Journal of Plant Biochemistry & Physiology
ISSN: 2329-9029

Rouse-Miller et al., J Plant Biochem Physiol 2013, 1:4
Doi: 10.4172/2329-9029.1000117

Research Article | Open Access

# Inverse Oriented Stem Cuttings Generate Tuberous Stems in Cassava *Manihot esculenta* Crantz; An Alternative Sink Site

**Judy Rouse-Miller[1]*, Valerie Bowrin[1], Grace Sirju-Charran[1] and Fedora Sutton[2]**

[1]*Department of Life Sciences, University of the West Indies (UWI), St. Augustine, Republic of Trinidad and Tobago*
[2]*Department of Plant Science, South Dakota State University (SDSU), USA*

**Abstract**

Cassava (*Manihot esculenta*) is normally propagated by stem cuttings planted in a slanted, vertical or horizontal orientation. Axillary buds produce aerial shoot and adventitious roots are produced at the base (proximal end) of the cutting, some of which develop into tuberous roots. However, when cuttings are planted in an inverse, slanted or inverse straight orientation, the buried bases of the shoots which arise from underground buds swell. In this study, we determined that the stem swelling accumulates and store starch as do the tuberous root tubers. This phenomenon designated as inversion-induced stem tuberization, first detailed here, provides a system which could be used to study the roles of phytohormones and light.

**Keywords:** Anatomy; Cassava; Cutting orientation; Sink sites; Tuberous stem; Tuberous roots

## Introduction

Cassava is an important staple of the tropics and sub-tropics. The crop is propagated using multimode stem cuttings planted vertically (with the buds pointing upward), slanted at a thirty-forty-five degree angle (with buds pointing upward) or buried horizontally two to four centimeters below the soil surface. The distally positioned buds on the stem cutting develop into aerial stems and adventitious roots develop at the proximal end of the cutting, with some of roots developing into the marketable tuberous roots. Planting cassava stem cuttings in an inverted orientation-buds pointing downward, as initially reported, resulted in increased toughness, lignification and swelling of the submerged stems, events indicative of tuber formation [1]. A more detailed explanation of this phenomenon was later offered using sweet potato, a naturally root tuberizing crop [2]. The observed tuberization of sweet potato stems was attributed to meristematic activity occurring in buried (dark grown) stems, produced when tubers used as the planting material were buried in an inverted orientation, distal end in the soil. In this study, we refer to this phenomenon as "inversion induced stem tuberization".

Tuber formation is a developmentally complex phenomenon that includes processes, such as: (a) inhibition of longitudinal growth and initiation of radial growth in the tuberizing organ via continued cell division and expansion; (b) formation of new xylem vessels (xylogenesis) with simultaneous inhibition of lignification of the other cells produced during normal secondary growth [3,4]; (d) synthesis of starch (amylose and amylopectin); (e) synthesis of storage proteins (patatins, sporamins and dioscorins), and (f) synthesis and accumulation of low levels of other secondary metabolites, including linamarin, scopoletin, lycopene [5].

Typically, tubers have storage and propagative function, as is the case with *Solanum tuberosum* (potato) in cassava, however, the storage roots have no propagative function, and as such are referred to here as tuberous roots. We report on the formation of tuberous stems in cassava and compare the anatomy of the tuberous stem to the tuberous root. We propose that tuberous stem formation in an otherwise non-tuberizing organ can serve as a model system to study the integration of physiological, molecular and cellular processes that allow for the transition of stems/organs into tubers.

## Materials and Methods

### Plant material

Stem cuttings of cassava cultivar MCOL22 were obtained from the cassava germplasm collection (Field Station, The University of the West Indies St. Augustine, Trinidad and Tobago).

### Planting conditions for stem cuttings for stem and root tuber formation

Cassava cuttings of cultivar MCOL22 were established singly in 40 L tins containing a mixture of garden top soil and sand in the ratio 3:1, using stem cuttings (20-25 cm in length), taken 10-15 cm from the base of the primary stem of the mother plant. Ten cuttings were planted in each of five orientations (a-e): (a) vertical with (3-5) nodes below and above soil level with buds pointing upwards (b) slanted with (3-5) nodes below and above soil level with buds pointing upwards, (c) vertical with (3-5) nodes below and above soil level with buds pointing downward (d) slanted with (3-5) nodes below and above soil level with buds pointing downward (e) cutting planted horizontally buried 2-3 cm below soil level. Orientations (a-e) had 2-4 nodes greater than 5 cm below soil level. Plants were harvested after 16 weeks and the below ground organs were described.

