Postharvest Biology and Technology 145 (2018) 10–14

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Correlation of scopoletin-induced fluorescence with visible PPD symptoms in greenhouse cassava

Nor Mahmod[a,*], John Beeching[b]

[a] AGROPOLIS, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia
[b] Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

ARTICLE INFO

Keywords:
Cassava
Scopoletin
Post-harvest physiological deterioration

ABSTRACT

Post-harvest physiological deterioration (PPD) is spoilage of cassava storage roots characterized by browning of parenchyma tissue and vascular streaking. A striking feature of PPD is an accumulation of hydroxycoumarins, such as scopoletin, scopolin, esculetin and esculin, which are products of phenylpropanoid pathway. PPD, which renders the roots unpalatable and unmarketable, leads to significant yield loss in global cassava production. Extensive studies have been conducted to understand the biochemical activities and pathways involved in PPD in which the result requires an evaluation of PPD symptoms which is usually based on a visual scoring system. However, high variations are consistently observed between biological replicates making analysis difficult. Therefore, there is urgent need to find a reliable marker since the generation of reliable data that can be shared or compared between institutions is desirable. The use of scopoletin-induced fluorescence as a PPD marker was tested in greenhouse-grown cassava storage roots by comparing fluorescence measurements and the PPD scores generated from normal light photographs of the harvested roots. It was found that all freshly harvested root samples lacked fluorescent but after 24 h they considerably fluoresced to a high level. However, the occurrence of intense fluorescence was not accompanied by intense PPD symptoms as fluorescence level declined when visible PPD symptoms developed. Hence, the use of fluorescence or scopoletin as an alternative marker for PPD remain unconvincing, mainly because it did not parallel with PPD symptom accumulation observed.

1. Introduction

Cassava is the sixth most important food crop in the world, where it is the staple food for over 800 million people (Sánchez et al., 2013). Its ability to grow in marginal soil conditions and under minimal care makes cassava a vital food security crop for resource-poor farmers. However, cassava production is constrained by post-harvest physiological deterioration (PPD), a storage root disorder characterized by vascular streaking and discoloration of parenchymal tissue.

PPD consists of biochemical and physiological changes in the cassava storage root, some of which are manifested as visual symptoms that can render them unacceptable as food or as input to processing (Beeching et al., 1998). The main PPD symptom is vascular streaking, but typically it is accompanied by browning of parenchymatic tissue (Hirose et al., 1983). The streaks or occlusions which tend to initiate at the root periderm are formed from pigment accumulation in the xylem vessels, which then spread to the adjacent parenchymatic xylem. Browning of parenchymatic tissue, which is initiated as a bluish pigmentation occurs when the pigments finally spread to parenchyma cells.

During PPD, a range of secondary metabolites accumulate, including the hydroxycoumarins, scopoletin, scopolin (the glycosylated form of scopoletin), esculetin and esculin (the glycosylated form of esculetin); it is the oxidation of some of these that leads to the blue-black vascular streaking symptoms (Wheatley and Schwabe, 1985; Reilly et al., 2004). These compounds are suitable as an alternative visual assessment mainly because they fluoresce under ultra-violet (UV) light. Amongst these compounds scopoletin fluoresces most intensely. Scopoletin and the other hydroxycoumarins derive from general phenylpropanoid metabolism, the key entry enzyme to which is phenylalanine ammonia-lyase (Vogt, 2010), which increases in activity during the early response of PPD (Tanaka et al., 1983). In freshly harvested cassava roots scopoletin is barely detectable but its abundance increased rapidly after one day of harvest.

Quantification of hydrocoumarins in storage period over six days at 2-day intervals revealed high differences between varieties. However, it can be generalized that scopoletin concentration peaked at day 2 and gradually decreased by day 4 and 6 in highly susceptible cultivars while

* Corresponding author.
E-mail addresses: norhasima@unisza.edu.my (N. Mahmod), J.R.Beeching@bath.ac.uk (J. Beeching).

https://doi.org/10.1016/j.postharvbio.2018.05.019
Received 14 November 2017; Received in revised form 27 May 2018; Accepted 27 May 2018

in less susceptible cultivars it peaked at day 6 (Buschmann et al., 2000). On the other hand, scopolin, esculetin and esculin concentrations fluctuated with storage time but were found in lower concentrations than scopoletin making them less reliable as potential markers of PPD. Scopoletin also lends itself as a preferred biochemical marker over other alternatives because its distribution across along the root length is more homogenous. Measurement of fluorescence from the proximal to the distal end from 25 different cultivars showed a higher consistency of scopoletin than the PPD symptoms (Salcedo et al., 2010).

