

Short Communication

Starch accumulation in cassava roots: Spatial and temporal distribution

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The relationship between starch and morpho-physiology aspects of cassava plant has not been identified. Considering this aspects, the present study showed the temporal and spatial distribution of starch grains in storage roots of cassava, variety IAC 576-70 with 90 and 120 days after planting. From these evaluations, a concentration of starch around the secondary xylem vessels of the cassava tuberous roots was observed, which may be related to the movement of organic solutes in the xylem vessels, water flow and the occurrence of the symptoms of the physiological deterioration in these roots.

Key words: *Manihot esculenta* Crantz., anatomy, histochemical test.

INTRODUCTION

Cassava is a plant of the Euphorbiaceae family and it is the only cultivated specie of the *Manihot* genus (Carvalho and Guerra, 2002). Although, it is commercially grown for one or two years, aiming the production of tuberous roots, botanically, the cassava plant is perennial, alternating periods of vegetative growth and dormancy (El-Sharkawy, 2006; Alves, 2002).

According to Viégas (1976) and Alves (2002) the growth of cassava plant during the first cycle have five distinct developmental phases as: emergency of sprouting, shoot development, development of stems and leaves, high carbohydrate translocations to root and dormancy.

During cassava growth, the leaf provides carbohydrate which is translocated to the tuberous roots by the phloem vessels, however this pattern and the transport pathway

of the organic sap may be inverted during the new vegetative growth as occurs with some perennial species, like grapevine and apple (Loescher et al., 1990; Zapata et al., 2004).

The cassava plant has two types of roots: fibrous and tuberous. The fibrous root has the function of fixing and absorbing nutrients and water, while the tuberous one, stores carbohydrate in starch form (Viégas, 1976). The tuberous root develop to a maximum of 0.30 m depth soil, and their ability to absorb nutrients and water decrease considerably (Souza, 2006; Alves, 2002).

Dias and Leonel (2006) reported that the starch content in the dry matter of cassava roots varies from values of 81.02 to 91.56%, however, this content of starch limits cassava's economic and industrial potential since it's biochemical reactions undergo rapid postharvest

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physiological deterioration, resulting in a vascular streaking, and the occlusion of the xylem vessels (Wenham, 1995; Hirose, 1986).

While all this results, the relationship between starch and morpho-physiology aspects of cassava plant has not been identified. In this study, we examine the temporal and spatial sequence of starch grains accumulation in storage roots of cassava, variety IAC 576-70 at 90 and 120 days after planting.

MATERIALS AND METHODS

The cassava plants were grown under field conditions, at the Universidade Estadual Paulista UNESP – Botucatu – São Paulo – Brazil (22° 49' 31" S Gr and 48° 25' 37" W) in Alfisol soil type (soil structured loam) using conventional tillage.

At 90 and 120 days after planting (DAP), basal tuberous roots (Lowe et al., 1982), from the middle third portion, which, anatomically, represents the region of cell maturation (Apezatto-da-Gloria and Carmello-Guerreiro, 2003), were collected from six cassava plants.

After collection, the roots were brushed and washed with water for disposal of land. Then the samples were fixed in FAA 50 (50% ethanol: 10% formalin: 5% acetic acid), and the air in the tissue was evacuated using a vacuum pump and subsequently, the samples were stored in ethanol 70% (Johansen, 1940). After the fixation, root fragments were dehydrated in ethyl series (Johansen, 1940) and embedded in Leica® Histo-resin (Gerrits, 1964). Transverse sections were cut on a Leica® microtome, with 12 µm thickness.

The histochemical detection of starch was done using lugol solution in order to stain the starch grains (Johansen, 1940). The starch localization was verified thru a light microscope, Zeiss® optical microscope and the images were taken by the microscope camera, Zeiss® optical microscope.

RESULTS AND DISCUSSION

The pulp of the cassava tuberous root results of the adaptation of the secondary xylem to store starch grains. The secondary xylem is formed from meristematic tissue, called vascular cambium, which also give rise to the secondary phloem (Indira and Kurian, 1977; Lowe et al., 1982; Moraes-Dallaqua and Coral, 2002; Wheatley et al., 2003).

