International Journal of Food Science +Technology

Original article

Post-harvest physiological deterioration in several cassava genotypes over sequential harvests and effect of pruning prior to harvest

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(Received 6 April 2020; Accepted in revised form 1 July 2020)

Summary Consumers, traders and processors consider post-harvest physiological deterioration (PPD) an important constraint. In Experiment 1, PPD was assessed three consecutive years in roots from five genotypes through seven storage days. PPD, scopoletin and dry matter content (DMC) was recorded during storage. Year, genotype, duration of storage and their interactions were significant. PPD was associated with duration of storage period, DMC and scopoletin contents. Ambient moisture and temperature during storage influenced PPD. In Experiment 2, roots from seven clones were harvested 10 months after planting from 30 consecutive biweekly plantings. PPD was assessed 0, 2 and 7 days after harvest. In 13 harvests, roots from plants pruned six days earlier were also evaluated. Results indicated large seasonal variation across genotypes. Pruning reduced PPD and DMC. Complex and contrasting relationships among the variables analysed were found. There is no uniform model explaining the relationship between PPD and the independent variables considered.

Keywords Dry matter content, fresh market, post-harvest losses, scopoletin, shelf life.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important food security crop for millions of people, particularly in Sub-Saharan Africa. It is also an important industrial crop reliably providing raw material for the starch, animal feed and ethanol industries. Predictions suggest that as result of climate change, cassava cultivation will expand in the years to come (Ceballos *et al*., 2011; Mbanasor *et al*., 2015; Pushpalatha and Gangadharan, 2020).

Several factors affect the ability of cassava to satisfy new and increasing demands. One of them is post-harvest physiological deterioration (PPD) in the roots (Djabou *et al*., 2017; Liu *et al*., 2017; Zainuddin *et al*., 2018). PPD rapidly renders the roots unpalatable and unmarketable few days after harvest. Consequently, cassava roots need to be consumed soon after harvest unless they are preserved in some manner (van Oirschot *et al*., 2000).

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A short shelf life makes roots a risky product to market and an inconvenient and expensive food for the urban dweller (Prakash, 2008). Several studies report significant losses (up to 30%) due to PPD, particularly in Sub-Saharan Africa (FAO/IFAD, 2002; Ndunguru *et al*., 1998; Nweke *et al*., 2002; Manu-Aduening *et al*., 2005; Fakoya *et al*., 2010; Thompson, 2013). Value chain surveys in Africa found out that the fresh market of cassava is almost exclusively done by women. These surveys indicated that deterioration of roots after harvest was the second most important constraint for marketing and processing cassava (Thompson, 2013). In cases where cassava is an important commodity for industrial processing (i.e. Thailand), PPD has also been demonstrated to result in up to US $35 million economic losses annually (Vlaar *et al*., 2007). More recently, it has been estimated that nearly a third of total fresh roots harvested worldwide are lost to PPD (Saravanan *et al*., 2016).

Different treatments (waxing their surface to reduce oxygen influx into the root, refrigeration, freezing,

doi:10.1111/ijfs.14711

drying, exogenous application of melatonin, pruning plants before harvest, etc.) can extend the shelf life of cassava roots (Booth, 1977; Wheatley, 1989; van Oirschot *et al*., 2000; Hu *et al*., 2016). However, these methods are expensive or logistically difficult to implement. There are several reports on genetic variation for PPD (Sánchez *et al*., 2006; Morante *et al*., 2010; Moyib *et al*., 2015). Different laboratories have used a transgenic approach to attempt to overcome PPD (Xu *et al*., 2013; Zainuddin *et al*., 2018). A major factor affecting research on PPD is the large influence of environmental conditions (particularly near harvest time), age of the plant, handling of the roots during harvest and thereafter, and storage conditions (Zainuddin *et al*., 2018). Experimental errors, therefore, are typically large and mask true genetic differences that may exist (Mahmod & Beeching, 2018). The accumulation of fluorescent compounds (mainly scopoletin) has been associated often with PPD (Saravanan *et al*., 2016; Mahmod & Beeching, 2018; Zainuddin *et al*., 2018).

The present study focuses on PPD, DMC and scopoletin in cassava roots from several genotypes. Additionally, the impact of pruning before harvest was tested. The main strength of our research is the large volume of data generated and analysed and the fact that responses of several genotypes through many harvesting times have been assessed.

Materials and Methods

Geographic location

Roots were harvested from two experiments at the Centro Internacional de Agricultura Tropical (CIAT) headquarters in Palmira, Colombia. In Experiment 1, planting and harvesting took place once each year from 2013 through 2015. For Experiment 2, stem cuttings from different genotypes were planted every other week through a period lasting two years (2016–2017). Plants were harvested 10 months after planting (also every other week), resulting in 30 harvesting dates. In addition, for some of these harvesting dates, a few plants from each genotype were pruned (the top of the plant was removed and left without leaves) six days before harvesting.

