Chapter 4

Storage Root of Cassava: Morphological Types, Anatomy, Formation, Growth, Development and Harvest Time

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Abstract

Cassava (*Manihot esculenta*, Crantz) is considered a starchy root crop that provides staple food for millions of people in tropical and subtropical regions of the world. Research efforts are directed toward genetic breeding and cultivation of cassava to improve cassava storage root starch production, nutritional values, and industrial utilization. Cassava storage root (CSR) is a vegetative storage organ with indeterminate type of growth that has a central cylinder (edible part) originated by the swelling of primary root and crown roots. Comprehensive studies on thickened primary root (secondary growth) are rare, incomplete, and to a certain extent, missing. In this chapter, we review and forward studies that move our knowledge on cassava storage root (CSR). CSR generally forms up to 12–14 storage root (SR) per plant, which can originate from three sources of propagating plant materials as well as being induced *in vivo* and *in vitro*. Types of storage root (morphologically defined), CSR physiology, tissue anatomy/histology (secondary growth), chemical composition of the edible part, biochemical features, gene expression and proteomics as secondary growth proceeds are of major importance in order to breed cassava plant for agriculture utilization. Storage root morphology varies in shape from cylindrical to globular. Time to initiation of storage root formation varies from 45 to 90 days after planting (DAP), depending on the leaf auxiliary bud position in the vegetative propagating material at the plant source. Storage root growth, starch accumulation, and nutrient contents are largely dependent on genotypes. Storage root anatomy can be identified by eight characteristics common to a root with secondary growth and starch reserve variants. Histological characterizations can be used to identify cell types of primary and secondary meristems, procambium, vascular cambium, phellogen, phelloderm, primary and secondary xylem and phloem, storage parenchyma and sclerenchyma. Three types of meristematic cell differentiations occur as secondary growth proceeds; one due to cork cambium with plane perpendicularly oriented cell division, second due to plane longitudinally oriented cell division in the root apex, and third longitudinally oriented in the epidermal cells. Chemical composition of the storage root varies in the central cylinder (edible part) depending on the sample position in the root and the plant genotype. Therefore, biochemical characteristics are known to change with tissue age as secondary growth proceeds. Moreover, the

composition of stored starch varies with tissue age across the central cylinder and may be used as a physiological indicator for bulk storage root maturation and storage root harvest time.

Keywords: storage root, secondary growth, physiology, development, maturation

1. Introduction

Cassava (*Manihot esculenta*, Crantz) is a starchy root crop that provides a staple food source for millions of people in tropical and subtropical regions of the world. Worldwide, research efforts are directed toward genetic breeding and cultivation of cassava to improve cassava storage yield, root starch production, nutritional quality, and industrial utilization. Cassava storage root (CSR) is an indeterminate, vegetative storage organ that results from the swelling of primary root crown root, with the central cylinder as the edible part. Studies on secondary growth of cassava storage root (CSR) are rare, incomplete, and to a certain extent, missing. In this chapter, we review our comprehensive studies related to (CSR) morphology, storage root (SR) formation, SR physiology (growth analysis, development and maturation), anatomy/histology (secondary growth), and biochemical (carbohydrate, carotenoids, proteins, and gene expression) characteristics as secondary growth proceeds in order to understand yield of CSR.

2. Storage root of cassava features

2.1. Storage root utilization, shape and diversity

The practical utilization of CSR can be described in relation to 11 features that vary in importance, depending on the end use. These important characteristics are ranked (**Table 1**) in relation to their utilization for fresh consumption and industrial use (two most common uses of cassava by mankind).

2.2. Storage root of cassava diversity

Diversity in CSR morph types (**Figure 1**) is considered important cassava breeding traits when considering mechanical harvest.

Diversity in central cylinder of CSR (**Figure 2**) for carotenoids (**Figure 2A**), and carbohydrate and starch iodine staining pattern (**Figure 2B**) indicate a large genetic [1] and are the most popular traits used for genetic breeding proposes [2–4].

2.3. Cassava storage roots formation and induction

A cassava plant can form up to 14 storage roots per plant, depending on the genotype. Storage root can initiate from three distinct sources (**Figure 3**) of plant propagating material. These include direct embryonic root formation at the seed germination event (**Figure 3A**) to form a single-tap SR (**Figure 3B**), the leaf axillaries bud in stem cuttings forming a single SR

Storage root features/utilization	Fresh consumption	Industrial cassava
Storage root format	+++++	+++++
Early harvest storage root	+++++	+++++
Storage root HCN content	+++++	+
Storage root color of central cylinder	+++++	+
Storage root high fiber content	+	++
Storage root high starch content	++	+++++
Storage root starch quality	+++++	+++
Storage root vitamins	+++++	+
Storage root easy peel off	+++++	+++++
Storage root cooking	+++++	–
Storage root rotting	+++++	+++++

Table 1. Features of cassava storage root and its importance ranked in association with practical utilization by mankind.

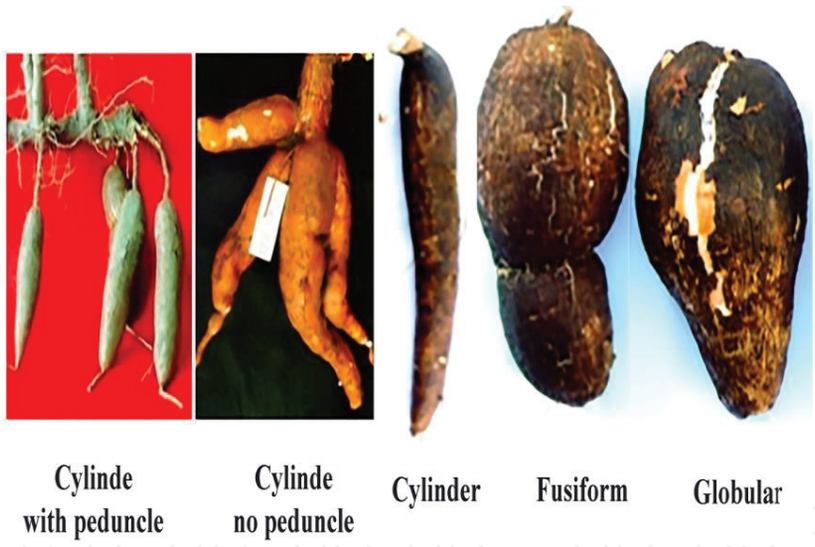

Figure 1. Cassava storage root morphological types.