### Stem and root tuber anatomical analyses

To determine whether the basal swelling of the underground stems was tuberous, sections of both swollen stem and tuberous storage roots were compared anatomically and stained for the presence of starch. Swollen stem and root sections were harvested after 16 wk and tissues

***Corresponding author:** Judy Rouse-Miller, Department of Life Sciences, University of the West Indies (UWI), St. Augustine, Republic of Trinidad and Tobago, E-mail: judy.rouse-miller@sta.uwi.edu

**Received** April 24, 2013; **Accepted** November 06, 2013; **Published** November 14, 2013

**Citation:** Rouse-Miller J, Bowrin V, Sirju-Charran G, Sutton F (2013) Inverse Oriented Stem Cuttings Generate Tuberous Stems in Cassava *Manihot esculenta* Crantz; An Alternative Sink Site. J Plant Biochem Physiol 1: 117. doi:10.4172/2329-9029.1000117

fixed in formalin-acetic acid (FAA) then dehydrated in an ethyl alcohol series and wax infiltrated [6]. Cross sections of 10 µm thickness were prepared and mounted on slides before staining with Periodic acid-Schiff (PAS), which stains starch a bright fuchsia. Slides were viewed using an Olympus BX50 microscope and digital images taken using a Pixera 5.8 megapixel 48-bit CCD camera.

## Results

All cuttings within each orientation responded similarly. Vertical and slanted stem cuttings with buds pointing downward (inverted) (c and d) produced new stems with a photosynthetic canopy, similar to that obtained with stem cuttings planted vertically or slanted with buds pointing upward (upright) (a and b) and horizontal, buried stems (e). Tuberous roots were observed in all orientations (a-e), and swollen stems were observed in orientations (c and d). The adventitious root initials in c and d developed at the proximal end of the stem cutting, however, these soon dried out. The adventitious roots which formed underground developed from nodal points on the mother cutting and later at the nodal positions of the underground swollen stem. Figure 1A and 1B depict the results of growing the stems in the inverted position (c). Note that the axillary buds on the mother stem face down confirming that the stem was planted in the inverse orientation. The resulting tuberous stems (st) are those emanating directly from the base of the mother stem. Three tuberous stems can be seen in Figure 1A. They appear curved at the base as the new stem re-orientates itself to grow upwards and break the soil surface where new foliage is generated. Stems originated from both aerial and underground buds, but only the

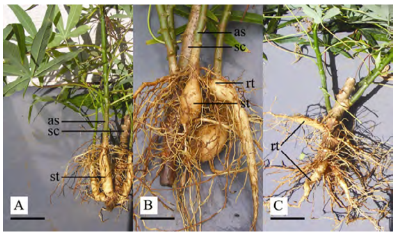


**Figure 1:** Morphological features of cassava tuberous organs formed on stem cuttings grown in tins (A) tuberous stems on inverted stem cuttings (orientation (c)) (B) tuberous stem with root tuber attached formed on inverted stem cuttings (orientation (c)) (C) tuberous roots formed on vertical upright stem cuttings (orientation (a)). Scale bars: A, C=3 cm; B=6 cm Legend: as-aerial stem above tuberous stem, sc-stem cutting, st–tuberous stem, rt–tuberous root.

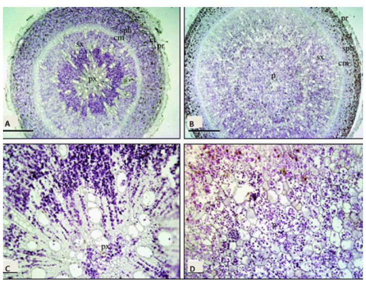

**Figure 2:** Cross sections of cassava tuberous organs stained with Periodic acid-Schiff (PAS): tuberous root tissue (A,C) (A) from periphery inwards; periderm (pr), cortex (ct), secondary phloem (sph), cambium (cm), secondary xylem (sx) and core of primary xylem(px) (C) primary xylem consisting of mainly xylem vessels with ray parenchyma containing starch grains. Tuberous stem tissue (B, D), (B) layers from periphery inwards; periderm (pr), cortex (ct), secondary phloem (sph), cambium (cm) ,secondary xylem (sx) and pith (p) (D) pith cells containing starch Scale bars: A, B=100 µm C, D=5 µm;