Cassava genotypes

Seven cassava genotypes were selected for their contrasting PPD levels. MNIG11, MPER183, MCOL22 and MCOL1505 are accessions from the germplasm collection at CIAT. AM206-5 is a genotype that produces amylose-free starch (Morante *et al*., 2010). CM523-7 is a bred variety released for the eastern savannas of Colombia in the 1990s. SM1219-9 is an elite clone developed by CIAT and used often as progenitor. HMC-1 is a released variety grown at mid-altitudes in Colombia. These genotypes were selected because of their expected contrasting reaction to PPD based on observations by the CIAT Cassava Breeding Program across many years and locations.

Experiment 1

Roots from AM206-5, MPER183, HMC-1, MCOL22 and CM523-7 were screened for three consecutive years (2013, 2014 and 2015). Forty-eight roots of commercial size (>5 cm in diameter and 15 cm in length) were selected for each genotype at each harvesting date. The storage period lasted for up to seven days. Six roots were selected randomly from each genotype and evaluated each day from day 0 through day 7.

Experiment 2

Roots from seven genotypes were screened (SM 1219-9, CM523-7, HMC-1, MNIG11, MPER183, MCOL22 and MCOL1505) for two consecutive years (2016 and 2017). Three important changes distinguish this experiment from the first one: i) PPD was assessed only at 0, 2 and 7 days after harvest; ii) the sequential planting of each genotype allowed up to 30 harvesting dates during the two years the experiment lasted; and iii) PPD was also assessed in roots from plants that had been pruned (the top of the plants was cut and removed) before harvest in 13 of the harvesting dates.

PPD Score

The evaluation of PPD in cassava roots is a destructive procedure. It was carried out according to the method developed by Wheatley in 1982, with some modifications (Sánchez *et al*., 2013). Roots were stored on shelves at room temperature under roof, protected from the sun and rain, but without walls. The environmental conditions during storage were monitored continuously with temperature and relative humidity data loggers. Every day (including harvest day, or *day zero*), roots of each genotype were selected randomly and evaluated for PPD. The duration of the storage period (**DAY**) was one of the independent variables considered in this study.

For evaluation, roots were transversely cut into seven slices (thickness 0.02 m), starting at the proximal end. Each slice was assigned a PPD score between 0 and 10, corresponding to the percentage of the cut surface that showed discoloration of the parenchyma (0 = 0%, 1 = 10%, 2 = 20%, etc.) (Sánchez *et al*., 2013). The average PPD score for each root was calculated by averaging the scores of the seven slices. Roots, that showed symptoms of microbial

decomposition or were affected by insects, were discarded (no data were recorded for discarded roots).

Root processing

The slices of each genotype were peeled and cut into small pieces. The pieces were homogenised using a stainless-steel food processor (SKYMSEN Food Processor MODEL PA-7SE. Brusque, Brazil) until a uniform mass was obtained.

Dry matter content

Three independent samples (aliquots of 40 g) were taken from the mass obtained after root processing. The samples were dehydrated in an oven (BINDER Class 2.0, serial no. 0848115, D-78532 Tuttlingen, Germany) at a temperature of 105 °C for 24 h. Dry matter content (**DMC**) was expressed in per cent as the ratio between dry weight and fresh weight of the sample (Sánchez *et al*., 2013).

Determination of scopoletin by high performance liquid chromatography (HPLC)

The methodology described by Sánchez *et al*. (2013) was used, with some modifications. Five grams of homogenised cassava obtained after root processing was weighed and used for analysis. Scopoletin was quantified using a standard calibration curve with eight levels of concentrations (5, 25, 50, 75, 100, 125, 150, 175 x 10^{-3} g.L^{-1}) and was measured in triplicate.

Environmental conditions during storage of roots

The experimental facilities at CIAT in Palmira have a weather station that provides many different climatic parameters. Temperature and relative humidity in the air at different hours during the day were obtained for the periods in which roots were stored in the first experiment.

Statistical analysis

The arcsine transformation for binomial proportions was applied to PPD data for the analysis of variance (Snedecor & Cochran, 1980). Regression analyses were performed on the data generated. The dependent variable was PPD, and the independent traits were DAY (i.e. duration of the storage period), DMC and scopoletin expressed on a fresh- (**SFW**) or dry-weight (**SDW**) basis. Scopoletin quantifications were included in the regression analyses in different ways. In Experiment 1, PPD scores were related to the daily evolution of scopoletin. For example, PPD measured in roots that had been stored for five days was linked to SFW and/or SDW also measured on the fifth day. The impact of initial levels of scopoletin (e.g. as soon as roots were harvested) on the onset and evolution of PPD through the different lengths of storage periods was also tested. Therefore, regression analyses were also performed linking PPD at different days with scopoletin levels at harvest day (**SFW(0)** and **SDW(0)**).

In Experiment 1, regression analyses were conducted across clones but also individually for each clone. A stepwise process defined the sequence of parameters in the model. A *lack of fit* test was then used to identify the best reduced model (Allen & Cady, 1982):

$$\text{Lack of fit} = \frac{(\text{SS Full Model} - \text{SS Reduced Model})/\text{Difference in degrees of freedom}}{\text{Residual Mean Square of Full model}}$$

where SS stands for sum of squares.

Results

Experiment 1

During storage, several roots began to show symptoms of microbial rotting, and hence, the number of roots remaining for evaluation seven days after harvest was limited. Therefore, data from roots stored for seven days were not considered in order to maintain the balance of the data. Scopoletin data in year 2015 were only taken in one root per storage day and clone rather than the six roots evaluated in the previous years.