(**Figure 3C**), and a number of nodal callus from the bases of stem cuttings forming more than one SR (**Figure 3D**), and buried nodes at the base of stem cuttings forming SR or induced "*in vitro*" plants [5]. The effect of leaf bud position on the stem cutting from a 1-year old mother plant is observed in **Table 2.**

2.4. Storage root anatomy and histology features

The anatomy of cassava storage root was first described by Rateaver [7] and more recently at [6]. From the basic secondary growth of CSR shown in **Figure 4**, it is possible to recognize at least 12 cell types in the storage root associated to secondary tissues including primary

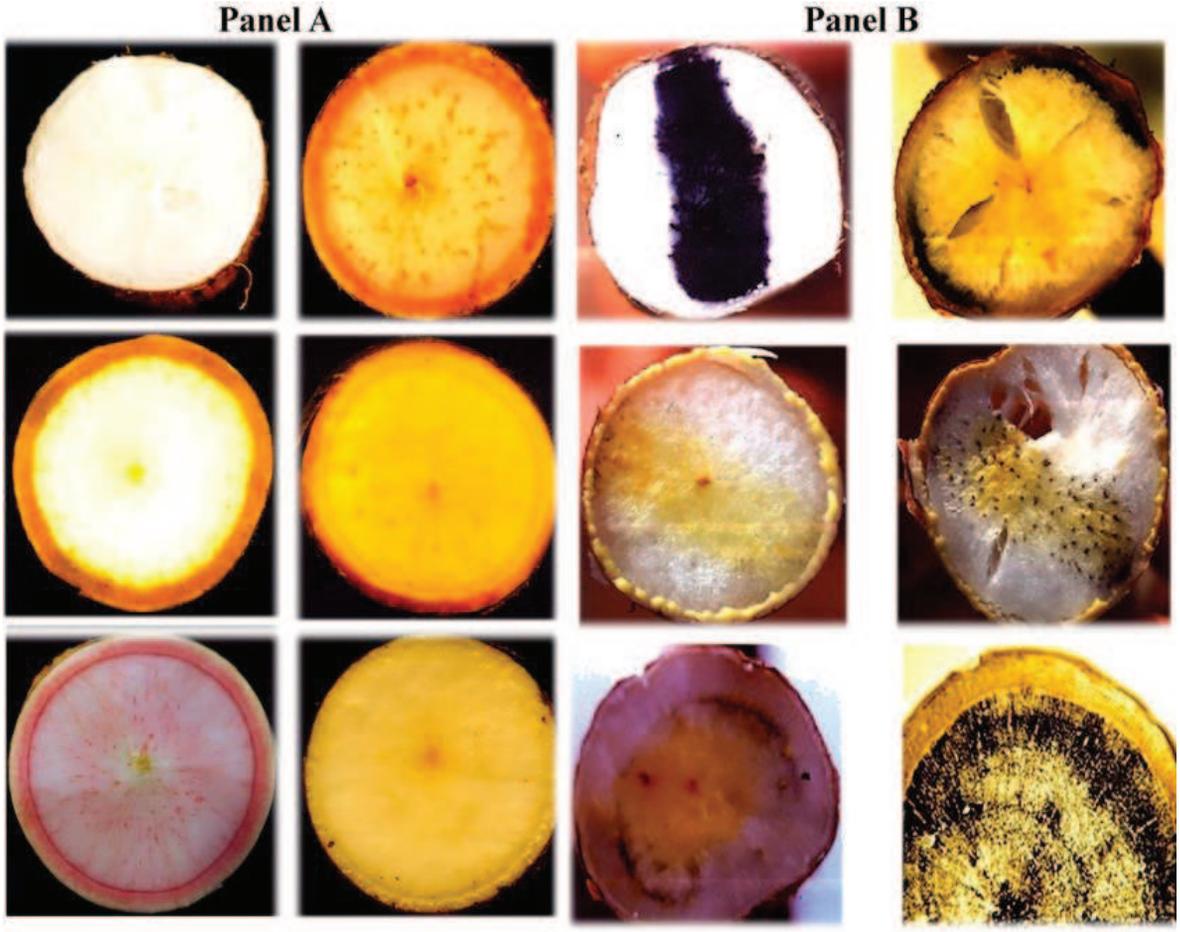

Figure 2. Diversity of cassava storage root in the central cylinder (edible part) related to carotenoid types and content (Panel A) and carbohydrate types as stained with iodine solution (Panel B).

meristem cells, secondary meristem cells, vessels, primary xylem, secondary xylem, primary phloem, secondary phloem, parenchyma cells, sclerenchyma, and epidermal cells.

Cassava storage roots grow in length from the apical meristem forming new cells continually, as generally observed in other plants carrying root secondary growth. In addition to the primary tissues (**Figure 4F)**, cassava storage root has secondary tissues that add thickness to a primary root (**Figure 4B–E**). Secondary tissues develop from two types of meristems. Based on these observations, from fibrous root, we defined six stages of CSR growth (**Figure 4G**). The cork cambium, originates beneath the epidermis, generally by pericycle dedifferentiation, producing cork cells and pushes them toward the outside of the root. As the cork expands outward, the endodermis, cortex, and epidermis die and peel off. The cork replaces them and becomes the outer covering of the root. The other secondary meristem, the cambium, lies between the primary xylem and the primary phloem. It produces secondary xylem cells toward the center of the root, and secondary phloem cells toward the outside. Qualitatively (**Figure 5**) and quantitatively (**Figure 6**), this pattern of tissue and cell type distribution in CSR over DAP as secondary growth proceeds indicates that CSR peel (secondary phloem, phellogen, and phelloderm), vascular cambium, and secondary xylem showed in **Figure 6A**, and

