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Full Length Research Paper

Comparative evaluation of physiological post-harvest root deterioration of 25 cassava (*Manihot esculenta*) accessions: visual vs. hydroxycoumarins fluorescent accumulation analysis

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Cassava (*Manihot esculenta*) is the most important root crop in the tropics and due to its drought tolerance, ability to grow in poor soils, and resistance to herbivore, cassava is well suited for cultivation by subsistence farmers. However, its use and expansion is constrained by rapid physiological post harvest deterioration (PPD), which often starts within 24 hours after harvest. PPD is a complex process that involves changes in the metabolic process and accumulation of secondary metabolites, such as hydroxycoumarins. The quantification of the fluorescence emitted by these hydroxycoumarins has been proposed as a quantifiable tool to evaluate PPD. Traditionally, the evaluation of PPD has been performed by more subjective methods based on the visual analysis of deterioration. Presented here is the use of a standard subjective rating in comparison to the accumulation of hydroxycoumarin fluorescence during PPD. PPD evaluation of ten month old tuberous roots from 25 accessions of cassava after five days of storage at room temperature shows that there was no correlation between the florescent accumulation of hydroxycoumarins and the visual symptoms. This suggests that the accumulation of hydroxycoumarins is not a reliable marker for evaluation of PPD response in different cassava accessions.

Key words: Cassava root, hydroxycoumarins, physiological post harvest deterioration.

INTRODUCTION

Cassava (*Manihot esculenta*), along with maize, sugar cane and rice, are the most important sources of dietary energy of tropical countries (Ceballos et al., 2004) and constitute an essential part of the diet of more than 500 million people (FAO, 2000). Every part of cassava can be used, but the starchy root is by far the most common food source. As a subsistence crop, cassava is the fourth most important crop in the developing countries as a source of calories surpassed only by maize, rice and sugarcane (Bradbury, 1988). When cultivated under optimal conditions cassava is one of the most efficient producers of edible carbohydrates among all of the world's major food crops. The production of cassava is estimated to reach 290.8 million metric tons per year by 2020, an increase from 165.3 million metric tons in 1996 (Scott et al., 2000). At 142 trillion kilocalories per year, cassava ranks first in edible energy production among major root and tuber crops and ranks fifth among all crops directly consumed by humans (Scott et al., 2000). Cassava is second only to sugar cane in caloric production per hectare (Scott et al., 2000).

Though the root is the main edible organ of cassava, the leaves also are consumed by many African cultures and are an excellent source of protein (8 - 10% FW) and vitamins (Bokanga, 1994). The main deficiency of

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Abbreviations: PPD, Post-Harvest Physiological Deterioration.

cassava roots is the lack of appreciable amounts of protein and with 0.9 grams per 100 grams of edible portion cassava has the lowest amount of protein content among major crops (Scott et al., 2000). Due to the low protein content (1-2%) additional food sources are required to ensure a diet balanced with proteins (Cock, 1985). Furthermore, other than for the presence of high vitamin C content cassava roots is not a good source of vitamins. As for vitamin C content, a serving consisting of 200g of cassava provides 71% of the recommended daily amount of vitamin C. Cassava is also not a rich source of minerals, providing less than 16% of the daily requirement for any minerals per 200g serving of cassava root. Manganese is the only exception, where 40% of the daily requirement is provided by a 200g serving of cassava.

In spite of the nutritional deficiencies cassava remains a key source of calories to the poverty stricken people in the world, due primarily to its agronomic properties. Cassava grows well under marginal conditions in degraded and acidic soils with minimal technical effort and has a flexible harvesting time ranging from 8 - 24 month after planting. Cassava is also amenable to partial harvest, which is the harvest of a couple of roots from a plant as needed, while the rest of the roots stay buried underground until the next need to harvest. A large proportion of cassava accessions are also drought tolerant, while being resistant to the most important diseases and pests (due to the presence of cyanogenic glycolsides). These attributes make cassava an attractive crop for small-scale farmers with limited resources particularly in sub-Saharan African populations (Ceballos et al., 2004; Wenham, 1995). Furthermore, cassava is also being grown and processed for animal feed and for various industrial applications (Beeching et al., 2002). However, because of its primary importance as a subsistence crop, research on cassava is often neglected by scientists in industrialized countries (Halsey et al., 2008).