There was a wide variation in the levels of PPD observed through the duration of this experiment. Environmental conditions during the storage period were correlated with PPD responses. There was a clear and negative association between PPD levels and average air relative humidity during the storage period. The strongest correlation (−0.997) was observed with relative humidity measured at 13:00 PM. Similarly, there was a positive relationship between air temperature during storage and levels of PPD. The strongest correlation was between PPD and maximum average temperature (0.986), followed by the average daily temperature (0.803) and average minimum temperature which was, as expected, negative (−0.624). It can be concluded from Experiment 1 that dry air and high temperatures tend to increase the levels of PPD.

Main sources of variation (YEAR, DAY, CLONE) and their interactions were highly significant (0.001 probability level), except for the year-by-day interaction for PPD, which was significant at the 0.052 probability level (Table S1). Average PPD in 2013 was about half of those values observed in 2014 and 2015 (Table 1). Interestingly, average DMC in 2013 was also significantly lower than that in the other years. SFW and SDW showed similar patterns, but the averages for 2015 were intermediate between those in 2013 and 2014.

As expected, PPD increased gradually with the duration of the storage period (Table 1); especially, we observed a major increase in the scores on the second day. There were significant differences for PPD among clones (Table 1). We note the large number of roots screened for each genotype compared with earlier reports. The mean PPD value for each clone showed a clear association with the respective DMC averages. The relationship between PPD and SFW and SDW was not so clear (Table 1). Figure 1 illustrates the relationship between PPD and the three independent variables analysed across years and genotypes. Although there are positive relationships, the dispersion of data points around the regression line explains the relatively low R^2 values. The relationships illustrated in Figure 1 suggest that more than one factor influences PPD outcome and that regression analysis with more than one independent variable may be necessary.

Figure 2 illustrates the evolution of PPD, DMC and SFW through the six days of storage. Only SFW is presented (SDW and SFW followed similar patterns). The increase in PPD through time is apparent. DMC remains more or less constant, with a narrow tendency for a reduction through time as previously reported (Sánchez *et al*., 2013). A sharp increase in SFW up to the second day of storage is conspicuous. Thereafter, scopoletin levels began to fall but in an oscillating fashion. The evolution of scopoletin through time behaves as previously reported (Buschmann *et al*., 2000; Sánchez *et al*., 2013; Mahmod & Beeching, 2018). SFW reached maximum levels at the second day of storage in every clone (Figure 3).

The top section of Table 2 shows a multivariate regression analysis across clones. The sequence in the full model was DAY, DMC, SFW(0), SDW, SFW and SDW(0) ($R^2 = 0.300$). However, the best model (based on the lack of fit test) included only DAY and DMC with a slightly reduced $R^2 = 0.292$. That is, SFW(0), SDW, SFW and SDW(0) were found to explain a negligible proportion of the variation observed for PPD across the five clones. The residual sum of squares remains large. This is not surprising as it is well established that experimental errors associated with PPD

Table 1 Least squares means for the four variables analysed in Experiment 1 for the three most relevant sources of variation (years, duration of the storage period and clones)

	PPD[a]			DMC[a]			Scopoletin FW[a]			Scopoletin DW[a]		
Class	***n***	**(%)**		***n***			***n***	**(nmol g^{-1})**		***n***	**(nmol g^{-1})**	
Year												
2013	210	7.4	b	210	33.5	c	210	17.9	c	210	53.5	c
2014	209	15.5	a	209	40.4	a	194	52.3	a	194	127.8	a
2015	209	14.6	a	209	38.5	b	35	33.0	b	35	84.4	b
Day (Duration of the storage period in days)												
0	90	0.0	e	90	37.1	f	65	3.5	g	65	9.8	g
1	90	1.7	d	90	37.7	c[b]	65	26.3	f	65	67.4	f
2	90	12.9	c	90	37.7	b[b]	65	52.9	a	65	137.9	a
3	89	13.7	c	89	37.4	e	64	39.3	d	64	100.8	d
4	90	17.7	b	90	36.9	g	60	41.9	b	60	108.8	b
5	90	19.1	ab	90	37.6	d	60	36.1	e	60	93.9	e
6	89	22.6	a	89	37.8	a	60	41.6	c	60	106.5	c
Clone												
AM 206-5	125	6.7	d	125	33.4	e	91	25.8	d	91	76.4	c
MCOL22	125	8.5	cd	125	37.6	c	90	24.1	e	90	61.8	e
MPER183	126	9.2	c	126	35.9	d	76	28.5	c	76	79.6	b
HMC-1	126	13.3	b	126	40.0	b	91	62.7	a	91	149.9	a
CM523-7	126	24.7	a	126	40.4	a	91	29.6	b	91	74.6	d

[a]Duncan's multiple range and LSD tests for contrasts among averages yielded the same conclusions regarding the statistical differences among them. For each category and response variable, averages with the same letter are not significantly different. PPD, post-harvest physiological deterioration; DMC, dry matter content; FW, fresh weight; DW, dry weight. In the case of PPD, significances presented are those based on the ANOVA for ArcSin (PPD)
[b]Actual dry matter values were 37.67 and 37.72 and significantly different.