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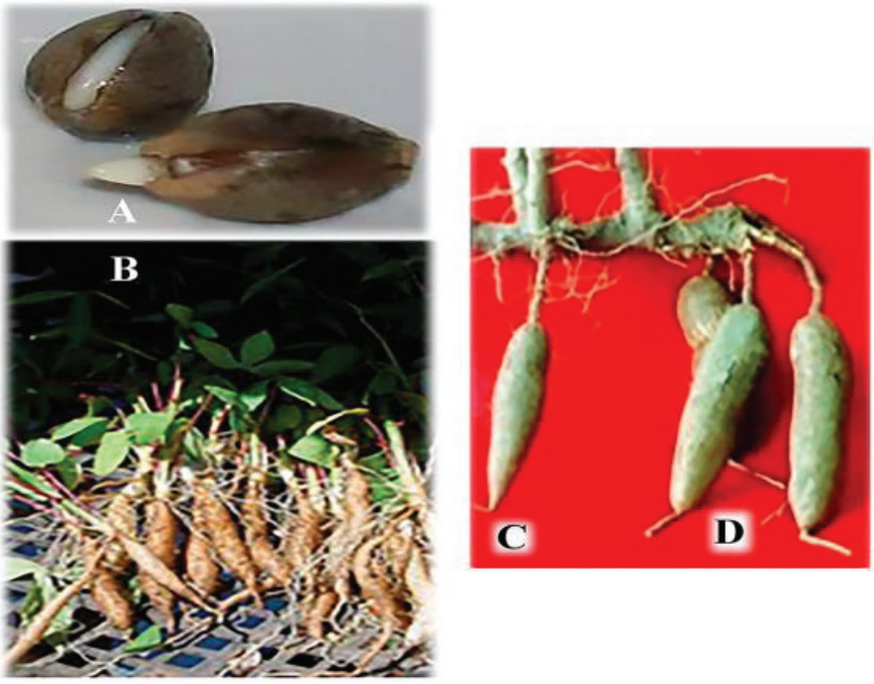

Figure 3. Source of storage root from cassava planting material. Germinating seeds (A) forming single-tap storage root (B). Leaf bud in the stem cutting from plant material forming single-tap storage root from leaf axillary bud germination (C) and callus on the stem cutting forming multiple storage roots (D).

Stem cutting bud position (bottom to top)	Counting bud age (DAP)	Fibrous root (S1)	Swelled root (S2)	Swelled root (S3)	Swelled root (S4)
StP1	30	32	1.2 (cm)	3.9 (cm)	11.3 (cm)
StP2	45	66	1.9 (cm)	3.0 (cm)	0
StP3	52	28	1.0 (cm)	3.2 (cm)	0
StP4	59	42	0.65 (cm)	0	0
StP5	66	14	0	0	0
StP6	73	23	0	0	0
StP7	87	50	0	0	0
StP8	94	21	0	0	0
StP9	115	29	0	0	0
StP10	122	0	0	0	0

Table 2. Cassava storage root formation in relation to leaf bud position in the stem cuttings from a 1-year old mother plant. Number of storage roots formed at leaf axillary bud from stem cuttings of the plant material. Initial fibrous root and defined stage of storage root S1. S2, S3, and S4 (as shown in **Figure 4G**) were based on root diameter (cm) starting 30 days after planting (DAP).

central cylinder (vessels and parenchyma cells in secondary xylem) shows opposite fashion. While secondary xylem peels, as well as vessels decrease with DAP, the secondary xylem and secondary parenchyma cells increases. Based on this analysis, we developed a tissue layer sampling system (**Figure 7**) and used the procedure for studies on biochemical features such as

