Development of Tuberous Cassava Roots under Different Tillage Systems: Descriptive Anatomy

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Abstract: The interaction between the roots of cassava (Manihot esculenta Crantz) and soil physical properties has previously been analyzed. This interaction results in differences in production of plant material and in the physicochemical features of the roots, suggesting that changes in soil physical conditions may be related to changes in root anatomy. This work described the anatomical development of the tuberous cassava roots (cv. IAC 576-70) under different tillage systems. Roots grown under three different tillage systems (minimum, conventional and no tillage) were examined at 15, 30, 60, 90, 120, 150 and 180 days after planting (DAP). The tillage systems did not appear to influence root anatomy during root development; at 15 and 30 DAP roots had early secondary growth; at 60 DAP the process of tuber formation had started; at 90 DAP the secondary xylem had completely differentiated to allow storage of starch; at 120, 150 and 180 DAP roots exhibited a similar anatomical structure to that observed at 90 DAP. From these results we conclude that the anatomical structure of cassava tuberous roots is established by 90 DAP and the sequence of establishment and development of tissues that make up the tuberous roots is not influenced by tillage systems during the first 180 DAP.

Key words: Cell, Manihot esculenta Crantz, Root anatomy, Soil physical properties.

The root system of the cassava plant, which is typically propagated through stem cuttings, arises adventitiously and consists of fibrous and tuberous roots (Moraes-Dallaqua and Coral, 2002). Moraes-Dallaqua classifies this type of root system as an adventitious fibro-tuberous (personal communication).

According to Viegas (1976) these adventitious roots arise from the basal cut surface of the stem or from buds in the cuttings. Lowe et al. (1982) mentioned the roots those arise from the basal cut surface generally differentiate into tuberous roots and are called as basal roots, and those that arise from buds present at internodes are called as nodal roots.

The differentiation of a fibrous root into a tuberous root designed to store carbohydrates is the result of a process called tuberization, which is morphologically characterized by a growth in thickness of the root. Anatomically, this thickening is called secondary growth and results from the activity of two secondary meristems, phellogen and vascular cambium (Williams, 1974; Indira and Kurian, 1977; Lowe et al., 1982; Moraes-Dallaqua and Coral, 2002; Wheatley et al., 2003).

The tuberous roots are the main part of the cassava plant possessing economic value; the interaction between roots and physicochemical characteristics of the soil results in differences in production (Oliveira et al., 2001; Figueiredo, 2012) and in characteristics related to cooking time and hardness (Maieves et al., 2011).

The physical properties of the soil can be managed through the tillage system used, affecting mainly soil bulk density, total porosity and soil strength (Oliveira et al. 2001; Fasinmirin and Reichert, 2011). The effects of soil strength and bulk density have been observed in studies of the root anatomy of corn, soybean and certain cereals (Baligar et al., 1975; Bergamim et al., 2010; Lipiec et al., 2012).

According to Silva et al. (2005) this effect of soil on root anatomy is best explained by the dynamics that exist between the plant body and the management practices used in agriculture, reflecting the production and physicochemical characteristics of crop plants. In this sense we considered the hypothesis that the differences in production and physicochemical characteristics on the
cassava tuberous roots may be associated with the interaction of the soil physical characteristics and the anatomy of these roots.

Therefore, this work describes the anatomical development of cassava tuberous roots, cultivar IAC 576-70, under different tillage systems and discuss about anatomical concepts and terms currently used in the literature to describe cassava tuberous root.

Materials and Methods

The experiment was conducted at the Faculty of Agricultural Sciences of UNESP, Botucatu, São Paulo, Brazil (22° 49′ 31″ S, 48° 25′ 37″ W), at an elevation of 770 m, slope of 3%, under field conditions in an Alfisol soil (soil structured loam). The climate is classified as Cfa with warm temperate (mesothermal) and wet conditions (Köppen, 1948), and based on the classification of Thornthwaite it is B2rB’3a, that is a humid climate, with little water deficit in April, July and August (Cunha and Martins, 2009).

The experiment was composed of three treatments (types of tillage systems) and six replications of each three treatments for a total of 18 test stems: minimum tillage (two passes with a rotary hoe), conventional tillage (one pass with disc plow and two with light harrowing) and no tillage. The treatments were made in 144 m² plots, with 0.9 m between rows and 1 m between plants, or a plant density of 11,111 plants ha⁻¹.

After treatments (different types of tillage) cuttings from the middle third of 12 months old cassava plants (cv. IAC 576-70) were planted. After planting, six roots per treatment (one root per plant per plot) were sampled at 15, 30, 60, 90, 120, 150 and 180 days after planting (DAP). The samples were taken from the middle region of the roots raised on the base of the cuttings.