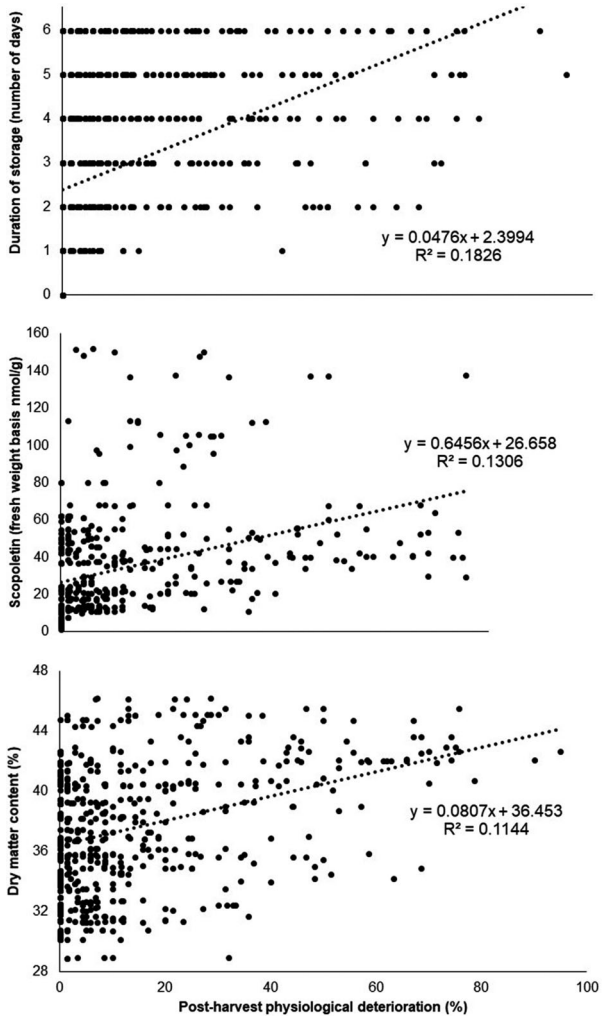

Figure 1 Relationship between PPD (%) and three independent variables in Experiment 1: duration of storage period (days after harvest), scopoletin levels and dry matter content. Each data point represents a single root.

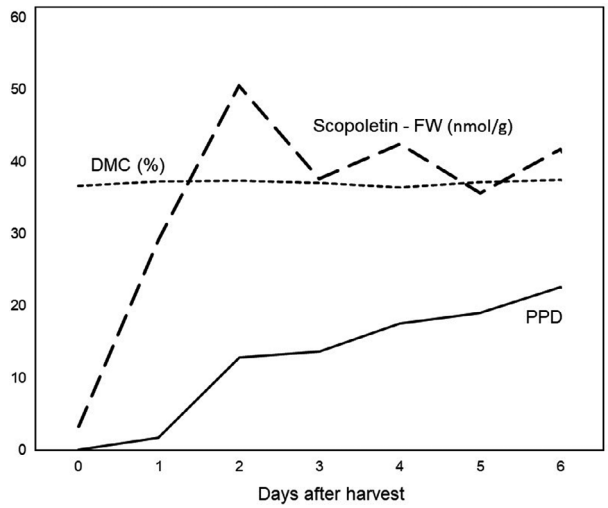

Figure 2 Evolution of DMC, scopoletin content (fresh-weight basis) and PPD after storing cassava roots for up to seven days (average across five contrasting genotypes) in Experiment 1.

studies are high (Mahmod & Beeching, 2018). Also, genetic variation could not be included in this model in spite of evidence of its relevance (Mahmod & Beeching, 2018; Morante *et al.*, 2010).

The significance of the genetic source of variation (clones) and the different interactions detected by the ANOVA suggested individual regression analyses for each clone (Table 2). The level of association between different independent traits and PPD changed for the different clones. The order in which each clone is presented in Table 2 goes from those with lowest (AM 206-5, 6.7% PPD) to the highest average PPD value (CM 523-7, 24.6% PPD). In each case, the best model is presented with parameters ordered in the sequence determined by the stepwise regression approach.

There was a notable contrast between the regression models for each clone (Table 2). Two or three parameters defined the best models. In most cases, the first parameter in the model was DAY. For MCOL22, however, scopoletin (SFW) was more important in explaining PPD values than the duration of storage. Initial levels of scopoletin at harvest time were important for AM206-5 (dry-weight basis) and MPER183 (fresh-weight basis). The coefficients of determination were low in clones with low PPD averages but relatively high for CM523-7 ($R^2 = 0.497$). It is clear that there is no single model to explain PPD and that factors affecting it, vary from clone to clone.