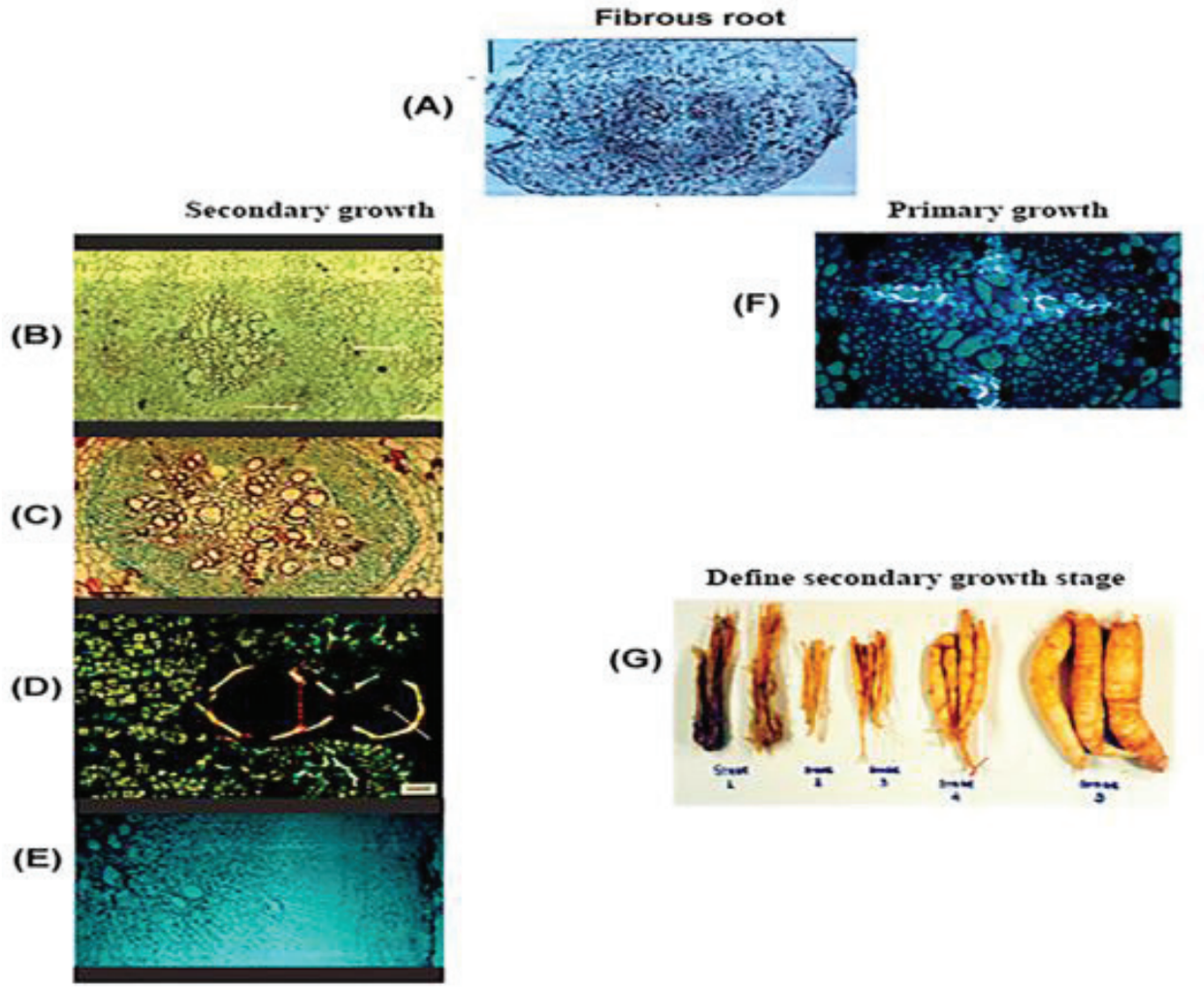

Figure 4. Recognizing storage root anatomy change initiation and advanced secondary growth stages in storage root of cassava. (A) refers to the initial fiber root; (B) refers to the initial pro cambium differentiation in fibrous root with pericycle dedifferentiation; (C) refers to the early events of secondary growth initiation; (D) refers to the complete secondary tissue formation with mature vessels; (E) refers to full secondary tissue formation; (F) refers to primary growth in fibrous root; and (G) defined six stages of storage root formation based on SR diameter.

carbohydrate (single sugar and starch) content [3], amylose percent variation [4], protein content variation [7], carotenoid content and type variation [1], and gene expression analysis [8–10].

2.5. Storage root growth, development, and physiological maturation

Storage root growth analysis was performed based on sampling SR at different time points after stem cuttings were planted in field plots at EMBRAPA Cerrados (Lat 15°35,769°) (Long 47°42,664°) and (Alt 977m) for a crop season of up to 170 days after planting (DAP) using genotypes for industrial use (cv.436) and fresh consume (cv. 982). Developmental stages of storage root (SR) were defined based on SR diameter (cm), SR length (cm), carbohydrate, carotenoid composition and content, protein content, fiber content, and fiber/starch ration to

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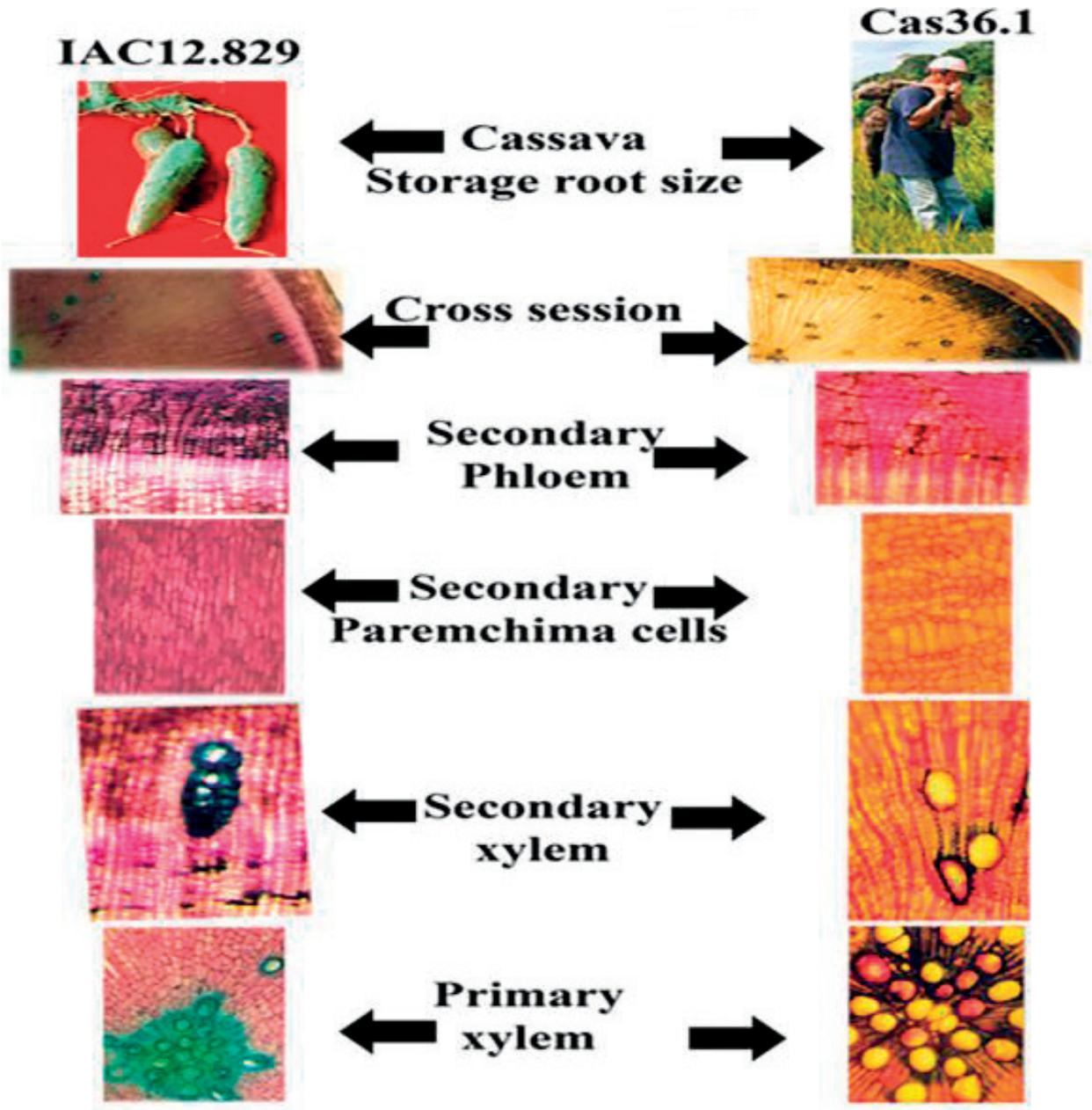

Figure 5. Visualization of cassava storage root morphology type (root size and shape) from two contrasting cassava genotypes. Cultivar IAC12.829 refers to commercial cultivar with the traditional type of storage root. Landrace Cas36.1 refers to a sugary cassava with giant storage root. Storage root tissues distinctions are observed. Cross session shows pattern of different stain with toluidine blue stain (traditional cassava) and iodine stain (sugary cassava). Microscopic observation for the major tissue types in both cassava types. Tylosis formation is observed only in sugary genotype.

accomplish harvest time (physiological maturation). Results shown in **Figure 8** indicate that CSR formation initiates 30 DAP, reaching a maximum number of SR (12–14) by 90 DAP, SR diameter increased linearly up to 170 DAP, while SR length reach a plateau around 40–70 DAP **(Figure 8 Panel A**) depending on the genotype. Either SR dry matter (%) or SR dry weight (gram/plant), and starch accumulation (gram/plant) extended up to 170 DAP and is largely dependent on the genotype (**Figure 8 Panel B**).