According to the FAO, delaying PPD up to two weeks would significantly improve the value of cassava, particularly its potential as an export commodity. Given this, extensive studies have been conducted to understand the biochemical activities and pathways involved in PPD including creating transgenic cassava overexpressing various anti-oxidant-related genes such as glutathione peroxidase (Vanderschuren et al., 2014) superoxide dismutase and catalase (Xu et al., 2013) and alternative oxidase gene (Zidenga et al. 2012). Also, there is an increasing number of PPD research teams which are based in countries where field cassava cultivation is not suitable. As an alternative, cassava is grown under greenhouse conditions on a smaller scale. Under such conditions space is at a premium, which leads to lower numbers of plants being grown, the use of small pots to encourage early rooting, and limiting the growth period of the plants to a few months. However, the evaluation of PPD symptoms remains unchanged which is usually based on a visual scoring system. The use of this scoring system is challenged by variation and inconsistencies in PPD symptoms which could be found even between sample replicates. The difficulty in assessing PPD has been acknowledged by many researchers in cassava PPD but has not been further investigated. The need to develop a reliable PPD marker is urgent, especially as the generation of reliable data that can be shared or compared. Where variations are least expected, the present study aims to evaluate the use of scopoletin as a visual marker using greenhouse-grown cassava.

2. Materials and methods

2.1. Plant material

Cassava plants (TMS 60444) were grown in 9 cm x 9 cm growing pots containing M2 Levington® compost. The plants were kept in a greenhouse with a temperature 26–30 °C under relative humidity of 40–80 % for six months.

2.2. Harvesting of cassava roots

Cassava plants were removed from compost and the storage roots were cleaned under warm running tap water and dried with tissue paper.

2.3. PPD assay 1

Two root sections were prepared from each plant replicate and placed on a Petri dish lined with a dry filter paper. The Petri dishes were kept covered and stored in a 27 °C incubator instead of the warm room. Humidity was provided by distributing approximately 100 mL distilled water in two Petri dishes which were left uncovered. The surface of the root sections was photographed at 0 DAH (day after harvest) and the same root samples were photographed again at 1, 3 and 4 DAH. Prior to photographing a thin layer of dried out surface (1 mm) was removed using a scalpel.

2.4. PPD assay 2

Four storage roots of a diameter 1.5–2.0 cm were obtained from each plant replicate. A pair of root sections of approximately 2 cm thickness was prepared from each storage root. A pair of root sections was assigned to a single time-point. The root sections were placed in a covered Petri dish with a dry filter paper used to support the base of Petri dish to avoid excessive transpiration. The Petri dishes were kept at 25–27 °C and photographing was carried out following Method 1.

2.5. White light photography

All photographs were taken using PENTAX K20D camera under uniform lighting and with the following settings; relative aperture f/5.6, exposure time 0.005 s, ISO 400.

2.6. UV light photography

All UV photographs were taken in a dark room using PENTAX K20D camera with the following settings; relative aperture f/2.8, exposure time 0.7 s, ISO 400.

2.7. Scoring of PPD

The root samples were subjected to computer-based scoring using image processing software called PPD Symptom Score Software [9]. Fluorescence accumulation was scored by visually rating the percentage of fluorescence produced on the surface of the root sections.

Data analysis

To find the association between fluorescence score and PPD score, the correlation of these scores were computed with Pearson Correlation at $p = 0.05$, two-tailed level of significance.

3. Results and discussion

3.1. Fluorescence profile is independent of PPD symptoms

This method is a modified method as the “whole root” method which is the most commonly used assay in the cassava research community working with field-grown material was found not suitable for storage roots produced in a greenhouse. Twenty-four pairs of storage root samples from six months old plants were subjected to PPD Assay 1 and photographs were taken at 0, 1, 3 and 4 DAH to track changes in PPD symptoms. The photographs were then used for estimation of PPD score using the image processing software. The same roots were photographed under UV light and scored using visual rating. It was observed that PPD symptoms were successfully induced using this method. Fig. 1 shows the development of PPD symptoms and fluorescence over the time course.

According to Figs. 1 and 2, neither PPD symptoms nor fluorescence was detected at 0 DAH. After 24 h, both the PPD symptoms and fluorescence started to significantly ($p < 0.05$) accumulate except that fluorescence accumulated more rapidly than the PPD symptoms. At 3 DAH most PPD symptoms became more visible, with brown staining being noticeably more prominent than the vascular streaking causing significant ($p < 0.05$) increase in PPD scores. On the contrary, the fluorescence level was maintained. At 4 DAH the roots fluoresced significantly less ($p < 0.05$) than at 3 DAH but PPD symptoms decreased non-significantly ($p = 0.24$).

PPD Assay 2 was different from PPD Assay 1 in which individual root sections were assigned to each PPD times. Compilation of 18 pairs of root sections taken over the storage period revealed PPD Assay 2 did not effectively produce PPD symptoms. The surface of all the root sections appeared white even until 4 DAH in which discoloration of root surface would have normally developed. In this method, water loss is reduced from the root sections and inadvertently delayed the development of PPD symptoms (Fig. 3).

On the other hand, fluorescence was produced following considerably similar trend as previously observed in PPD Assay 1. After 24 h, fluorescence level significantly peaked to the highest level ($p < 0.05$) but decreased significantly at 3 DAH. At 4 DAH there was a