However, there were sharp differences in the evolution of scopoletin through time in the roots of different genotypes. The maximum scopoletin level of HMC-1 (>90 nmol g^{-1}) was about twice as large as that of clones CM 523-7 and MPER 183 (45–50 nmol g^{-1}) and three times larger than for roots of MCOL22 and AM 206-5 (≈30 nmol g^{-1}). Roots from HMC-1, CM 523-7 and MPER 183 showed similar increases in SFW during the first day of storage. However, SFW values continued to rise at the same rate in the roots of HMC-1 through the second day of storage, whereas those from 523-7 and MPER 183 began to level off. Significant increase of SFW in MCOL22 and AM 206-5 could only be observed after two days of storage; thereafter, the levels remained more or less constant. On the other hand, scopoletin levels in HMC-1, CM 523-7 and MPER 183 gradually decreased after the maximum attained on the second day.

Table 2 Relevant results of the regression analyses conducted across clones (top) and for each individual clone in Experiment 1

Best model							
Model SS	**Error SS**	R^2	**Coefficient in best model**				**Full model**
Across the five clones							
58763	143239	0.292	Intercept	DAY	DMC		R^2
			-61.22	3.55	1.51		0.300
			(98236)	(36877)	(21886)		
AM 206-5							
3850	11024	0.261	Intercept	DAY	SDW(0)		
			−33.68	1.14	16.41		0.303
			(5663)	(2846)	(1004)		
MCOL 22							
9617	20456	0.320	Intercept	SFW	DAY	SDW	
			13.04	2.59	3.08	−1.00	0.323
			(9083	(6221)	(1012)	(2383)	
MPER 183							
6455	14146	0.316	Intercept	DAY	SFW(0)	DMC	
			115.26	2.22	−4.77	−1.69	0.324
			(10650)	(3615)	(1763)	(1078)	
HMC-1							
10014	21547	0.318	Intercept	DAY	SFW		
			2.42	3.15	0.35		0.321
			(22450)	(8027)	(1987)		
CM523-27							
38810	39846	0.497	Intercept	DAY	DMC	SDW	
			−153.16	6.78	1.22	0.28	0.523
			(7662	(24280)	(12278)	(2252)	

The R^2 values for the full model (six parameters) are at the right. Information from the best (reduced) model identified for each data set, including the R^2, sum of squares and regression coefficients is presented. Reduced models required two or three parameters.
(1/DAY stands for the duration (in days) of the storage period; DMC, dry matter content; SDW, Scopoletin levels on a dry-weight basis; SFW, Scopoletin levels on a fresh-weight basis.

Experiment 2

Average PPD values were significantly different for the 30 harvesting dates ranging from 1.9 to 29.5% (ANOVA not presented). In general, the highest PPD levels were observed in the later harvests. The analysis of variance indicated that all sources of variation (harvesting date, genotype, duration of the storage period and their interactions) were highly significant ($P < 0.001$). The roots-within-clone source of variation, however, was not statistically significant.

Figure 4 illustrates the large variation observed in selected genotypes from Experiment 2. The four genotypes represent very high (37.5% in CM 523-7), high (15.2% in MCOL22), moderate (9.3% in HMC-1) and low (4.4% in SM 1219-9) average levels of PPD. In three of the 30 harvesting dates, data from one of the four clones were missing and thus they were not considered in the plot. The salient information in Figure 4 is the large oscillations in PPD levels through the different harvesting dates. Acceptable confidence in genetic differences for PPD, therefore, can be achieved only through several harvesting batches. As was the case in Experiment 1, CM523-7 almost always had the highest PPD levels. Low PPD levels were observed in both experiments for MPER183. Results from HMC-1 and MCOL22 (the other clones included in both experiments), on the other hand, were more inconsistent.

The main effects of pruning plants six days before harvest relied on 13 harvesting dates and three clones that had been pruned (or not) before harvest. The main variables of interest were DMC and PPD. All sources of variation (harvesting date, clone and pruning) and their interactions were highly significant, except for the Clone-by-Pruning interaction for DMC, which was significant at $P < 0.074$ (ANOVA not presented). The most important

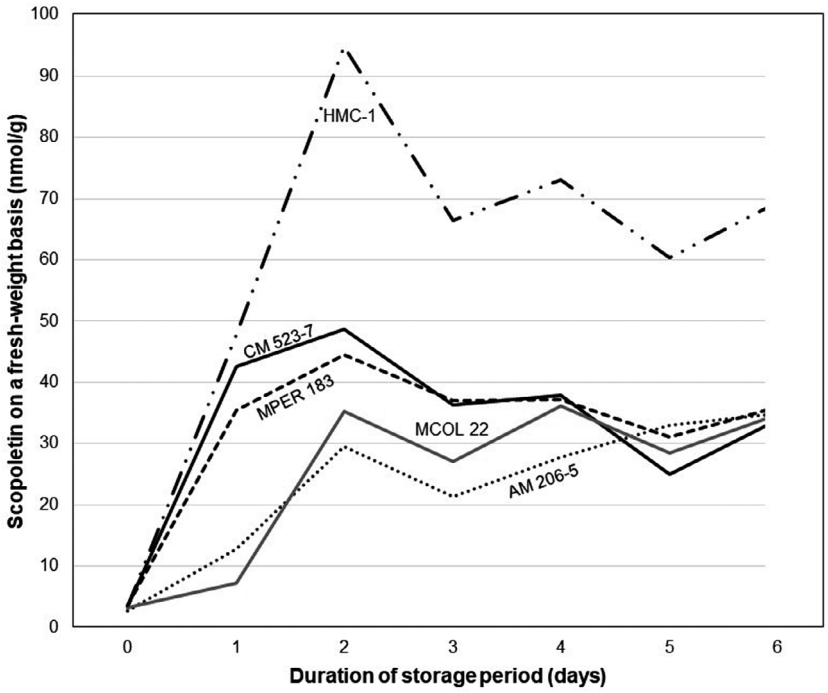

Figure 3 Evolution of scopoletin content (fresh-weight basis) through seven days of storage of roots from five different clones in Experiment 1.