A typical secondary xylem tissue is composed of parenchymal cells, vessel elements and fiber (Apezatto-da-Gloria and Carmello-Guerreiro, 2003). In the cassava tuberous root, this tissue is composed predominantly of parenchymal cells that are un lignified, which store the starch grains, few vessel elements and fibers only in the region of the central fiber strand (Figueiredo, 2012).

According to Viégas (1976) and Alves (2002) within 90 and 120 DAP cassava plants are on shoot development stage, tuberous root within 90 DAP presents the amount of starch, in parenchymal cells, smaller than the proportion of cells already differentiated from the vascular cambium (Figure 1A), this result is verified by the little evidence of starch using the reaction of lugol in the region of secondary xylem near the vascular cambium

(Figure 1A). It occurs due to the high energy demand for the shoot establishment, and the low but positive rate of net photosynthesis (Luo and Huang, 2011).

At 120 DAP, the parenchyma cells of the secondary xylem, near the vascular cambium, are already plenty of starch grains (Figures 1B and 1D), indicating the simultaneity between the differentiation of these cells from the vascular cambium, and the filling of them with starch grains, which is due to the positive net photosynthesis (EL-Sharkawy et al., 1990). It is also observed that, there is a concentration of starch grains around the xylem vessels of the secondary xylem (Figure 1C).

The transport of organic solutes by the root xylem vessels has been confirmed in perennial plants that present a period of dormancy and consequently a rapid seasonal grow, like grapevine, *Vitis vinifera* L. and woody plants like apple, *Malus domestica* Borkh, sweet cherry *Prunus avium* 'Bing' on 'Mazzard' and sugar maple *Acer saccharum* (Loescher et al., 1990; Zapata et al., 2004).

Considering that the cassava plant, as a perennial plant with dormancy period and that the xylem vessels compared with phloem vessels are spatially favored, it is possible that, the concentration of starch around the xylem vessel (Figure 1C) occurs due to the need of being transported in the form of soluble sugars to other parts of the plant.

The concentration of starch grains around the xylem vessels can be either related to the flux water in these roots, according to Larcher (2006) macromolecules as starch do not alter the osmotic potential of the water at the same intensity of small molecules as the soluble sugars, that is because the hydrolysis of macromolecules rapidly alters the osmotic potential, controlling water efflux and influx to the cell.

The control of water flow in the tuberous root is indeed important since their ability to absorb water and nutrients is considerably reduced (Alves, 2002) and why the dormancy period normally occurs under low temperature and water stress conditions (EL-Sharkawy, 2006), in this, sense the water contained in the vessels is necessary for hydration of the cells and tissues of the tuberous roots, since, according to Souza (2006), these roots develop up to 0.30 m depth, and that, during water stress, only the fibrous roots in deeper layers of the soil can absorb water.

Other hypothesis involving the concentration of starch grains around the xylem vessels may be related with the rapid symptoms noted near the vascular vessels during the physiological deterioration. Hirose (1986) identified a blue-black pigmentation within the xylem vessels in roots with physiological deterioration symptoms. This symptoms results, among other factors (Zidenga et al., 2012), from the rate increasing of cellular respiration, resulting in the degradation of starch into soluble sugars (Booth, 1976). This hypothesis presents the same physiological principle about the source-sink pattern and transport pathway of the organic solutes reversal during a greater energy

