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Effects of harvest period, storage, and genotype on postharvest physiological deterioration responses in cassava

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Abstract. *Rahmawati RK, Khumaida N, Ardie SW, Sukma D, Sudarso. 2021. Effects of harvest period, storage, and genotype on postharvest physiological deterioration responses in cassava. Biodiversitas 23: 100-109.* Postharvest physiological deterioration (PPD) is the major constraint in cassava root production. The breeding program to develop PPD tolerant cassava varieties requires a long period to complete. Although it is the first step in breeding for PPD tolerance, evaluating cassava germplasm responses for the PPD remains a major problem because it is a laborious process, and the evaluation often contains a high experimental error. This study aims to develop methods for evaluating PPD response, i.e., evaluating the effects of two harvest periods and storage on PPD responses of nine cassava genotypes. The developed scoring system based on cassava root discoloration could group the evaluated cassava genotypes into either PPD tolerant, medium tolerant, or sensitive. The tolerant varieties showed less than 10% root discoloration areas, while the medium tolerant was between 11-20%, and the sensitive was larger than 20%, respectively. ADR-24 and GJ-11 were identified as PPD tolerant, while ML-19 was sensitive using the developed scoring system. This study showed that PPD is a complex phenomenon associated with genetics and environmental factors. Root dry matter content and maximum root diameter traits may play an important role in PPD development in cassava. We have developed procedures for identifying genotype responses to PPD and showed that cassava roots harvested as early as eight months after planting and stored as late as five days under the control relative humidity were good conditions for studying PPD. Evaluating the percentages of root discoloration was a good measure for PPD response prediction. Moreover, we showed that less than 10%, 20%, and more than 20% of root discoloration might be used to group cassava into PPD tolerant, medium tolerant, and sensitive for Indonesian accessions. Therefore, the methods developed in this study may support the Indonesian cassava breeding program and provide biochemical and molecular analysis materials to elaborate the mechanisms of PPD in cassava roots.

Keywords: *Manihot esculenta*, storage-root, shelf-life, PPD, dry-matter-content

Abbreviations: DAH: days after harvest; DMC: dry matter content; MAP: months after planting; MRD: maximum root diameter; PPD: postharvest physiological deterioration

INTRODUCTION

Cassava is an essential crop in many countries, and it is used as a staple food, animal feed, industry materials, and biofuels (FAO 2018). Indonesia also has significant potential as one of the biggest cassava producers in the world (FAO 2019). However, this advantage was constrained by the short shelf-life of cassava roots because of postharvest physiological deterioration (PPD). The PPD resulted in either blue, black, or brown discolorations and a bitter taste of the cassava roots. Therefore, the PPD makes cassava unmarketable shortly after harvest (Hu et al. 2016; Liu et al. 2017). In Indonesia, the cassava production losses due to PPD amounted to more than 25% (Ginting 2002). Moreover, PPD has caused a significant economic impact in cassava-producing regions (Naziri et al. 2014). Therefore, solving the PPD problem will be beneficial.

The breeding program positively solves the problem due to PPD in cassava production (Salcedo and Siritunga 2011; Tumuhimbise et al. 2015). Breeding for cassava requires the availability of germplasm resources carrying the desirable traits and reliable methods for evaluating the target phenotypes (Morante et al. 2010). In the previous research, genetically diverse cassava germplasms have been regenerated through gamma-ray irradiation of six cassava varieties to induce random genetic mutations (Subekti 2013; Maharani 2015). The previous report also identified two putative mutants that were PPD tolerant (Rina 2019). Those two putative mutants showed fewer PPD symptoms during the cassava root storage period of 28-days (Rina 2019). Therefore, the two putative mutants could be used as references for developing the standard method for PPD tolerant cassava germplasms. The availability of simple techniques for evaluating PPD responses among cassava accessions is essential to support cassava breeding programs (Rahmawati et al. 2021).

PPD evaluation using visual appearance to quantify the root discoloration was still preferred due to easy observation. However, this method is destructive and uses different cassava roots in every storage period. Therefore,

to generate accurate data associated with PPD responses, many commercially uniform sizes of cassava roots were needed (Morante et al. 2010; Tumuhimbise et al. 2015). Moreover, the cassava roots should have minimum root damage (Garcia et al. 2013; Tumuhimbise et al. 2015). The PPD is probably also associated with agronomic traits and biochemical processes in the stored cassava roots. A complex biochemical process has also been reported (Uarrota et al. 2016). Consequently, the PPD evaluation process is laborious and time-consuming (Luna et al. 2020). The available methods for PPD tolerance evaluation have some disadvantages, which affect the accuracy of the results (Garcia et al. 2013; Tumuhimbise et al. 2015). Moreover, the analysis results for PPD responses using the available methods are also contained a high experimental error (Morante et al. 2010; Gracia et al. 2013; Mahmod and Beching 2018). According to Morante et al. (2010), Tumuhimbise et al. (2015), and Verturini et al. (2015), the PPD response trait is controlled genetically. However, environmental factors also played an essential role in PPD response, especially to the onset of the PPD responses (Zainuddin et al. 2018).