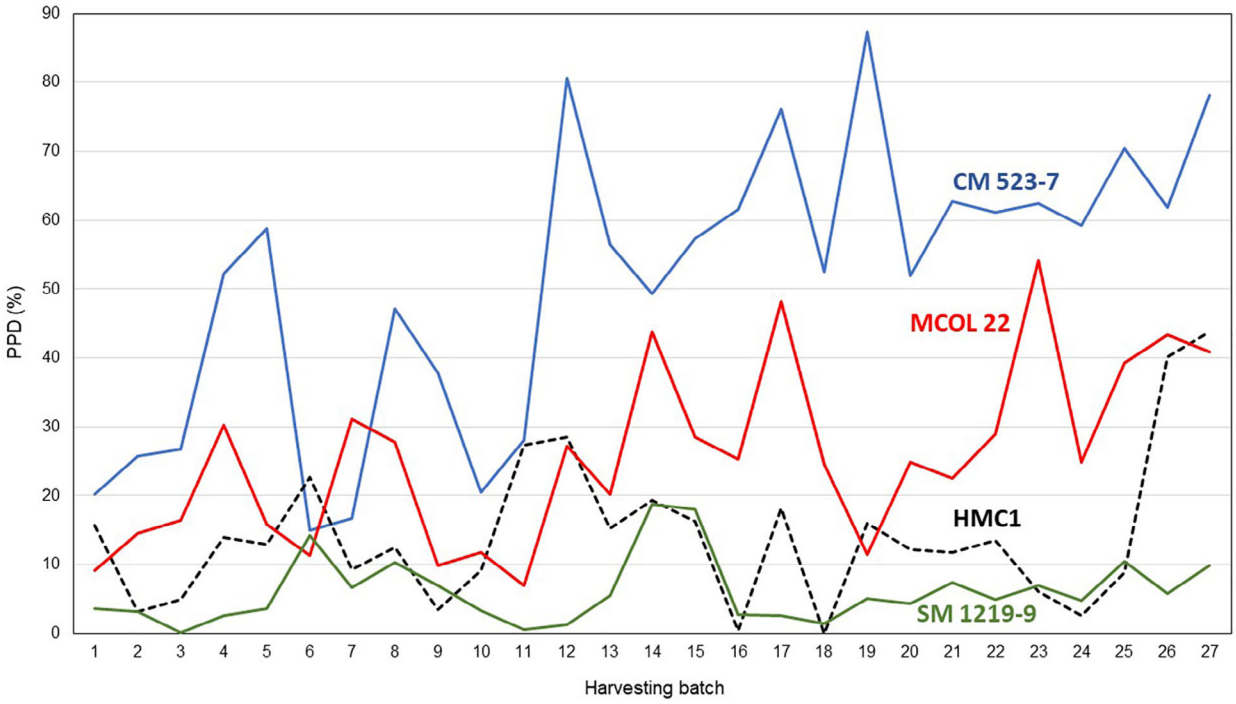

Figure 4 Variation in PPD levels from four of the seven genotypes (to reduce complexity of the graph) screened in Experiment 2. Data presented are the average PPD after seven days of storage for roots from each clone at each harvesting date.

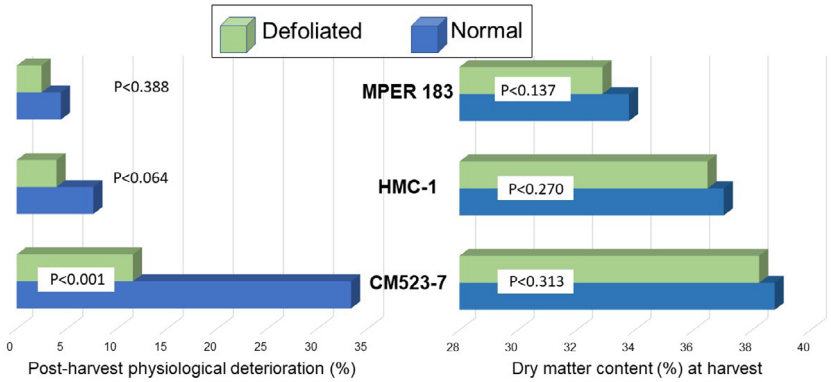

Figure 5 Impact of pruning seven days before harvest on dry matter content and post-harvest physiological deterioration in three clones. Results combined across 13 harvesting batches.