After collection, the roots were brushed and washed with water to remove any loose soil. Then the samples were fixed in FAA 50 (10% formalin, 50% ethanol, 5% acetic acid) and subsequently, the air in the tissue was evacuated with water to remove any loose soil. Then the samples were stored until use (Johansen, 1940). After fixation in 70% ethanol, root fragments were dehydrated in ethanol series (Johansen, 1940) and embedded in Historesin (Leica®, Germany) (Gerrits, 1964).

After being embedded in resin, 8 μm-thick sections were cut on a microtome (Leica RM 2025; Leica®, Germany). The sections were stained with 0.05% toluidine blue at pH 4.7 (O’Brien et al., 1964) and mounted on permanent slides with Entellan (Merck Millipore®, Germany).

In addition, histochemical tests and maceration techniques were used. The histochemical test was performed to detect lignin, using phloroglucinol (Sass, 1951) from the material stored in 70% ethanol sampled at 120, 150 and 180 DAP. The maceration technique was performed on fresh material sampled at 180 DAP, using hydrogen peroxide and acetic acid (Franklin, 1945) for three-dimensional observation of the tuberous root cells.

The samples subjected to maceration were divided to represent different stages of root development as described in Cereda et al. (2002). Samples used for histochemical tests and maceration were mounted on slides. These slides, as well as permanent ones, were studied using a light microscope (Zeiss®, Inc., NY, USA) and the images were taken by a digital camera (Olympus®, Japan), connected to a microscope (Zeiss®, Inc., Thornwood, NY, USA).

Results

No anatomical differences were observed between cassava roots grown under different tillage systems at all stages of development. At 15 DAP only fibrous roots were observed and these had no thickening. Anatomically, these roots had already initiated secondary growth, marked by the formation of the vascular cambium, with a continuous and irregular shape between the primary phloem and primary xylem (Fig. 1).

At this time, cross sections had a one-cell layer of epidermis covered with cuticle layers and immediately below were parenchyma cells making up the cortex, whose innermost layer is the endoderm (Fig. 1a and 1b). Internally the endoderm contains the vascular cylinder with six poles of protoxylem differentiated from the outside with alternating groups of primary phloem; the center of the vascular cylinder is filled by metaxylem cells.

At 30 DAP the tuberization process could still not be observed in the roots. At this stage cross sections show the establishment of secondary growth marked by the collapse of the epidermis along the circumference of the root as well as compression of cortex layers at the end of the body caused by the formation and activity of the vascular cambium and phellogen (Fig. 2a).

The activity of the underlying phellogen can be observed in the cells with suberin deposited in cell walls in the region just below the epidermis and cortex; the activity of the vascular cambium is shown by the production of secondary xylem and phloem (Fig. 2a). The secondary xylem produced in this stage, 30 DAP, is composed mainly of vessel elements, fibers and few parenchyma cells (Fig. 3); these cells along with those formed at the beginning of primary growth are compressed at the center of the root constituting the fibrous central cord (Fig. 4b).

At 60 DAP some roots were observed with thickening in diameter, marking, morphologically, the tuberization process. Anatomically, cross sectional observation (Fig. 2) revealed this thickening results from the establishment of the periderm, which is formed by suber, phellogen and phellogen, secondary phloem, and more intense activity of the vascular cambium in the production of secondary xylem with a higher proportion of parenchyma cells. At this stage, the periderm is the new dermal tissue of roots.
and secondary xylem produced is composed mainly of parenchyma cells with scattered vessel elements (Fig. 2b).

At 90 DAP all basal roots had already formed tuberous roots with cross sections showing the typical structure of a storage root, with secondary xylem composed of abundant parenchyma cells and vessel elements dispersed throughout the root (Fig. 2c), and with the periderm easily detachable from the secondary xylem, in the region near the vascular cambium.

At 120 DAP roots presented the same anatomical structure of roots at 90 DAP. The vessel elements present in the secondary xylem have a rounded contour, punctuated cell walls with simple perforation plates and oblique ends (Fig. 3c), which are aligned in axial and radial rays (Fig. 4b and 4d).

The outer layer composed mainly of suber is easily detachable of the phellogen, which lies immediately below a narrow layer of phelloderm. Below the phelloderm the secondary phloem composed of parenchyma cells could be observed along with sieve tube elements and fibers (Fig. 4).

At 150 and 180 DAP the cross sections showed a structure similar to roots observed at 90 and 120 DAP. However, a greater amount of secondary xylem was

Fig. 1. Cross sections of cassava root at 15 DAP. a: General aspect of the root in early secondary growth (scale bar: 100 μm). b: Details of the endoderm (scale bar: 100 μm). c: Vascular cambium (scale bar: 50 μm). Ep, epidermis; Co, cortex; Pt, protoxylem; Mx, metaxylem; En, endoderm; Vc, vascular cambium.