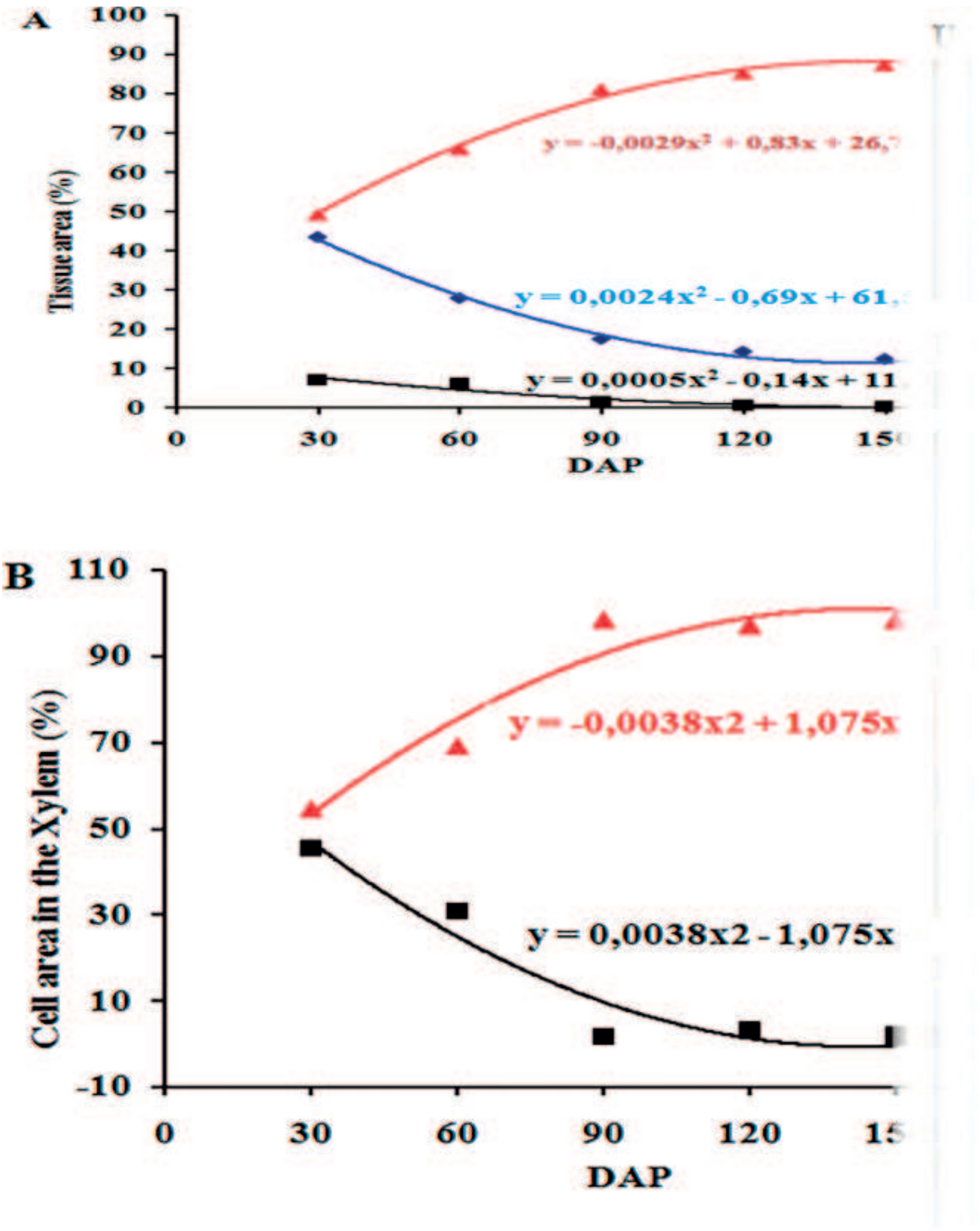

Figure 6. Changes in proportion distribution of tissue and cells type in cassava storage roots as secondary growth proceeds. (A) Refers to tissue of peel (secondary phloem, phellogen, and phelloderm), vascular cambium, and secondary xylem. (B) Refers to vessels and parenchyma cells in secondary xylem.