Unlike others roots, such as yam or sweet potato, once cassava is detached from the plant it deteriorates rapidly and cannot be kept in satisfactory condition in storage for long periods. This phenomenon, known as post-harvest physiological deterioration (PPD) depends on the accession as well as the environmental conditions and is due to the onset of two modes of deterioration: physiological and pathological (Reilly et al., 2004).

Cassava root PPD, which often begins rapidly at the wounded proximal terminal of the root, is a complex abiotic process that is still not fully understood (Aristizabal and Sanchez, 2007). The initial symptoms are blue/black vascular streaking, brownish occlusions, and chemical deposits from wound sites along the roots xylem strands, followed by discolor-ration of the storage parenchyma, and accompanied by an unpleasant flavor and odor (Reilly et al., 2001, 2007). The initial visual symptoms of PPD are accompanied by a rapid accumulation of fluorescent compounds under UV light in the root parenchyma. These compounds have been identified as hydroxycoumarins such as scopolin, scopoletin, and esculin (Buschmann et al., 2000). Scopoletin, which has been identified as the most fluorescent compound (Rickard, 1982), is absent or has very low occurrence in fresh roots, However, during the first 24 to 48 hours after harvest its concentration increases 150 to 200-fold and is then followed by the accumulation of scopolin and esculin (Uritani et al., 1983; Aristizabal and Sanchez, 2007). A second smaller increase of hydroxycoumarins has been reported at 4-6 days post-harvest with a differential accumulation bet-ween accessions with low and high susceptibility to PPD (Buschmman et al., 2000). This finding led to the assumption that hydroxycoumarin accumulation is closely related with PPD, but more cassava accessions need to be tested to determine whether this is indeed correlation of significance (Oirschot et al., 2000).

Cassava root PPD limits the expansion of cassava production in developing countries, and has become a major constraint compared to other root crops, due to root discounting, waste and added production costs (Wenham, 1995; Westby, 2002; Reilly et al., 2004). The traditional practice of partial harvesting of roots in subsistence farming communities can delay the complete use of the roots per cassava plant, but the practice remains unfavorable in commercial fields. Furthermore, the remaining roots after partial harvest are subjected to a loss of starch content, decline of palatability due to the increase of fiber content and an associated increase in cooking time (Rickard and Coursey, 1981; Wheatley and Gomez, 1985).

Increasing the storage life of cassava roots to a minimum of two weeks by means of breeding or biotechnological approaches could have substantial effects on cassava utilization and potentially resolve constraints associated with marketing and storage practices (Oirschot et al., 2000). Efforts have been made to identify QTLs associated with resistance to PPD in cassava (Cortes et al., 2002).

These evaluations were carried out using a qualitative measurement based on the visible changes observed in the parenchyma (Wheatley et al., 1982). These efforts identified a few markers with poor association to PPD, presumably due to lack of an objective and systematic methodology to evaluate the PPD level in cassava, as well as the existence of a high variation in cassava root PPD response. The objective of this study was to compare two methods to evaluate cassava root PPD: visual analysis and the accumulation of florescent compounds associated to PPD in parenchyma root tissue using image analysis software.

MATERIALS AND METHODS

Plant material

Twenty-five cassava accessions from the Caribbean, Africa, Central America and South America (Table 1) were grown 1 m apart at the Isabela Agricultural Experimental Station of the University of Puerto

Table 1. Morphological data and PPD scores of 25 cassava accessions using a fluorescence accumulation method and a visual inspection method (Average ± Standard error).