Fig. 2. Cross sections of the middle third of cassava tuberous root at a: 30, b: 60 and c: 90 DAP (arrows shows the vessel elements). Scale bar of a, b, c: 500 μm. Ep, epidermis; Co, cortex; Pl, phellogen; Px, primary xylem; Sp, secondary phloem; Vc, vascular cambium; Sx, secondary xylem; Cc, central cord.

Fig. 3. Cells of the secondary xylem of a cassava tuberous root at 180 DAP (scale bar: 100 μm). a: Parenchymal cells. b: Fiber present only in the central cord. c: Vessel elements present in all regions of secondary xylem.

Fig. 4. Cross (a, b), longitudinal (c) and radial (d) sections of the middle third of a cassava tuberous root at 120 DAP, showing the lignification of cell wall as indicated by the reaction of phloroglucinol (scale bar: 150 μm). a: The bark. b: The central cord showing vessel elements and fibers. c: Secondary xylem with vessel elements. d: Details of vessel elements. Su, suber; Pl, phellogen; Pd, phelloderm; Sp, secondary phloem.
observed at these later stages with a large amount of parenchyma cells providing reserve storage, which was morphologically realized through the larger diameter of the tubers.

**Discussion**

The anatomical sections described here, based on the scheme proposed by Viégas (1976), were at different physiological developmental stages. At 15 DAP plants were emerging and sprouting. At 30 DAP the root system was beginning to develop and form. At 90 and 120 DAP shoots were developing and at 150 and 180 DAP rapid carbohydrate translocation to the roots was occurring.

During all these stages, roots grown under different tillage exhibited no qualitative anatomical differences. At the end of this study, the physical characteristics of the soil were: 46.7, 47.6 and 47.7% total porosity, 28.5, 28.0 and 28.4% moisture content and 2.02, 1.52 and 2.73 MPa penetration resistance; to minimum, conventional and no tillage, respectively [More details of methodologies utilized to check the soil physical conditions can be found in Figueiredo (2014)].

Besides the tillage promoted statistical differences in soil physical characteristics, those differences were not enough to promote anatomical changes in tuberous roots. It is important to highlight that we cannot say the values above of those, observed in Figueiredo (2012), will not bring about different anatomical responses in tuberous roots.

The tuberous root has a secondary growth, where the secondary xylem is differentiated to storage starch and the cortical cells disappear from 60 DAP. In this sense, it is possible to say that secondary xylem is less sensitive to physical characteristics of the soil when compared to roots with primary growth whose cortical cells are prevalent, as concluded by Baligar et al. (1975), Bergamim et al. (2010) and Lipiec et al. (2012).

The results obtained in this study corroborate those obtained by Indira and Kurian (1977), Lowe et al. (1982), Moraes-Dallagu and Coral (2002) and Wheatley et al. (2003) and contribute to the elucidation of the anatomical process of differentiation of fibrous roots into storage roots as well as to the unification of terminology used to describe the botanical aspects of cassava roots.

These results demonstrate that the process of tuberization in cassava roots occurs in a manner similar to carrot (Daucus carota), which according to Esau (1940) vascular cambium is formed between the xylem and phloem, and the storage region consists of secondary vascular tissue with a large proportion of parenchyma cells.

According to Silva et al. (2005) the use of consistent and accurate botanical terms contributes to the advancement of research in a particular plant since each morphological part of a plant responds to a particular way to environmental conditions. Thus the term cortex (Fogaça et al., 2010) should not be used to describe the inner bark of tuberous cassava roots, since, according to Esau (1974), tuberous roots are a result of secondary growth and the cortex is a tissue present only in organs in primary growth or at the beginning of the secondary growth (Fig. 1 and 2a).

Another term commonly used to explain the uneven cooking and chemical characterization of tapioca pulp is amount of “fiber” in the root. According to Apezzato-da-Glória and Carmello-Guerreiro (2006), in anatomical sense fiber is a long sclerenchymatic cell type with lignified and more or less thick secondary walls; fiber usually provides a support function in plant parts that no longer stretch and is also found in the forms of strands or bundles in various parts of the plant body and in the primary vascular tissues in xylem and phloem of eudicotyledonous plants (Fig. 3b and 4b).

Thus the “fibrous” portion of cassava tuberous roots is composed of cell wall substances (Raupp et al., 1999), which include cellulose, hemicellulose, pectin and lignin (Pourchet-Campos, 1990). The results of this study demonstrate that anatomical fiber is present only in the central cord of the roots, meaning that the composition and quantity of this “fiber” varies based on the morphological age of the plant and environmental conditions (Pereira and Beléia, 2004).

**Conclusions**

The anatomical structure of tuberous cassava roots is well established by 90 DAP. The sequence of development and establishment of the various tissues that form these tuberous roots are not influenced by tillage for up to 180 DAP.

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