The confounding effect of the environmental factors makes the accurate measurement of genetic factors controlling PPD traits less accurate (Tumuhimbise et al. 2015). Plant age was assumed to affect PPD because the scoring of PPD responses increased with months of planting, and roots harvested at ten months showed lower PPD symptoms than ones harvested at 13 months (Coelho et al. 2019). Therefore, according to Luna et al. (2020), evaluation in several harvest periods can be a strategy to minimalize the variance.

This research aimed to evaluate the consistency of PPD responses of cassava roots harvested at two different harvesting periods, identify the tolerance of nine cassava genotypes to PPD, and conduct correlation analysis among cassava root agronomic traits and PPD responses. The evaluation results developed more objective selection criteria for identifying PPD tolerance in cassava breeding programs and selecting potential PPD tolerant cassava genotypes.

MATERIALS AND METHODS

Plant materials and planting locations

Roots were harvested from cassava grown in Sukamantri (560 m asl) and Cikabayan Field Experiment (240 m asl). The cassava roots were harvested at either eight or 13 months after planting (MAP) in 2020 (M8 generation mutants) and 2021 (M9 generation mutants). The harvested cassava roots were handled and evaluated in the Postharvest Laboratory, Departement of Agronomy and Horticulture, Bogor Agricultural University, Indonesia. The experiments evaluated the PPD responses of nine cassava genotypes (Table 1).

Planting, harvesting, and storage of cassava roots

The vegetative propagules were planted at 1 x 1 m^2 planting distances (1 m within and among rows). Urea (200 kg ha^{-1}), SP36 (150 kg ha^{-1}), and KCl (150 kg ha^{-1}) were used as fertilizers and applied four weeks after planting. All plants were maintained according to standard cassava agronomic practices until ready to harvest either eight or 13 months after planting (MAP).

The harvesting and handling of cassava roots were carefully processed to minimize any mechanical damages. Thirty commercial cassava roots per genotype were selected and stored five or ten days after harvest (DAH). Therefore, fifteen cassava roots (biological replications) for each genotype were evaluated every storage period. The criteria for a commercial size for cassava roots include the root length is at least 20 cm (Fukuda et al. 2010), the diameter is ≥ 5 cm (Luna et al. 2020), and the size is uniform. The evaluated cassava roots were stored under the ambient air temperature ranging from 25 to 28 °C and relative humidity from 70 to 95% before PPD evaluation.

Qualitative and quantitative traits evaluation

The harvested cassava roots from each tested genotype were evaluated for several qualitative and quantitative traits. As described by Fukuda et al. (2010), the recorded cassava root qualitative traits include (i) presence and extent of peduncle; (ii) existence of storage root constrictions; (iii) root shapes; (iv) epidermis color; (v) parenchyma color; (vi) cortex color; (vii) ease of cortex peeling; (viii) epidermis texture; and (ix) bitterness taste. For the quantitative traits, the collected data include (i) root length (cm); (ii) maximum root diameter (cm); (iii) weight losses (%) after storage; and (iv) dry matter content (DMC) (%). As much as 20-30 g of cassava root samples were chopped into small pieces and dried in an oven at 105 °C for 24 hours to estimate DMC (Qin et al. 2017: Luna et al. 2020). The DMC was calculated as the dried cassava root sample weight divided by the fresh weight (Sanchez et al. 2013).

Table 1. List of nine cassava genotypes evaluated in this study and their genetic background

Cassava genotype codes	Remarks
ADR-24	Mutant #24, M8, and M9 generation derived from Gamma-ray irradiation of cassava cv. Adira 4
GJ-8	Mutant #8, M8, and M9 generation derived from Gamma-ray irradiation of cassava cv. Gajah
GJ-11	Mutant #11, M8, and M9 generation derived from Gamma-ray irradiation of cassava cv. Gajah
ML-0	Malang 4, an Indonesian commercial cassava variety (cassava cv. Malang 4)
ML-18	Mutant #18, M8, and M9 generation derived from Gamma-ray irradiation of cassava cv. Malang 4
ML-19	Mutant #19, M8, and M9 generation derived from Gamma-ray irradiation of cassava cv. Malang 4
RTM-0	Ratim, a local cassava variety from Halmahera
RTM-26	Mutant #26, M8, and M9 generation derived from Gamma-ray irradiation of Ratim
UJ-0	UJ-5, an introduced cassava variety from Thailand