buds positioned greater than 5 cm below the soil level produced shoots that were swollen at the base (Figure 1A). Tuberous roots developed from the swollen stem as a result of swelling of adventitious roots present on the stems (Figure 1A and 1B). Outgrowths from buds close to the soil surface in orientations c and d did not swell as was the case for shoot developed from the stem cutting buried in the horizon orientation (e).

The stem swellings developed periderm with pigmentation typical of root tubers, and were thus indistinguishable at the morphological level, except that the buried lateral bud initially grew downward before exhibiting negatively geotrophic shoot growth compared to the positively geotrophic root tuber (Figure 1A-1C).

### Stem and root tuber anatomical analyses

Figure 2 depicts PAS stained cross sections of tuberous root (A and C) and swollen stems (B and D). The root tubers were characterized by a central core of primary xylem (px) vessels and xylem ray parenchyma, which accumulated starch (Figure 2 A). In the root, starch accumulation was observed earliest in the first formed cells of the secondary tissue and radiated outward with tuber development (Figure 2C). The tuberous stem (B and D) lacked the central core as the root. However, it did accumulate starch as reflected by the fuchsia staining throughout. Common to both tuber types was the contribution of the secondary xylem as the main starch storing tissue and increased girth of the organ (Figure 2 C and 2D).

## Discussion

The swelling of the inverted stem can be referred to as tuberization, since the increase in girth observed was accompanied by an increase in secondary xylem which formed the major starch storing tissue, in addition to the pith. The tuberous stem can, therefore be considered an alternative sink site different from that reported in cassava due to tuberization of rooted petioles [7].

The difference between the stems that resulted in tubers and those that did not produce tubers appears to be mainly due to the degree of absence of light. It can be agreed that the stem region in the soil further away from the surface received no light, whereas that close to the soil surface would be exposed to some quantity and quality of light. The observation of tuberization of the petioles [7] was only in portions of the petioles, which were buried in sand and therefore not exposed to light. This result adds to the inference that particular wavelengths of light may inhibit tuberization in cassava as was hypothesized [2].

In this study, we determined that the base swelling of the inverted stem accumulated starch, and thus could be designated as a tuber. This phenomenon designated as inversion-induced stem tuberization provides a model system which could be used to study the roles of phytohormones and light in stem tuber induction. Additionally, future studies will determine whether these tuberous stems have similar or different nutrient profiles to the tuberous roots and their potential as a food source.

**Acknowledgement**

This work was supported by funding from a Research and Publications Grant CRP.3.NOV.07.15. The University of the West Indies. Collaboration between SDSU and UWI was facilitated by funding from the SDSU New Ideas for Research Funds.

The authors wish to thank Miss Ann Soo Ting and Mrs. Maggie Mootoosingh for technical assistance.

## References

1. Onwueme IC (1978) The tropical tuber crops: Yams, cassava, sweet potato, cocoyams. Chichester Wiley, Germany.
2. Sirju-Charran G, Wickham LD (1988) The development of alternative storage sink sites in sweet potato *Ipomoea batatas*. Ann Bot 61: 99-102.
3. Wilson LA, Lowe SB (1973) The anatomy of the root system in West Indian sweet potato (*Ipomoea batatas* (L.) Lam.) Cultivars. Ann Bot 37: 633-643.
4. Dabydeen S, Sirju-Charran G (1990) The developmental anatomy of the root system in Yam bean, *Pachyrhizus erosus* Urban. Ann Bot 66: 313-320.
5. Shewry PR (2003) Tuber storage proteins. Ann Bot 91: 755-769.
6. Johansen D (1940) Plant Microtechnique. Mc Graw Hill, New York, USA.
7. Duncan EJ, Mc Pherson GI (1974) Changes in the petiole of leaves of *Manihot esculenta* (Crantz), on rooting. Ann Bot 38: 261-268.