Cassava accessions	Root length (cm)	Max.root diameter (cm)	PPD fluorescent (%)	PPD visual (%)
SM 494	33.8 ± 8.8	3.9 ± 1.0	68 ± 6.8	1 ± 1.3*
Amarillo	26.8 ± 9.7	4.8 ± 1.0	89 ± 9.9**	2 ±1.0*
PI12900	32.2 ± 11.9	6.8 ± 0.7	45 ± 7.6	3 ± 0.4*
Brava	28 ± 17.9	3.2 ± 0.5	34 ± 5.7	5 ± 3.4
CM 3380	30.6 ± 4.7	5.0 ± 1.8	76 ± 6.9	7 ± 3.4
PI2902	22.6 ± 2.5	6.9 ± 0.8	30 ± 3.6	9 ± 2.0
Abuelo	25 ± 4.6	6.5 ± 2.7	85 ± 3.1	13 ± 3.3
Jamaica 18	24.2 ± 4.6	5.8 ± 0.9	31 ± 4.0	13 ± 4.5
Senon	27.4 ± 7.6	6.7 ± 6.4	83 ± 5.9	13 ± 8.6
Tremesiana	29.6 ± 8.8	4.1 ± 0.4	40 ± 12.5	22 ±9.6
TMS60444	22.8 ± 7.2	4.7 ± 0.9	70 ± 9.9	24 ± 2.6
PI12903	58.2 ± 17.9	5.6 ± 0.6	33 ± 11.2	24 ± 8.4
Forastera	37.6 ± 17.5	4.6 ± 0.6	15 ± 4.7*	27 ± 6.1
Chilena	24.6 ± 5.1	5.2 ± 1.0	62 ± 8.0	30 ±10.8
Serralles	22 ± 2.9	4.8 ± 0.3	27 ± 5.2	32 ± 5.5
CM 4484	33 ± 14.7	6.4 ± 1.3	79 ± 5.3	32 ±16.3
Seda	23.4 ± 4.2	2.8 ± 0.5	39 ± 4.1	33 ±5.1
CM 3311	22.4 ± 9.0	5.4 ± 1.6	66 ± 5.2	38 ± 6.1
Cubana	27.2 ± 9.8	4.0 ± 0.4	39 ± 7.1	51 ± 5.3
CM 3064	24.2 ± 6.5	4.8 ± 0.5	64 ± 6.8	57 ±17.7
Trinidad	28.2 ± 15.5	5.0 ± 1.6	33 ± 8.3	58 ± 17.3
SM 523	19.0 ± 8.8	3.5 ± 0.6	69 ± 3.2	61 ± 14.9
Valencia	28.2 ± 5.1	4.3 ± 0.5	69 ± 8.9	64 ±8.2
Llanera	20.4 ± 5.2	3.0 ± 0.6	45 ± 6.7	67 ± 13.2
Mcol 2215	20.2 ± 5.5	4.3 ± 0.8	64 ± 5.8	70 ±7**

* Accessions with significantly different (P<0.05) low PPD. ** Accessions with significantly different (P<0.05) high PPD.

Rico, Mayagüez, located on the Northeast coast of Puerto Rico. Five cassava roots (replicates for each accession from different plants) from 10-month old plants were harvested by digging the rhizosphere area and carefully removing the roots from the soil while avoiding any wounding. Root peduncles were removed and entire roots were stored for five days under ambient conditions and protected from sun light. Root length and maximum root diameter were measured.

PPD evaluation

After five days, six transverse sections at 15, 30, 45, 60, 75 and 90% of the total length were cut from each root, starting from the proximal end. A slice (0.3 cm average thickness) was cut from the distal end of each transverse section. Digital pictures of each slice were obtained at 50 cm of distance from the slice with and without ultraviolet light exposure (302 nm). Visual inspection was done according to Wheatley et al. (1982) by assigning a score between 0 and 100% to each slice based on the observed physiological deterioration of the central parenchyma surface of each slide root.

