Fluorescence Measurement of Postharvest Physiological Deterioration (PPD) in Cassava Storage Roots

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[Abstract] Cassava (*Manihot esculenta* Crantz) is a perennial root crop in the tropics. Within 24-72 hours of harvest the storage roots deteriorate rapidly, thereby necessitating their prompt processing or consumption. Postharvest physiological deterioration (PPD) of cassava storage roots is the result of a rapid oxidative burst, which leads to discoloration of the vascular tissues. The various fluorogenic probes available for *in vivo* reactive oxygen species (ROS) imaging could reveal complex spatial and temporal dynamics in plant tissues. Fluorescence measurement of PPD became widely used assay for ROS. Most of the ROS probes passively diffuse across cell membranes localize in the mitochondria, and exhibit fluorescence. Due to its high sensitivity to ROS and ease of loading and detection, the Dihydrorhodamine123 probe has been widely used in plants to monitor ROS accumulation in response to various stimuli and range of developmental processes.

Materials and Reagents

1. DMSO (Sangon Biotech, catalog number: D0231)
2. Dihydrorhodamine123 (Invitrogen, Molecular Probes®, catalog number: D632) (see Recipes)
3. MitoTracker Deep Red FM (Invitrogen, Molecular Probes®, catalog number: M22426) (see Recipes)
4. 0.1 M sodium phosphate buffer (pH 7.0) (see Recipes)

Equipment

1. 48-well plate
2. Razor blade
3. Slides
4. Centrifuge

5. Confocal laser scanning microscope (Zeiss, model: LSM 510 META)

Procedure

1. Cut fresh cassava storage roots into smaller segments about 5 x 5 x 0.5 mm in length, width and height by razor blade. The areas (Figure 1):
   a. The center: vascular tissue found within the center of the root cross-section.
   b. The middle: the tissue filled with storage cells with streaks of xylem throughout.

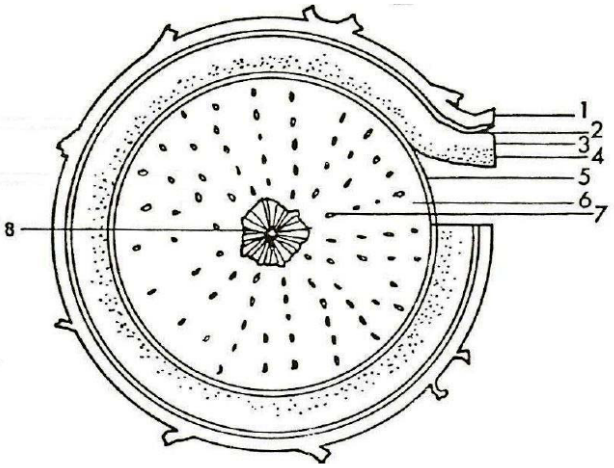

Figure 1. Cassava storage root cross-section (Rickard, 1985). 1: periderm; 2: sclerenchyma; 3: parenchyma; 4: latex tubes; 5: cambium; 6: parenchyma (The middle); 7: xylem vessels (The middle); 8: xylem bundles (The center).

2. Immersed in the sodium phosphate buffer with Dihydrorhodamine123 or MitoTracker Deep Red FM, stain for 10 and 20 min, respectively in the dark at room temperature.
3. Afterwards, wash with sodium phosphate buffer once before viewing under the microscope. Use sodium phosphate buffer to mount the sample on slides.
4. Use a Zeiss LSM (Laser Scanning Microscope) 510 META Confocal with the capturing program LSM 510 equipped with a10x and 20x objective.
5. Allow the microscope and lasers to warm up for about 20 minutes before use.
6. The settings used for each stain are as follows:
   a. Dihydrorhodamine123 excitation/emission 488/515 nm.
   b. MitoTracker Deep Red FM excitation/emission 635/680 nm.
7. First use the visible spectrum to find the sample, after that change to fluorescence spectrum (Figure 2).

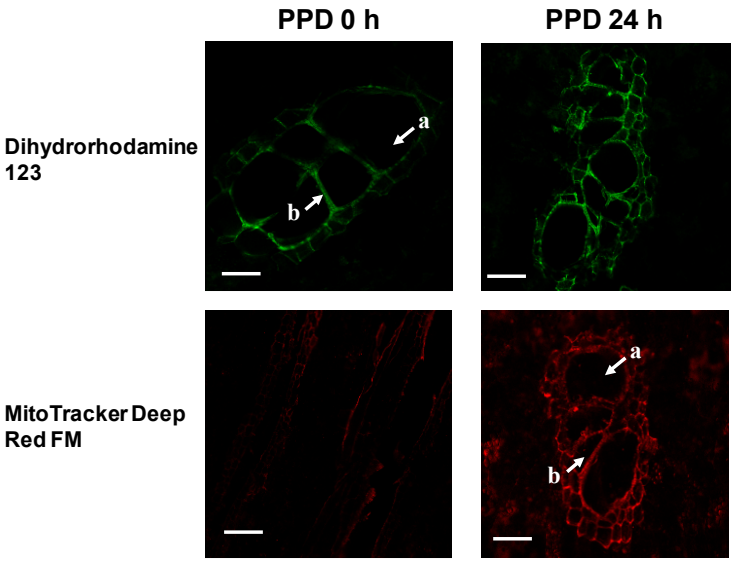

Figure 2. Fluorescence determination of PPD in cassava storage roots. a, xylem vessel; b, bundle sheath; Scale bar = 20 μm.

Recipes

1. 0.1 M sodium phosphate buffer, pH 7.0 (100 ml)
   Mix 61.5 ml 1 M K_2HPO_4 and 38.5 ml 1 M KH_2PO_4
   Filter sterilize (0.45 μm)
   Store at 4 °C
2. Dihydrorhodamine123 working solution
   a. 50 mM Storage solution
      10 mg Dihydrorhodamine123 with 577.4 μl DMSO
   b. 50 μM Working solution
      Sodium phosphate buffer dilution
      Store at -70 °C
3. MitoTracker Deep Red FM
   a. 1 mM Storage solution
      50 μg MitoTracker Deep Red FM with 91.98 μl DMSO
   b. 250 nM Working solution
      Sodium phosphate buffer dilution
      Store at -70 °C

Acknowledgments

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