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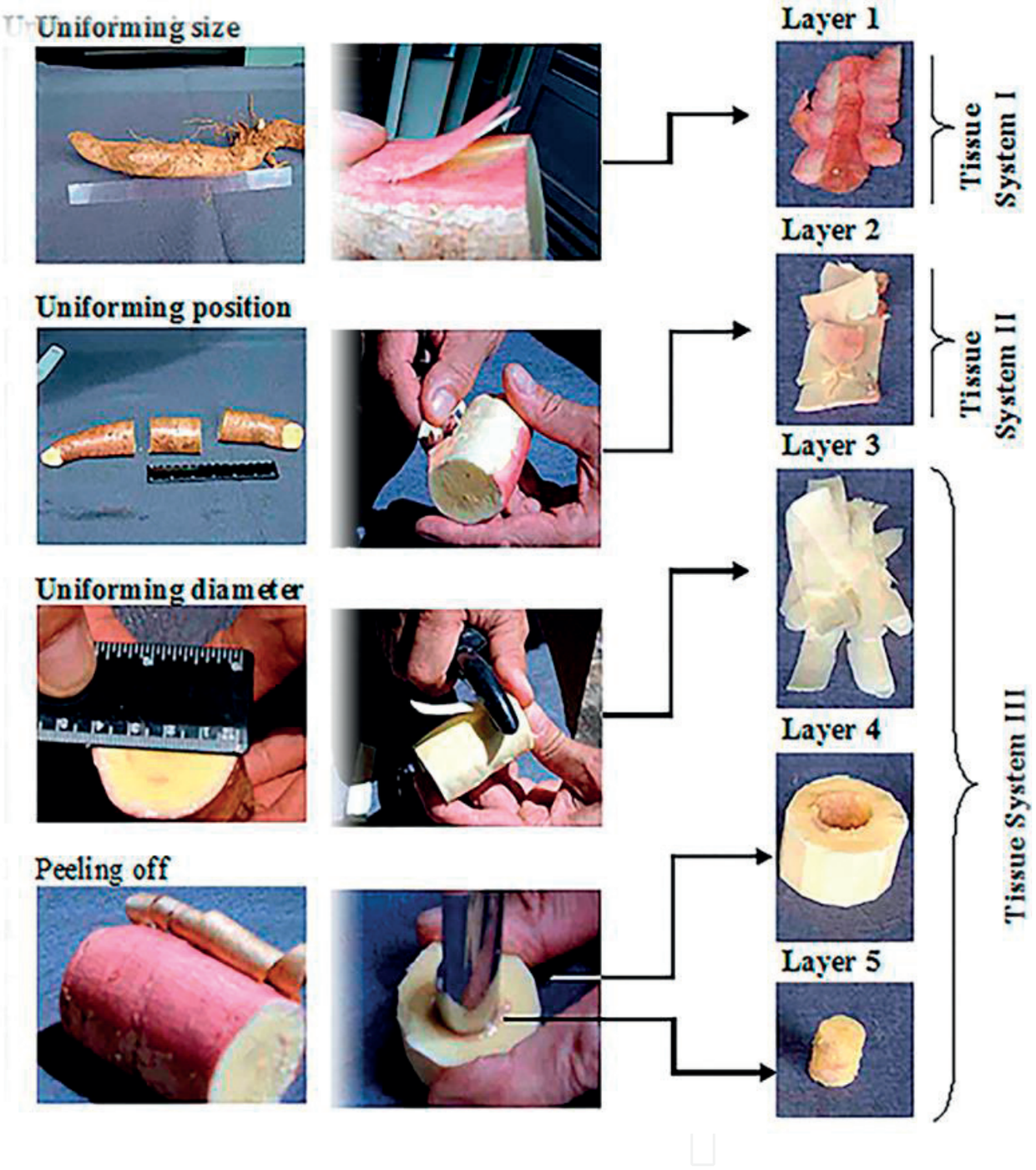

Figure 7. Step by step for storage root tissue sampling system used to further biochemical feature studies of cassava storage root as secondary growth proceeds. Tissue sample I (layer 1), tissue sample II (layer 2), and tissue sample III (layer 3, layer 4, layer 5). Tissue cell compositions are as described in **Figure 5**.

The SR maturation (physiological maturation), as taken by the rate of CSR growth, starch accumulation, and crude fiber accumulation, and crude fiber/starch ratio vary in relation to conventional utilization of the crop (**Table 3**). The major differences occurring are early harvest

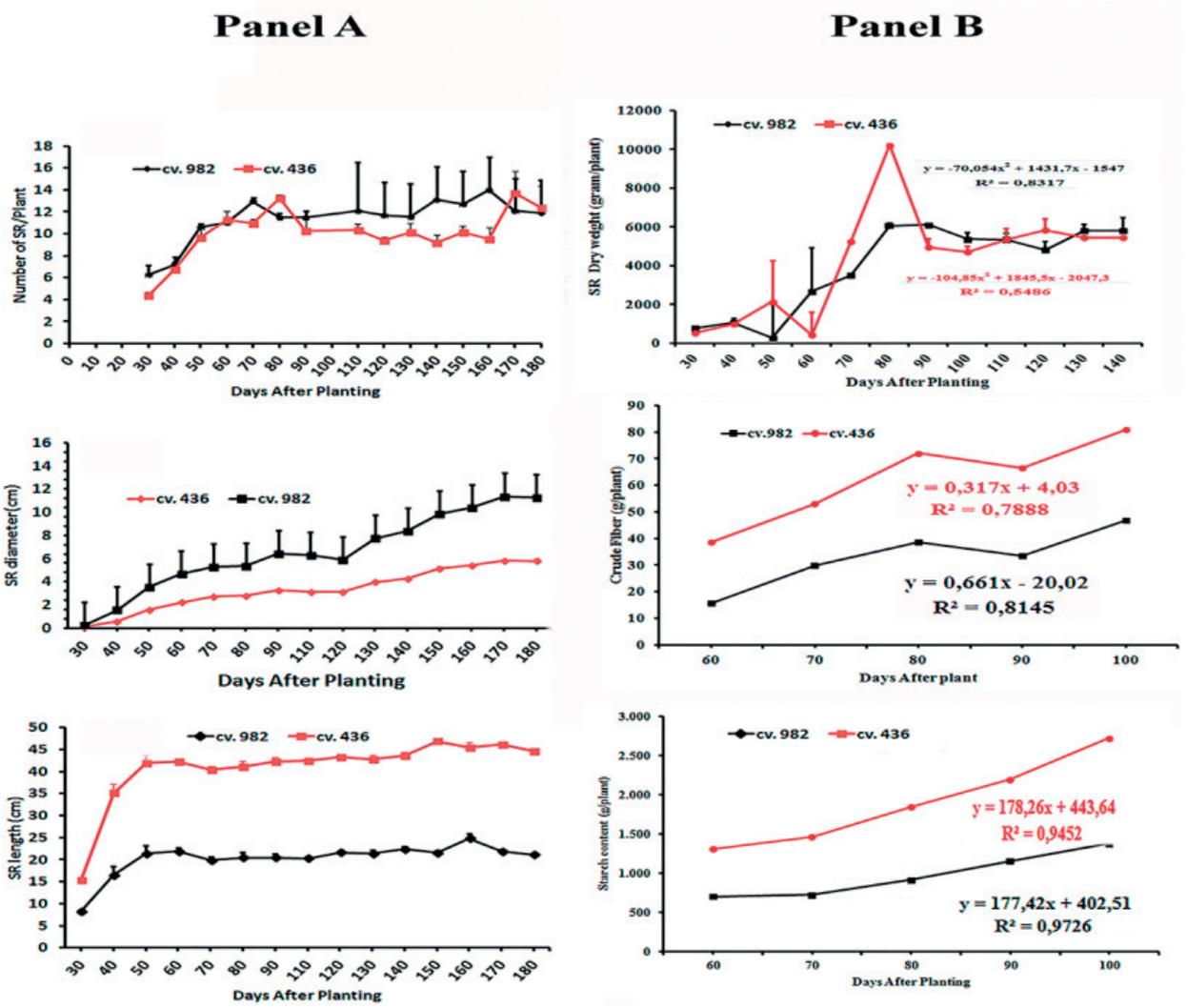

Figure 8. Storage root formation, growth, and development analysis. Panel A—storage root formation, referring to number of storage root per plant, storage root central diameter, and storage root length. Panel B—referring to total dry matter, starch, and crude fiber accumulation over time. Plants were grown at EMBRAPA Cerrados (Latitude 15°35,769°) (Longitude 47°42,664°), and (Altitude 977 m) for a crop season of up to 170 days after planting (DAP).