Note: M8 and M9-8th and 9th generation of vegetative propagation mutants derived from Gamma-ray irradiation treatments

Postharvest physiological deterioration (PPD) evaluation

PPD symptoms in the cassava roots were evaluated for both storage periods using a destructive method (Morante et al. 2010). The cassava roots were transversally sliced (3-5 mm) at four relative positions (25.0, 37.5, 50.0, or 75.0% of the root proximal end), as illustrated in Figure 1. The root slice surfaces were scanned using a Canon MG2570S scanner to capture the presence of root discoloration. The scoring of PPD symptoms was expressed as percentages of black or brown discolorations areas to total areas using ImageJ software (Zidenga et al. 2012; Xu et al. 2013; Qin et al. 2017). Cassava roots infested with microbes were excluded from the PPD symptom evaluation as suggested by Quevedo et al. (2014).

Data analysis

The qualitative root trait data were analyzed using PBSAT-CL to construct a cophenetic dendrogram with the Gower dissimilarity method and clustering based on the average linkages. The score of PPD symptoms was transformed using Arcsine square before analysis (Garcia et al. 2013)**.** Subsequently, all data were analyzed using analysis of variance (ANOVA), and mean comparisons were made using Duncan's multiple range tests (DMRT) at $\alpha = 0.05$. The correlation among PPD symptoms to other quantitative traits was evaluated using Pearson's correlation, and the regression model was generated using stepwise multivariate analysis. Statistical analysis was conducted using either statistical functions of Microsoft Excel or Statistical Tools for Agriculture Research (STAR) software.

RESULTS AND DISCUSSION

Cassava root qualitative traits

The visual characterization of the cassava roots can easily be differentiated by observing the root epidermal color, the cortex color, and the epidermis texture. Representative of three classes based on these qualitative characters was presented in Figure 2. The tested nine cassava genotypes were grouped into three groups based on the cassava root qualitative traits. Group I c rough and dark brown epidermis and purple cortex. Group II consists of the rough and dark brown epidermis and pink cortex. Meanwhile, Group III consists of the light brown or yellow color and a smooth epidermis and white or cream cortex. The tested cassava genotypes belonging to group I was GJ-8 and GJ-11; Group II was ADR-24, ML-0, ML-18, and ML-19; Group III was RTM-0, RTM-26, and UJ-0 (Table 2).

Meanwhile, only the ADR-24 cassava genotype consistently showed the presence of peduncles in all the storage roots (Table 2). On the other hand, the eight genotypes showed a mixture of cassava roots with and without peduncles (Table 2).

The PPD response evaluations

Environmental conditions during the root storage were summarized in Table 3. The storage temperatures ranged from 25.5 to 28.8 °C, and the relative humidity was from 74.0 to 95.0%. The average temperature in both evaluations (roots harvested either from eight or 10 MAP) was similar. The average temperature ranged from 26.6 to 26.8 °C, while the relative humidity from 80.3 to 85.3% (Table 3).

Previous studies have shown that temperature and relative humidity were two factors associated with PPD symptoms development. Dry air and elevated temperature promoted the PPD symptoms (Garcia et al. 2013; Luna et al. 2020). However, in this experiment, the correlation between environmental conditions and PPD symptoms was weak (Table 3). Pearson's correlation coefficients were smaller than 0.2, and they were not significant statistically (Table 3). Therefore, environmental conditions should not cause PPD response differences during cassava root storage.

Results of the ANOVA showed that the genotype and the interaction between the genotype by the harvest period effects were significant ($p < 0.05$) (Table 4). Therefore, the tested cassava genotypes were probably responded differently at the eight or 13 MAP. However, only the cassava genotype ML-19 showed differential PPD responses between roots harvested at eight to those at 13 MAP (Table 5). Moreover, the PPD symptoms occurred during either the storage periods (5 or 10 DAH). Storage periods did not significantly affect PPD symptoms, indicating that five days of storage is as good as ten days in inducing PPD symptoms.

The five days storage period resulted in more accurate PPD symptoms evaluation. Microbial infections occurred when the roots were stored for 10 days. In this experiment, the data showed no microbiological infections at five days after root storage. Moreover, at 10 DAH, microbiological infections occurred more frequently than at 5 DAH. The microbial infection resulted in more discoloration in the evaluated cassava roots (Figure 3). Therefore, the cassava roots infected with microbes were discarded, and no PPD evaluation was conducted because of the confounding symptoms.