Image data analysis

Digital images for each slice were analyzed using Pixcavator Image Analysis Software v 3.1 (Intelligent Perception, Huntington, WV, USA) with the following settings: JPG format, green-blue channel, shrink factor 4.

Contrast and growing rate were set until fitting non-deteriorated area and total area of the root slide. PPD score for each slide were assigned according the following equation:

%PPD = [1-(Non-deteriorated area/Total area)] x 100

The % PPD is the percentage of deterioration per slice. The deteriorated area and total area were measured in pixels. PPD percentage deterioration for each cassava accession was obtained by averaging each independent PPD percentage from biological replicates (the average of slices 15, 30, 45, 60, 75 and 90% of total length) (Figure 1).

Statistical analysis

All statistical analyses were carried out using Infostat v 2009 (Grupo Infostat, Cordoba, Argentina. Analysis of variance was used to evaluate the differences between cassava root accessions. The relationship between visual and fluorescence accumulation scores were assessed using linear regression, by taking the mean score of each accession.

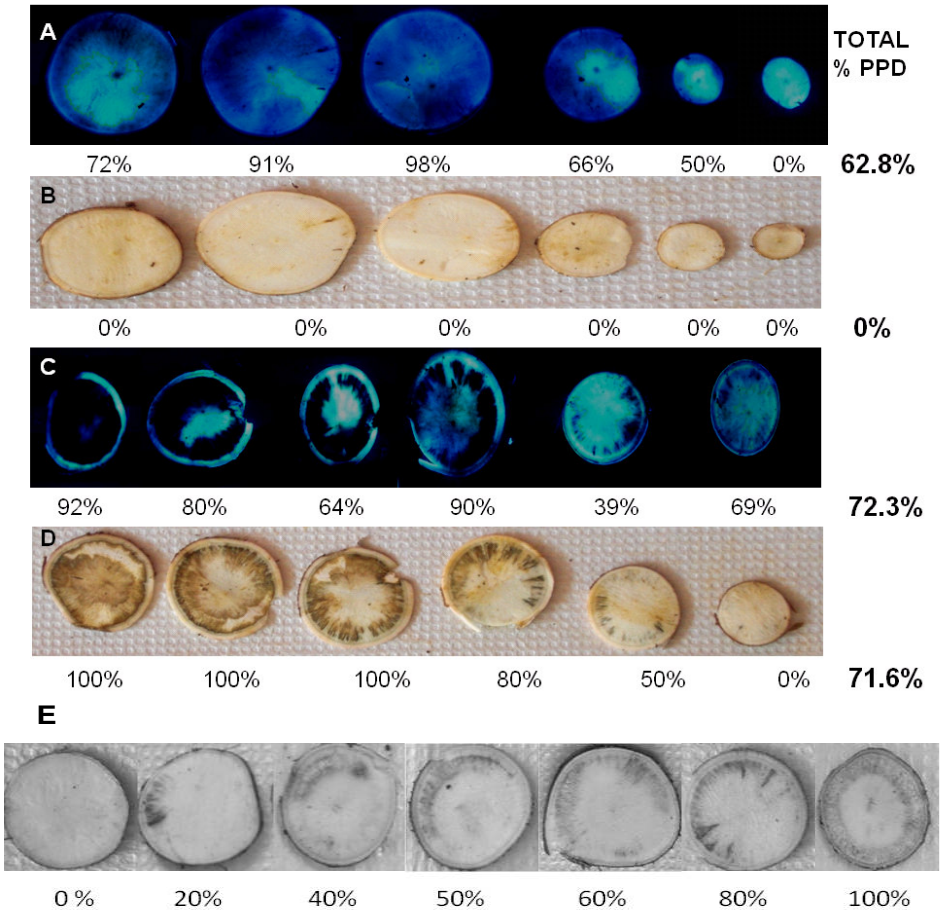

Figure 1. Comparison between fluorescence accumulation and visual symptoms of PPD, one replication is shown. Fluorescence accumulation scores were obtained by means of image analysis software (Pixcavator ver 3.1). Visual scores based on methodology described by Wheatley et al. (1982). A. SM 494 fluorescence accumulation scores under ultraviolet light. B. SM 494 visual scores. C. Mcol 2215 fluorescent accumulation scores under ultraviolet light. D. Mcol 2215 visual scores. E. Scale used for the visual PPD analysis.