time for the fresh consumption genotype (cv. 982) and late harvest time for the industrial use genotype (cv.436).

The overall chemical composition of CSR has recently been reviewed [1]. The major conclusions indicate that fresh peeled cassava storage roots are rich in carbohydrates (30–35%), low in protein (1–2%), and fat (<1%). In addition, CSR has nutritionally significant amounts of calcium (50 mg/100 g), phosphorous (40 mg/100 g), and vitamin C (25 mg/100 g), and poisoned values of cyanogenic glycosides upon the hydrolyses of linamarin [11, 12, 15, 16]. In this chapter, we forward our knowledge on nutritional values of CSR based on three major biochemical features that lead to more precise natural variation in the composition and accumulation of carbohydrates (free sugar and starch), carotenoids (type and content), and proteins (content and exploratory functionalities) in the CSR central cylinder.

Storage root growth parameters/genotype	cv. 982	cv. 436
Days to form storage root (DAP)	60	90
Root growth rate (g/plant/day)	1431.70	1845.5
starch accumulation rate (starch gram/root/day)	177.42	178.26
fiber accumulation rate (fiber gram/root/day)	0.661	0.317
Fiber/starch ration	0.0037256	0.0017783
Harvest time	Early season	Late season

Table 3. Storage root growth and development parameters of an early (cv. 982) and late season (cv.436) harvest time in cassava crop. Plants were grown at EMBRAPA Cerrados (Latitude 15°35,769°) (Longitude 47°42,664°) and (Altitude 977 m) for a crop season up to 170 days after planting (DAP).

2.6. Storage root biochemical features and natural genetic variation

This chapter focus on the identification of spontaneous mutations in two biochemical pathways (sucrose/starch conversion and carotenoid biosynthesis), as well as mechanisms of carotenoid and proteins accumulation, and gene expression analysis.

Carbohydrate composition, content, and genetic variation: *Sugary* cassava is a unusual SR phenotype as observed in **Figure 2** (**Panel B**) for the cross session of SR stained with iodine solution,

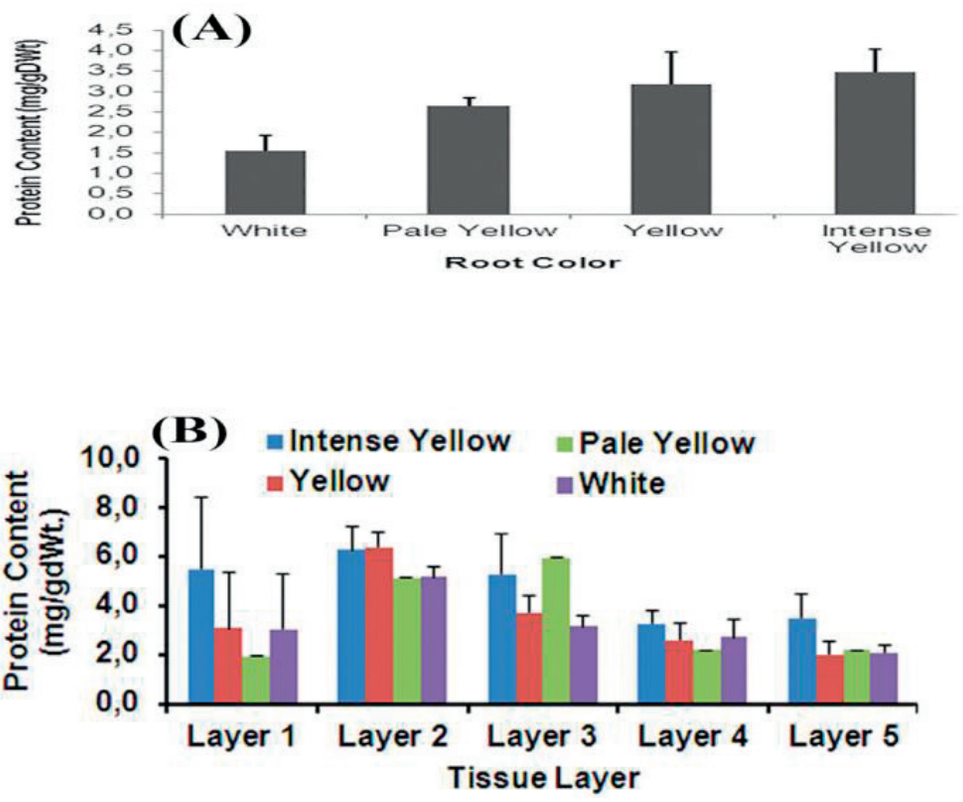

Figure 9. Variation in total protein content of storage roots (mg/gDWt) in relation to four categories of central cylinder color genotypes (A) and tissue age (B).

cells morphology, free sugar composition, and sucrose/glucose content in relation to normal genotypes and SR tissue age [3].

Carotenoid biosynthesis, accumulation, and genetic diversity: Landraces diversities (**Figure 2** (**Panel B**)) have been studied to understand carotenoid biosynthesis [6, 12] mechanisms of carotenoid accumulation [1, 7, 13], identification of mutants [13], and breeding commercial varieties [2].