For cassava roots harvested at eight MAP, the microbial infected root samples ranged from 0 to 5 from a total of 15 samples, and with an average of infected roots across genotypes was 2.7. No microbial infection was seen at ten days after harvesting for two samples of cassava genotypes (GJ-11 and UJ-0) harvested at eight MAP. On the other hand, for cassava roots harvested at 13 MAP, the microbial infected root samples ranged from 0 to 1 from a total of 15 samples, with an average of infected roots across genotypes was 0.6. No microbial infection was seen at ten days after harvesting for samples of cassava genotypes (ADR-24, GJ-8, GJ-11, and UJ-0) harvested at 13 MAP. The examples of augmented discoloration because of confounding effects of PPD and microbial infection were presented in Figure 3. Because of the microbial infection, the cassava genotype supposed to be medium tolerant to PPD (ML-0) with the score of root discoloration supposed to be less than 20%, showed a more severe discoloration score of >20% (74.3%).

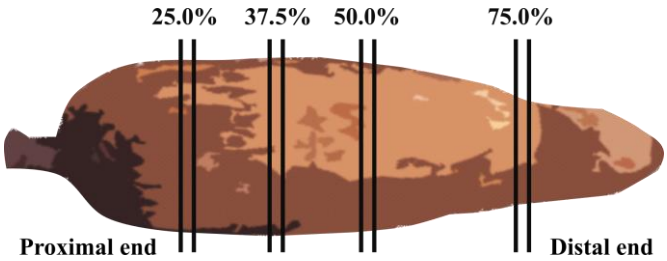

Figure 1. Position of root slice cutting positions to evaluate PPD from the proximal to the distal ends. The cassava root cutting was conducted at four relative positions, such as 25.0, 37.5, 50.0, and 75.0% of the root proximal end