RESULTS AND DISCUSSION

PPD scores after five days of storage for 25 cassava root accessions were measured by using image analysis of fluorescence accumulation and visual inspection.

Entire roots were selected to induce the natural conditions of post harvest deterioration, which is different from conventional procedures that use a method based on 15 cm root block tissue (Wheatley et al., 1982). Roots after five days of storage were used to evaluate PPD since it has been previously reported that at 5 days after storage metabolic changes associated with PPD occur (Hiroshe et al., 1984), including the accumulation of hydroxycoumarins (Buschmman et al., 2000). On average, the lengths of the selected roots were 27.6 cm with an average maximum root diameter of 4.9 cm. There were no correlations between either the root length or the maximum root diameter with PPD analysis using either the fluorescent accumulation method or the visual method (data not shown).

After averaging the transverse cross sections, accessions SM 494, Amarillo and PI12900 under visual inspection and the accession Forastera under the fluorescent accumulation method showed the lowest levels of PPD. Comparatively, accession Mcol 2215 under visual inspection and the accessions Senon, Amarillo and Abuelo under the fluorescent accumulation method showed the highest levels of PPD (Table1). This contrasting results were clearly confirmed when regression analysis showed no association between the two methods (R_2=0.007) (Figure 2a). Only six accessions showed comparable percentages of PPD between both the fluorescence accumulation and visual inspection methodologies (Serralles, Seda, CM 3064, Mcol 2215, SM 523 and Valencia). Similar comparative analysis performed for each transverse cross section of 15, 30, 45, 60 75 and 90% also showed no association between the two methods (Figure 2b). It is interesting to note that