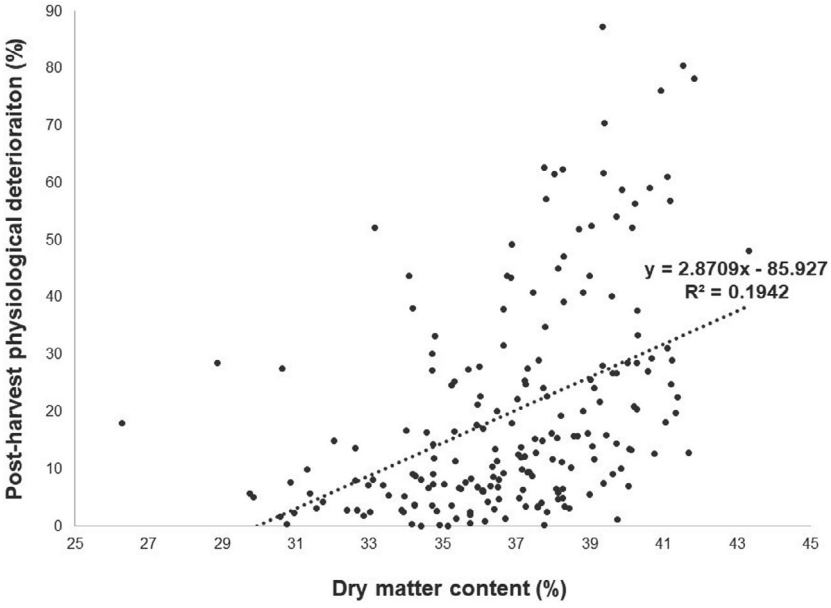

Figure 6 Relationship between average PPD and DMC for seven clones in 30 harvesting batches from Experiment 2.

result is that pruning plants before harvest had a highly significant ($P < 0.001$) effect on DMC and PPD (averages across clones).

Figure 5 presents a comparison of PPD and DMC averages from normal husbandry versus pruning before harvest (average of three clones and 13 harvesting dates). Pruning significantly reduced PPD (from 33.3 down to 11.6%, $P < 0.001$) in CM523-7. It also reduced PPD in MPER 183 and HMC-1, but differences failed to reach statistical significance. Pruning reduced DMC across clones ($P < 0.001$). Although pruning also reduced DMC at the individual clone level, differences failed to reach statistical significance (Figure 5).

Environmental conditions affect dry matter content in the roots (Ceballos *et al.*, 2011). Pruning the plants is an artificial modification of the conditions under which plants grow. Figure 5 shows that pruning had an effect not only on PPD, but also on DMC. It is possible, therefore, that some of the variation of PPD depicted in Figure 4 is due to an indirect effect of the environmental conditions on DMC. Figure 6 presents the relationship between PPD and DMC for each clone through the 30 harvesting batches of Experiment 2. The R^2 value of the regression of PPD on DMC was 0.19. This is considerably higher than the similar plot in Figure 1. In part, this is because genetic differences have been taken into consideration for Figure 6.

Discussion

Dry matter content had an impact on PPD levels in the analysis across genotypes (Table 2). This is in agreement with previous reports (Sánchez *et al.*, 2006; Morante *et al.*, 2010). The inclusion of DMC in the regression models for individual clones was only justified in two cases (MPER183 and CM523-7). In the

remaining clones, however, the influence of DMC on PPD was negligible (Table 2). The relevance of DMC in the individual regressions for each clone was not as clear as in the regression across clones. This makes sense because differences in DMC are much larger between clones than among roots from the same clone.

The contrast between CM 523-7 and HMC-1 in Experiment 1 deserves a closer analysis. These two clones had high (24.7%) and intermediate (13.3%) PPD levels, respectively. Average DMC of these clones was similar (40.4 and 40.0%, Table 1). The most conspicuous difference between these two clones was for scopoletin content. There was a positive relationship between scopoletin and PPD across the entire experiment (Figure 1). However, this overall trend contradicts the specific situation of these two clones. HMC-1 had much higher SFW levels (average = 62.7 and maximum = 97.2 nmol g^{-1}) than CM 523-7 (mean = 29.6 and maximum = 45.6 nmol g^{-1}), yet it had lower PPD score (Table 1).

The comprehensive review on PPD by Zainuddin and co-workers in 2018 highlights the complexities and challenges that research on this topic entails. PPD is conditioned by pre-harvest cultural practices, such as pruning (Ravi & Aked, 1996; van Oirschot *et al.*, 2000; Figure 5), environmental conditions during growing and harvest (Figure 4), injuries to roots during harvest and handling, environmental conditions during the storage (as suggested by data from Experiment 1), duration of the storage period, genetic variation and experimental procedures to assess it. Age of roots and heterogeneity in their size may also affect precision of results. The standard procedure based on the visual assessment of PPD is laborious and prone to large experimental error. Alternative approaches such as sugar/starch ratio or fluorescence screening have been evaluated, but so far offer little promise (Mahmod & Beeching, 2018).

Our results complement those in the literature. It can be summarised that control of PPD includes four strategies: i) modifying storage conditions; ii) selection of tolerant genotypes; iii) preconditioning cassava tissue before harvesting; and iv) preventing active gene expression during storage. Some of these strategies can be combined.