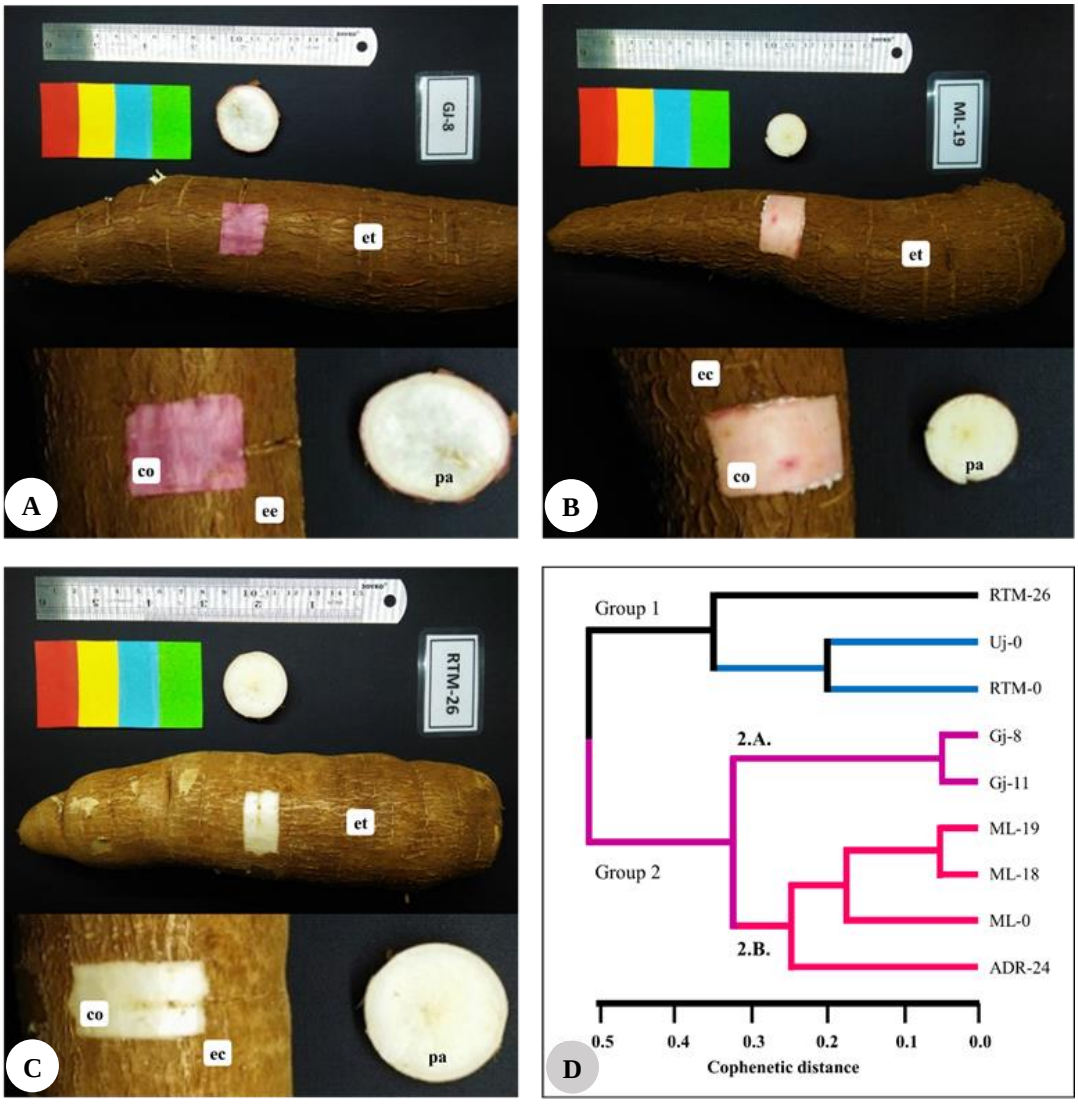

Figure 2. The representative of three groups of cassava root qualitative traits, epidermis texture (et) and color (ec), and cortex (co) color as represented by GJ-8 (A) with the dark-brown and rough texture of epidermis and purple cortex, light-brown or yellow/smooth/white or cream, ML-19 (B) with the dark-brown and rough texture of epidermis and pink cortex, and RTM-26 (C) with the light-brown or yellowish and smooth texture of epidermis and white or cream cortex, respectively. All genotypes have white parenchyma color. (D) The constructed dendrogram using the qualitative trait data, i.e., epidermis texture (et) and color (ec), and cortex (co) color. The studied cassava genotypes were classified into two groups, such as group 1 and group 2. Subsequently, group 2 was divided into two subgroups, i.e., 2.A. and 2.B

Table 2. The qualitative traits of the roots of nine cassava genotypes in this research. Evaluated samples consisted of three plants per-genotypes.

Genotypes	The presence of peduncle	Cassava root traits		Color traits			Cortex peeling	Epidermis texture	Taste
		Constriction	Shape	External epidermis	Cortex	Paren-chyme			
ADR-24	100%	Some	Conical-cylindrical	Dark brown	Pink	White	Easy	Rough	Intermediate
GJ-8	Mixed	Few to none	Conical-cylindrical	Dark brown	Purple	White	Easy	Rough	Sweet
GJ-11	Mixed	Few to none	Conical-cylindrical	Dark brown	Purple	White	Easy	Rough	Sweet
ML-0	Mixed	Few to none	Conical-cylindrical	Dark brown	Pink	White	Easy	Rough	Bitter
ML-18	Mixed	Some	Conical-cylindrical	Dark brown	Pink	White	Easy	Rough	Bitter
ML-19	Mixed	Some	Conical-cylindrical	Dark brown	Pink	White	Easy	Rough	Intermediate
RTM-0	Mixed	Some	Conical-cylindrical	Light brown	White or cream	White	Easy	Smooth	Bitter
RTM-26	Mixed	Few to none	Cylindrical	Yellow	White or cream	White	Easy	Smooth	Sweet
UJ-0	Mixed	Some	Conical	Yellow	White or cream	White	Easy	Smooth	Bitter

Table 3. The ambient temperature and relative humidity during the root storage periods

Harvest periods (Months after planting)	Storage periods (Days after harvest)	Ambient temperature (°C)			Relative humidity (%)		
		8 AM	12 AM	3 PM	8 AM	12 AM	3 PM
8	5	25.8	26.0	26.5	90.0	77.0	73.0
	10	25.5	25.9	26.8	82.0	77.0	79.0
13	5	28.8	27.6	26.3	78.0	74.0	95.0
	10	27.1	26.8	26.7	91.0	93.0	84.0
Means		26.8	26.6	26.6	85.3	80.3	82.8
Pearson's correlation coefficient between ambient temperature or relative humidity and PPD responses		0.013	0.024	0.085	0.131	0.200	0.004

Table 4. Summary of the analysis of variance (ANOVA) results for the main effect of genotypes, harvesting periods, and storage periods and their respective interactions to PPD scores of cassava roots

Sources of variance	Df	F value	Pr (>F)
Harvest periods	1	2.92	0.1627
Block within Harvest periods	4	0,59	0.6703
Genotype	8	5.47	0.0000**
Storage period	1	2.30	0.1340
Genotype x Storage period	8	1.41	0.2092
Harvest periods x Genotype	8	2.28	0.0317*
Harvest periods x Storage period	1	2.38	0.1276
Harvest periods x Genotype x Storage period	8	1.06	0.4000
Error	68		
Total	107		

Note: *, **-significant ($p < 0.05$) and highly significant ($p < 0.01$)

