** Region 1 shows more layers, bigger cells diameter and more cell differentiation. **B.** Region 2. Secondary growth is slower, noted by lesser number of cells in the fascicular cambium (fc). **C.** The great amount of druses (black arrow) and starch grains in the cortical and medullar regions. **D.** Region 3. Interfascicular cells (ic) and phloem (ph) in differentiation. **E.** Region 4. Vascular cambium (vc) in intense activity, formation of laticifers from these cells. **F.** Region 4. Medullar region with cells in division (m). **G.** Region 5 with accentuated secondary growth: secondary phloem (sp) and secondary xylem (sx). **H.** Secondary xylem in detail: vessel elements solitary (ve) or clustered (*). Bar = 100 μm .

Fig. 5. Longitudinal cross-sections of *M. esculenta* cultivar. **A.** Phloem parenchyma rays continuous with the secondary xylem. **B.** Sieve plates in sieve elements. Laticifers (black arrow), sieve elements (se) and companion cells (*). Bar = 100 μm .