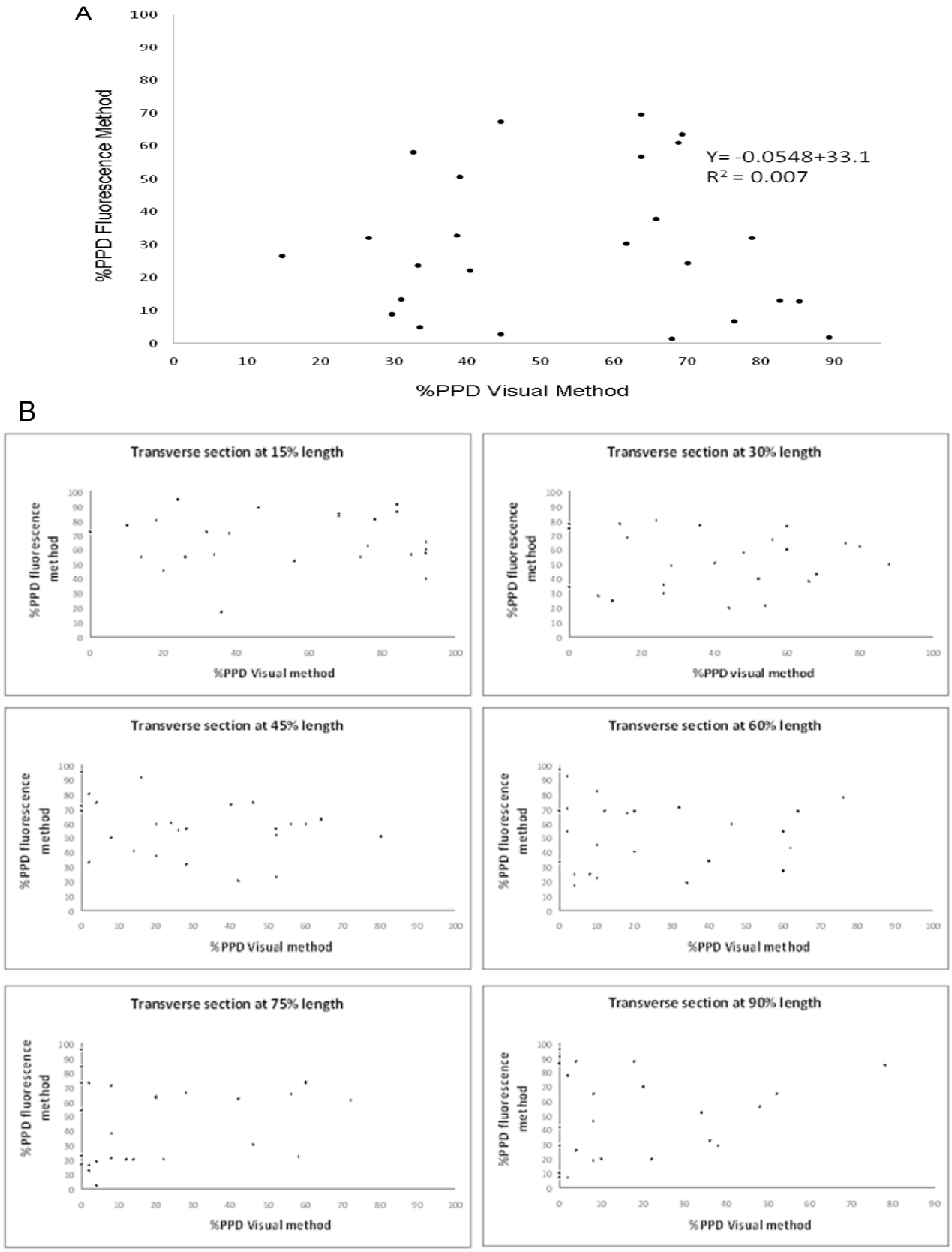

Figure 2. Association between percentage of PPD using fluorescence accumulation of hydroxycoumarins and the percentage of PPD based on the visual inspection method. A. Average of all transverse sections (regression analysis was based in on PPD scores transformed by Arcsin fuction). B. Individual transverse sections.