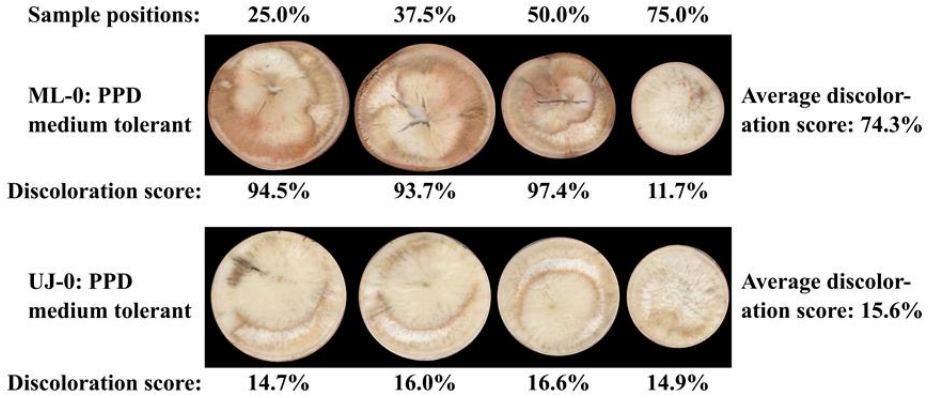

Figure 3. Comparison of cassava root discoloration because of microbial infection (above) and postharvest physiological disorder (PPD, below). The cassava genotype ML-0 and UJ-0 were both PPD medium tolerant. Sample positions (25.0, 37.5, 50.0, and 75.0%) indicated the relative positions from the distal end compared to the total length of the evaluated cassava roots. The observation was conducted ten days after harvest (DAH)

Figure 4 showed that the more distances from the proximal site of the cassava roots, the lower the score of root discoloration. Root samples sliced 25.0% from the distal end showed more root discoloration symptoms than those from the 75.0% position. Such a pattern of root discoloration occurred in all genotypes (Figure 4). The peduncle cuttings at the proximal end of the cassava roots may have caused more severe PPD reactions in the samples close to the proximal end than the distal ones. Previous research has also reported that cutting either the proximal or distal end of the cassava roots elevated PPD symptom development more than those without root cutting (Zainuddin 2016; Tomlins et al. 2021). Furthermore, mechanical damage can also serve as an entrance to microbe infection (Garcia et al. 2013). Therefore, causing as minor as possible mechanical damage to the cassava roots during harvesting and handling processes has caused either the reduction of PPD symptoms or microbe infection.

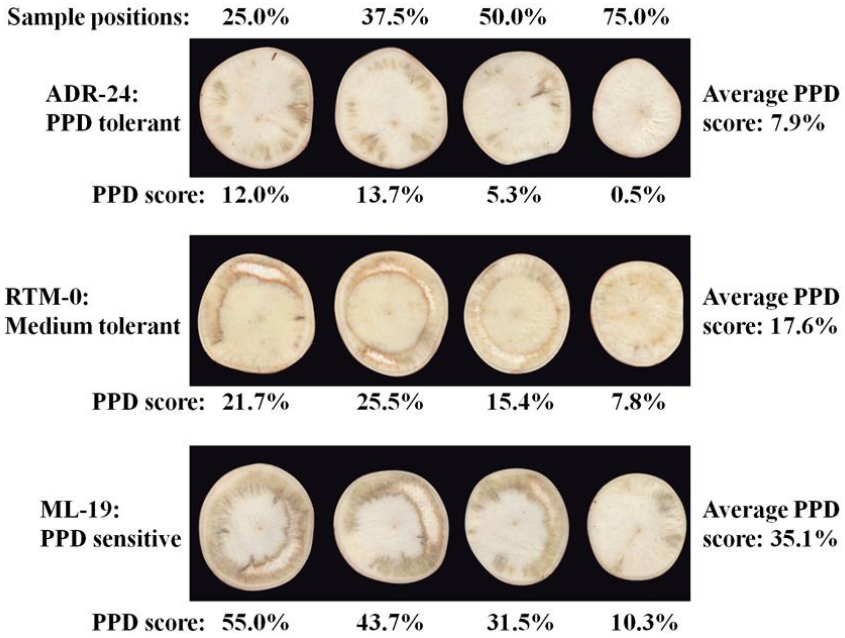

Figure 4. Representative root discoloration because of PPD in cassava genotype ADR-24 (tolerant), RTM-0 (medium tolerant), and ML-19 (PPD sensitive). The scoring indicated the measures of discoloration at four relative sample positions (25.0, 37.5, 50.0, and 75.0%) from the distal end of the evaluated samples. Subsequently, the average score of root discolorations among four sample positions was determined and used to group PPD responses. These images were from cassava roots harvested at eight months of planting, and the evaluated cassava roots were stored for ten days after harvest (DAH)

This study was conducted to develop methods for selecting potential PPD tolerant cassava genotypes. The PPD evaluation at two different harvesting periods showed the consistency of PPD responses among nine cassava genotypes. In general, there was an increase in PPD responses between cassava roots harvested at either eight MAP or 13 MAP (Table 5). These findings were in line with Coelho et al. (2019) report, which showed an increase in PPD symptoms in cassava roots harvested at 10 MAP after planting than that at 13 MAP.