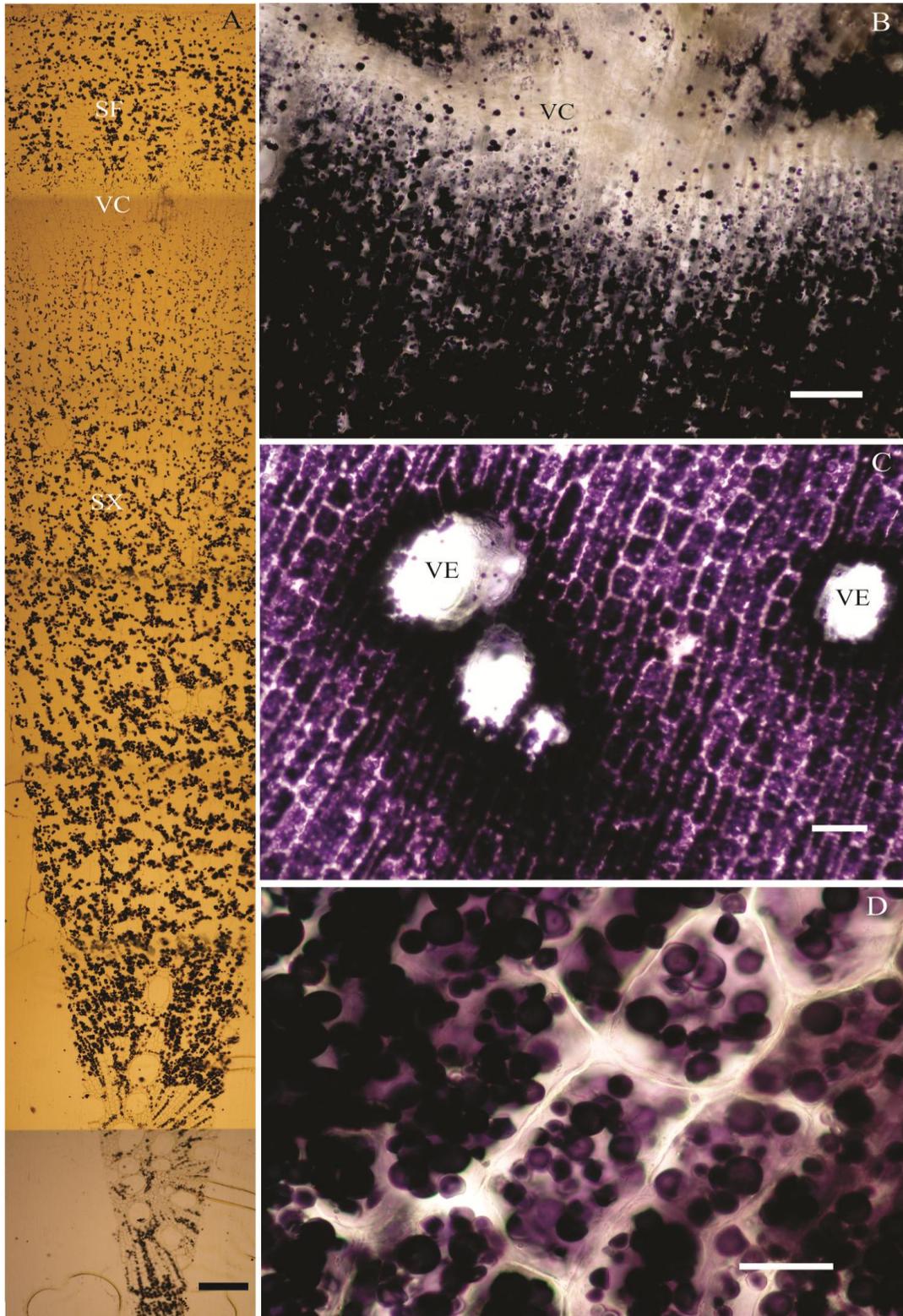


Figure 1. Cross sections of the middle region of *M. esculenta* Crantz cv. IAC 576-70 at 90 (A) and 120 (B, C, D) DAP, showing starch grains by the lugol reaction; A: Note starch grains in the secondary phloem (SF) and xylem (SX), note the filling process of the parenchymal cells of the secondary xylem near the vascular cambium (VC), scale bar = 500 μ m; B: Region near the vascular cambium (VC), where the parenchyma cells are filled with starch grains, scale bar = 150 μ m; C, D- Detail of B; C: Detail of the concentration of starch grains around the vessel elements (VE), scale bar = 150 μ m; D: Detail of the parenchyma cell of the secondary xylem filled with starch grains, scale bar = 50 μ m.

demand (Loescher et al., 1990; Zapata et al., 2004).