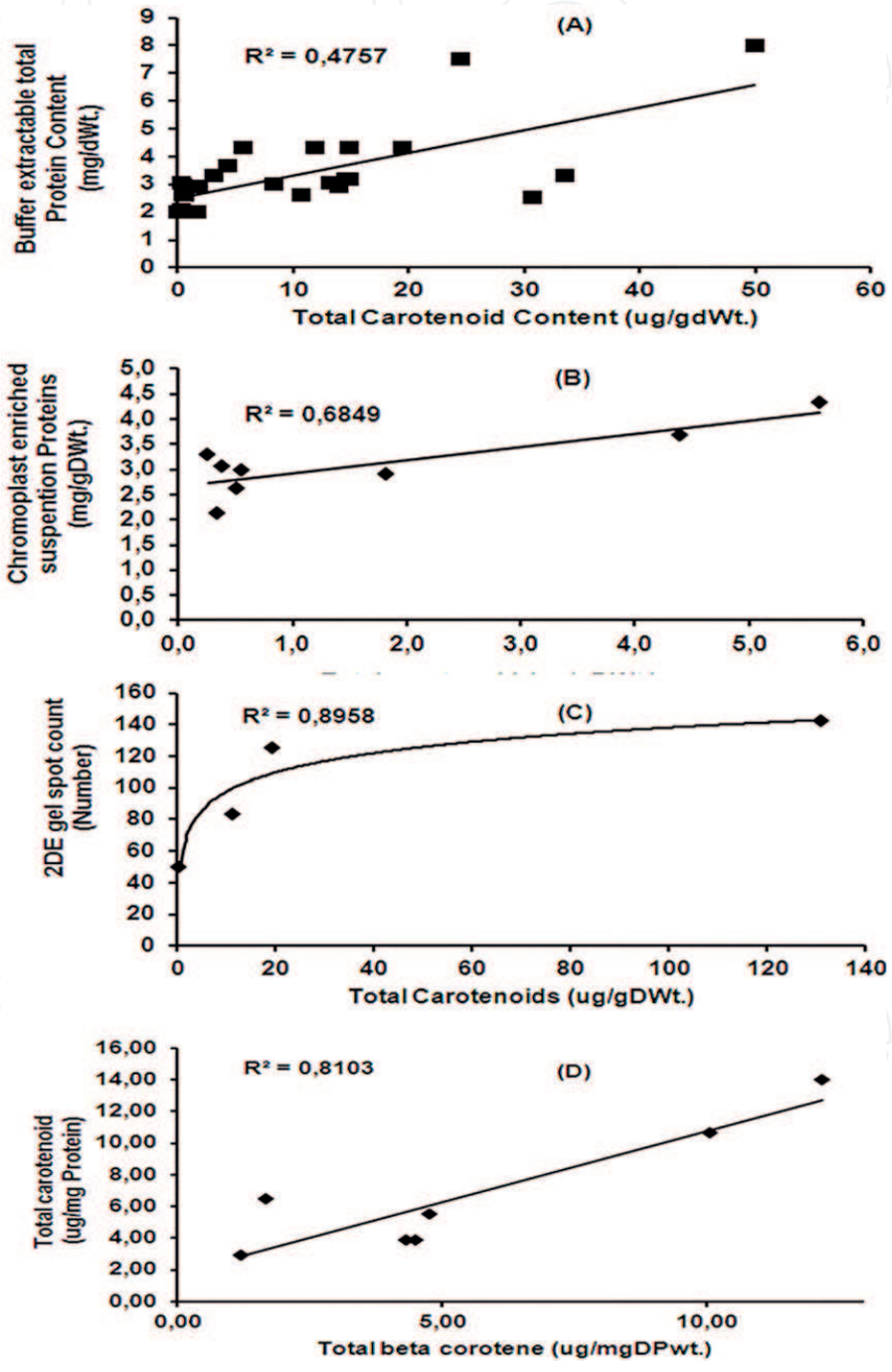

Figure 10. Correlations of total carotenoids (μg/mgDWt) and (A) buffer extractable proteins content, (B) chromoplast suspension proteins (mg/gDWt), (C) counting number of proteins in 2DE gel separated, and (D) total β-carotenoid content in cassava storage roots.

The major achievements, includes the discovery of a putative mutant for the gene LYCb that leads to the accumulation of solely lycopene in the landrace CAS51 and the discovery of a mutant for the gene HYDb that leads to accumulate mainly β-carotene in the landrace CAS64. Discovery of a single point mutation on the gene coding for protein SHSP that lead to the sequestration specifically of β-carotene in landrace CAS64. Six new commercial varieties were developed, registered, and protected in 5 years instead of 15 years as it is ordinarily done. It has been reported that sampling variation among plants and roots from the same plant is responsible for 20–25% [13] that causes uncertainty of values used for selection of clones in a breeding program. The sampling tissue system based on tissue age, as discussed above, could improve the accuracy of quantification of total carotenoid content for this propose.

Protein content and exploratory functionalities: Cassava storage root protein content variations predicted functionalities, patterns of distribution in source and sink organs, and post-harvest physiological deterioration studies using PROTEOMIC's technologies.

Cassava storage root proteins content in relation to color categories of genotypes (**Figure 9**): Similar to carbohydrate, protein content varies in two ways. One, higher protein content is observed in pigmented cassava rather than in white cassava (**Figure 9A**). Second, protein content varies according to tissue type and age across the central cylinder by decreasing from layer 3 to layer 4 to layer 5 (**Figure 9B**). In addition, protein content is strongly correlated with total carotenoid content (**Figure 10**). Heat shock proteins (HSPs) are the most abundant proteins types [13] in cassava storage root and are closely associated to accumulation of total carotenoid, with small shock proteins (SHSPs) being the major type of HSP [13].

3. Synthesis and conclusions

The studies discussed in this chapter highlight the importance of natural variation in landraces previously unknown for the cassava community in several ways. 1. Accurate estimation of the genetic of traits in landraces derived from alteration in two major metabolic pathways (starch and carotenoid) of great relevance for the two recognized practical utilization of CSR by using physiological concepts and sampling strategy. 2. Describing a CSR sampling procedure specific for CSR to estimate traits of agronomic importance for the two major practical utilization of CSR to improve product quality. 3. Incorporation of those genetic variants in a conventional breeding program, which reduced the time for obtain new commercial varieties. 4. Discovery of three putative mutants in the CSR. Further researches to dissect transcriptome and proteome of CSR are under way using the sampling system proposed in this chapter to elucidate molecular mechanisms regulating CSR formation, growth, development, and physiological maturation.

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