Three PPD responses were assigned based on the percentages of root discoloration (Figure 4). This classification is similar to Luna et al. (2020), who stated a low PPD response with 6.7%, medium-13.3%, and high-24.7%. The tolerant genotype was assigned as having a percentage of root discoloration of less than 10%. Two cassava genotypes (ADR-24 and GJ-11), using the evaluated criteria, were identified as PPD tolerant (Table 5). The root discoloration symptoms of the ADR-24 were consistently less than 10%, both in cassava roots harvested at eight MAP and 13 MAP (Table 5). On the other hand, the GJ-11 genotype showed a percentage of cassava root discoloration of more than 10% at 13 MAP and less than 10% at 8 MAP, respectively. However, GJ-11 still belonged to PPD tolerance based on the average root discoloration percentages (Table 5). One cassava genotype was identified as sensitive to PPD (ML-19), and this genotype has an average root discoloration percentage of more than 20% (Table 5). Most of the evaluated cassava genotypes were identified as medium tolerant to PPD. The root discoloration percentages for medium tolerant to PPD of the cassava genotypes (GJ-8, ML-0, ML-18, RTM-0, RTM-26, and UJ-0) ranged from 11-20% (Table 5).

Association among quantitative traits to PPD response

Pearson's correlation analysis results among the four agronomic traits to PPD responses were presented in Table 6, while those of the multivariate regression analysis were in Table 7. The Pearson's correlation analysis results for the combined of the two harvest periods showed that maximum cassava root diameter was highly correlated to PPD responses (Table 6). Moreover, neither dry matter content, root length, nor weight loss was associated with the PPD response characters (Table 6).

At the eight MAP, Pearson's correlation analysis results showed that only dry matter content was significantly correlated to PPD while the others were not (Table 6). Moreover, neither dry matter content, root length, or weight loss were associated with the PPD response characters. On the other hand, Pearson's correlation analysis results at the 13 MAP showed that only the maximum root diameter was significantly correlated to PPD while the others were not (Table 6). Therefore, the dry matter content and the maximum root diameter may be the only two agronomic traits correlated to the PPD responses.

Our result is slightly different from Salcedo et al. (2010) and Tumuhimbise et al. (2015) reports, showing no correlation between root diameter and PPD responses. However, our finding confirmed the Tumuhimbise et al. (2010) report, which showed no correlation between root length and PPD responses. Moreover, our result also confirmed Sanchez et al. (2013) and Luna et al. (2020) report, which showed a correlation between root dry matter content and PPD responses.

Table 5. Mean value of the root discoloration percentages as the measure of postharvest physiological deterioration (PPD) responses and the grouping of PPD responses among nine cassava genotypes harvested at either 8 or 13 months after planting (MAP)

Genotypes	8 MAP		13 MAP		Mean of root discoloration	PPD responses
	Cassava root discoloration (%)	Level of PPD responses	Root discoloration (%)	Level of PPD responses		
ADR-24	8.97 (A) cd	T	9.40 (A) c	T	9.19	T
GJ-8	12.92 (A) bcd	M	14.98 (A) bc	M	13.95	M
GJ-11	6.88 (A) d	T	13.18 (A) bc	M	10.04	T
ML-0	16.92 (A) ab	M	13.11 (A) bc	M	15.01	M
ML-18	22.09 (A) a	S	14.91 (A) bc	M	18.50	M
ML-19	17.80 (B) ab	M	29.36 (A) a	S	23.59	S
RTM-0	19.18 (A) ab	M	14.82 (A) bc	M	17.00	M
RTM-26	14.95 (A) abc	M	20.83 (A) ab	S	17.90	M
UJ-0	10.57 (A) bcd	M	14.08 (A) bc	M	12.33	M
Mean	14.48		16.08		15.28	

Note: Data in the same column followed by different lower case letters and those between 8 and 13 MAP followed by different capital letters indicated that the percentage of root discoloration is significantly different based on Duncan's multiple range tests (DMRT) at $\alpha = 0.05$.T-PPD tolerant; M-PPD medium tolerant; S-PPD sensitive

Table 6. Pearson's correlation coefficients among dry matter content, maximum root diameter, root length, and root weight loss after storage to postharvest physiological deterioration (PPD).