The first approach is to control the environmental conditions during storage. This study confirms the advantages of increasing relative humidity and/or reducing temperature. Evaporative cooling, refrigeration and freezing root sections remain the most widely used protocols for the reduction of PPD. A second economically feasible method is to reduce oxygen levels by waxing roots with paraffin. Modified atmospheres could be another alternative but has not been widely implemented at the commercial level. These methods, although effective, are expensive and require a basic infrastructure that is often missing, particularly in Sub-Saharan Africa.

This study provides evidence than selecting clones with tolerance to PPD and acceptable levels of DMC is possible. HMC-1 has similar DMC compared with CM523-7 but lower PPD levels (Tables 1 and Table S1). SM1219-1 showed very low levels of PPD in Experiment 2 (Table S2), while DMC was acceptable (35.9%). Although scopoletin and DMC have been shown to be related to PPD, this study also provides evidence on their inconsistent association. Screening for PPD reaction is still inefficient, prone to large experimental error and affected by strong genotype-by-environment interactions. Improvements in the screening protocol are, therefore, critical for the deployment of tolerant cultivars. A rapid, simple and non-destructive method to monitor the peroxidase activity in cassava roots, based on time domain nuclear magnetic resonance, has been evaluated (da Silva et al., 2018). Our group at CIAT is currently developing an image-interpretation algorithm to facilitate scoring of PPD. It may be useful to assess the association between PPD and respiration of roots during storage. Such study is currently underway at CIAT and showed promising preliminary results discriminating genotypes tolerant and susceptible to PPD. Root respiration may have a more consistent relationship with PPD than scopoletin or DMC.

The third strategy proposed above is preconditioning tissues before harvesting. This study demonstrates that pruning plants before harvest improves shelf life of roots, supporting this type of approach. Although pruning is effective, it also reduces DMC which can negatively impact market acceptability. The main effect of pruning plants before harvest is to reduce respiration of roots during the storage (Ravi & Aked, 1996). Pre-treatment of plants with products that can later reduce respiration of roots during storage may be the solution that the entire value chain demands. The cassava program at CIAT is exploring the possibility of spraying plants (before harvest) with chemical compounds such as 1-methylcyclopropene or silver thiosulphate (STS). These compounds regulate ethylene and plant tissue respiration. Preliminary results were promising but not conclusive. Considerable efforts need to be invested in determining dosage and timing of the applications.

The last strategy is preventing gene expression during root storage. There is an active modulation of transcriptome and proteome, in which more than 100 genes have been found to be up- or down-regulated (Zainuddin *et al.*, 2018) during storage. In an unpublished study, roots were exposed to high levels of gamma rays prior to storage but did not prevent PPD (G. Amenorpe and R. Thompson, personal communication). It had been assumed that the high levels of radiation used (1300 Gray) would severely affect DNA

and prevent the expression of PPD-related genes. Studies at higher radiation levels should be conducted. Irradiating roots is probably too expensive as a tool to control PPD, but could contribute to our understanding of the metabolic processes leading to it.

Experiments 1 and 2 are complementary in nature. The focus in the first study was the relationship between PPD and DMC, scopoletin and genetic differences through seven days of storage. The second experiment focused on the influence of environmental conditions through the growing season and at harvest and on the effect of pruning before harvest. Several conclusions can be drawn from the two experiments:

1 There are large genetic differences for the reaction to PPD. Proper assessment requires several batches in different seasons.
2 Differences in PPD levels among clones can be partially explained by differences in their DMC. Clones with low DMC tend to have low PPD.
3 Although scopoletin levels seem to be correlated with PPD across genotypes, there are exceptions when individual clones (particularly those with high PPD) are considered.
4 Environmental conditions during growth and at harvest strongly affect PPD. A separate study should associate a large set of weather and soil variables with Experiment 2 data.
5 Environmental conditions during the storage period affect PPD. This validates storage methods designed to reduce temperature and/or increase relative humidity and can explain some of the variation in Experiment 2.
6 Pruning plants before harvest reduces both PPD and DMC levels. The final costs of pruning can be reduced, if the foliage is sold for animal feeding.
7 PPD is a complex phenomenon which seems to depend on several factors acting differently in each clone. A single, universal model that explains PPD in cassava roots seems unlikely. Based on this study scopoletin, DMC and carotenoids should be reported in future PPD studies.

Acknowledgments

This work was supported by the CIAT Cassava Program (Colombia), CIRAD Qualisud Research Unit, and funded mainly by the CGIAR Research Program on Roots, Tubers and Bananas (RTB) with support from CGIAR Trust Fund contributors (https://www.cgiar.org/funders/) and the grant opportunity ID: OPP1178942: Breeding RTB products for end user preferences (RTBfoods), to the French Agricultural Research Centre for International Development (CIRAD), Montpellier, France, by the Bill & Melinda Gates Foundation (BMGF) https://rtbfoods.cirad.fr. The editorial comments and corrections by Clair Hershey improved greatly the quality of this manuscript. The valuable suggestions and corrections by reviewers also contributed significantly for the quality of this article.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.14711.

Data Availability Statement

Data available on request from the authors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Analysis of variance for Experiment 1. The ANOVA is based on Type III sum of squares.

Table S2. Average, across harvesting dates, for each of the clones evaluated in Experiment 2.