These results raise several hypotheses about the starch in cassava roots as the movement of organic solutes in the xylem vessels, water flow and the occurrence of the symptoms of the physiological deterioration in cassava roots, suggesting topics for research.

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REFERENCES

- Alves AAC (2002). Cassava: botany and physiology. In: Hillocks et al. (eds) cassava: biology, production and utilization, CABI, Wallingford, pp. 67-89.
- Appezato-da-Glória B, Carmello-Guerreiro SM (2003). Anatomia Vegetal, Editora UFV, Viçosa, P. 438.
- Booth RH (1976). Storage of fresh cassava (*Manihot esculenta*). I. Post-harvest deterioration and its control. *Exp. Agric.* 12(2):103-111.
- Carvalho R, Guerra M (2002). Cytogenetics of *Manihot esculenta* Crantz (cassava) and eight related species. *Hereditas* 136(2):159-168.
- Dias LT, Leonel M (2006). Caracterização físico-química de farinhas de mandioca de diferentes localidades do Brasil. *Ciênc. agrotec.* 30(4):692-700.
- EL-Sharkawy MA (2006). International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. *Photosynthetica* 44(4):481-512.
- EL-Sharkawy MA, Cock JH, Lynam JK, Hernandez ADP, Cadavid LLF (1990). Relationship between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. *Field Crop Res.* 34:183-201.
- Figueiredo PG (2012). Morfo-anatomia de raízes tuberosas de mandioca (*Manihot esculenta* Crantz) cultivar IAC 576-70 em diferentes preparos do solo 86 f. Master thesis. Universidade Estadual Paulista, São Paulo, Brasil.
- Gerrits PO (1964). The application of glycol metacrylate in histotechnology: some fundamental principles, Leica GmbH, Groningen, P. 80.
- Hirose G (1986). Physiological studies on postharvest deterioration of cassava roots. *Jpn. Agric. Res. Quart.* 19(4):241-252.
- Indira P, Kurian TA (1977). Study on the comparative anatomical changes undergoing tuberization in roots of cassava and sweet potato. *J. Root Crop.* 3:29-32.
- Johansen D (1940). *Plant microtechnique*, McGraw-Hill, New York. P. 523.
- Luo X, Huang Q (2011). Relationships between leaf and stem soluble sugar content and tuberous root starch accumulation in cassava. *J. Agric. Sci.* 3(2):64-72.
- Larcher W (2006). *Ecofisiologia vegetal*. Rima, São Carlos, P. 532.
- Loescher WH, Mccamant T, Keller JD (1990). Carbohydrate reserves, translocation, and storage in woody plant roots. *HortScience* 25(3):274-281.
- Lowe SB, Mahon JD, Hunt LA (1982). Early development of cassava. *Can. J. Bot.* 60(12):3040-3048.
- Moraes-Dallaqua MA, Coral DJ (2002). Morfo-anatomia. In: Cereda MP (ed.) *Agricultura: tuberosas amiláceas latino americanas*, Fundação Cargill, São Paulo. pp. 48-65.
- Souza LS (2006). Aspectos socioeconômicos e agrônômicos da mandioca, Embrapa Mandioca e Fruticultura Tropical, Cruz das Almas, P. 817.
- Viégas AP (1976). Estudos sobre a mandioca, IAC/BRASCAN Nordeste, Campinas, P. 214.
- Zapata C, Deléens E, Chaillouc S, Magne C (2004). Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *J. Plant Physiol.* 161(9):1031-1040.
- Zidenga T, Leyva GE, Moon H, Siritunga D, Sayre R (2012). Extending cassava root shelf life via reduction of reactive oxygen species production. *Plant Physiol.* 159(4):1396-1407.
- Wheatley NCC, Chuzel Z, Zakhia N (2003). Cassava: the nature of the tuber, *Encyclopedia of food sciences and nutrition*, Elsevier Science Ltd., London. pp. 964-969.
- Wenham JE (1995). Post-harvest deterioration of cassava: a biotechnology perspective. *Plant production and protection paper* FAO n. 130. FAO Plant Production and Protection, Rome Italy.