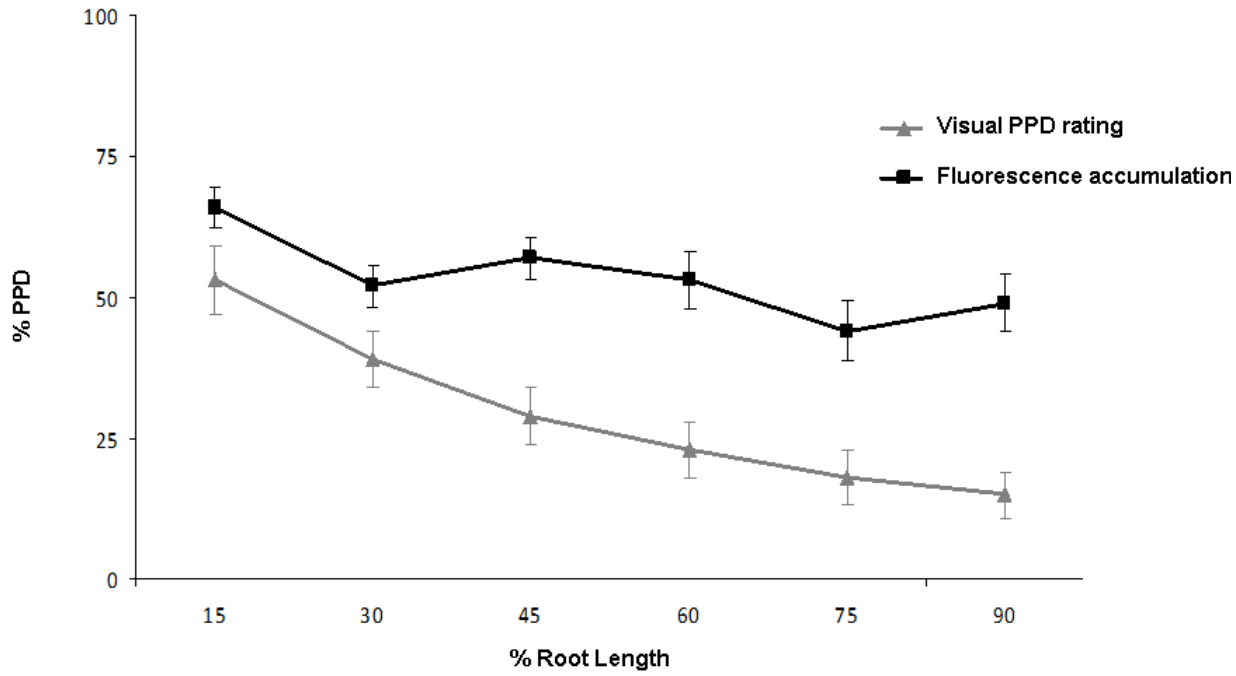

Figure 3. Average percentage of PPD using fluorescence accumulation and visual inspection in different sections of the root of the 25 cassava genotypes. Bars represent standard error.

accession Amarillo showed the highest level of PPD under the fluorescence accumulation method but demonstrated very low levels of PPD under visual inspection. The roots of Amarillo are light yellow in color and Sanchez et al. (2006) has shown a high association between parenchyma root color and carotenoid content. In turn, carotenoid content in cassava roots has been shown to be inversely correlated to the reduction or delaying of PPD (Chavez et al., 2007). This example demonstrates that hydroxycoumarin accumulation alone cannot explain the susceptibility to PPD in all cassava accessions.

Overall, the findings contradict the suggestion that an increasing of the fluorescence hydroxycoumarins (mainly scopoletin) between 4 - 6 days is related to PPD response in cassava roots, but corroborate the main results found by Wheatley and Schwabe (1985), Buschmann et al. (2000) and Oirschot et al. (2000), which did not report a clear correlation between scopoletin accumulation using spectroscopy and fluorescence methods with PPD susceptibility using visual inspection. The reason for this behavior can be attributed to a decreasing and stabilization of hydroxycoumarin content before the main visible symptoms appear. All the transverse sections would be accumulating equivalent contents. This phenolmenon was observed by Wheatley and Schwabe (1985) after three days of PPD and by Buschmann et al. (2000) after six days of PPD. Evidence for this can be seen when the average concentrations of fluorescence accumulation and the average score for the visual inspection at each length are compared. From the visual inspection, there was a progressive reduction in the percentage of PPDfrom the proximal (15%) to the distal (90%) slices, which is expected since the deterioration is progressive from the proximal to the distal end. However, this tendency was less evident using the fluorescence method after five days of PPD (Figure 3).

A post harvest loss in cassava globally is approximately 19% of production, with Latin America, Asia and Africa being 10, 8 and 29%, respectively. In 2005, the estimated loss of cassava due to PPD is 26 million tons, calculated to be an approximate loss of US$2.9 billion at $111/ton. Increasing the shelf-life of cassava storage roots is desirable not only to solve problems of utilization and marketing but also to facilitate the conversion of cassava from a traditionally famine reserve crop and rural food staple to a cash crop. To accomplish this, cassava breeders and biotechnologists need a method in which they have confidence to evaluate PPD, in order to use genotypic variation and gene-transfers to overcome the problem. This study shows that there is no association between the accumulation of hydroxycoumarins and PPD. Oirschot et al. (2000) has demonstrated that a significant negative correlation exists between sugar/starch ratio and PPD, which could be an alternative method to explore. Due to the lack of a proper quantitative method to evaluate PPD in a simple but confident way, visual inspection remains the preferred method, though subjective, to evaluate PPD in cassava roots.

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