Classes	Dry matter content (%)	Maximum root diameter (cm)	Root length (cm)	Weight loss (%)
Combined two harvest periods	0.0345	0.3573**	-0.0691	0.0798
8 MAP	0.2771*	0.2244	-0.0760	0.2109
13 MAP	0.2676	0.4668**	-0.0460	0.2371

Note: * and **-significant ($p < 0.05$) and highly significant ($p < 0.01$)

Table 7. Representative multivariate linear regression models using the stepwise method for the combined two harvest periods, for the harvest period at eight months and 13 months after planting (MAP)

Classes	R^2	Multivariate regression model
Combined two harvest periods	0.1619**	PPD =-4.8 + 0.2 DMC + 3.0 MRD
8 MAP	0.1283*	PPD =-6.4 + 0.4 DMC + 2.3 MRD
13 MAP	0.2179**	PPD = 8.5 + 2.6 MRD

Note: PPD-postharvest physiological deterioration; DMC-dry matter content; MRD-maximum root diameter; * and **-significant ($p < 0.05$) and highly significant ($p < 0.01$)

Model validation using multivariate regression analysis confirmed that the dry matter content and the maximum root diameter were associated with PPD response (Table 7). The multivariate analysis also showed the presence of different regression models for either eight MAP or 13 MAP harvest periods (Table 7). However, the regression model fitted for the combined two harvest periods, and the eight MAP includes dry matter content and maximum root diameter traits. On the other hand, the regression model for 13 MAP harvest periods only has a maximum root diameter (Table 7). Our regression analysis results supported Luna et al. (2020), which showed that no single regression model could explain the PPD responses.

In the multivariate regression analysis, the R^2 values for the fitted regression models were all significant, and they ranged from 0.1283 to 0.2179. The multivariate regression models calculated by Luna et al. (2020) also showed R^2 values ranging from 0.2 to 0.5. Therefore, our regression models (Table 7) may still be used to predict the association among dry matter content, maximum root diameter, and PPD responses.

Discussion

PPD is a complex physiological process affected by genetic and environmental factors (Zainuddin et al. 2018). However, we have demonstrated in this study that the storage periods have no significant effect on PPD. We also showed that the storage period affected the development of microbiological infections. Some of the evaluated genotypes have rotten at 10 DAH and were removed from PPD evaluation.

Morante et al. (2010) and Luna et al. (2020) have reported similar constraints in PPD evaluation because of microbial infection on long cassava root storage. Similarly, Garcia et al. (2013) also wrote that microbe infection limited PPD symptoms evaluation.

This research also confirmed the effects of wounding for inducing PPD. The PPD symptoms developed from the

proximal to the distal end of cassava roots since mechanical injuries usually occurred at the proximal end of the cassava roots. Injured cassava roots resulted in oxidative stress as indicated by the rapid increase of reactive oxygen species (ROS) in the form of increased singlet oxygen ($^1O_2$), superoxide O^{-2}, and hydrogen peroxide (H_2O_2) within 15 minutes after injury (Iyer et al. 2010; Xu et al. 2013; Vanderschuren et al. 2014; Hu et al. 2016; Qin et al. 2017).

One of the defense responses to alleviate oxidative stresses was increasing the content of phenolic compounds (Iyer et al. 2010). One of the phenolic compounds associated with oxidative stress in cassava roots is scopoletin (Uarrota and Maraschin 2015; Uarrota et al. 2016). On the other hand, scopoletin was suggested as the precursor of root discoloration associated with PPD in cassava (Uarrota and Maraschin 2015). The increased oxidative stress also results in storage root deterioration and cell death (Djabou et al. 2017). Therefore, carefully handle cassava roots to prevent mechanical injuries during harvesting and cassava root handling.

To obtain accurate measures in PPD evaluations is still troublesome because of the high experimental error (Morante et al. 2010; Garcia et al. 2013; Mahmod and Beeching 2018). However, in our experiment, we showed consistency of PPD scoring on two different harvesting periods (either eight or 13 MAP) among nine cassava genotypes. Therefore, potential cassava genotypes having PPD tolerance responses could be identified using the developed selection methods.

Morante et al. (2010) grouped the PPD responses into five classes. In our study, we standardized the grouping of PPD responses using the measured root discoloration percentages. The PPD responses of cassava genotypes were grouped into three classes, such as PPD tolerant (root discoloration <10%), medium (root discoloration ranged from 11-20%), and sensitive (root discoloration >21%). The grouping of the cassava genotype was based on the consistency of PPD response evaluation among two independent experiments.

The PPD responses were significantly affected by the interaction of genotype by harvesting periods. Therefore, the patterns of PPD responses among tested genotypes may be different when harvested in either eight or 13 months after planting, respectively. Such differences were also supported by the multivariate regression analysis, which showed different regression models for each of the tested genotypes. Two cassava mutants (ADR-24 and GJ-11) were identified as PPD tolerant, while others (ML-19) were identified as PPD sensitive. Morante et al. (2010) have reported that mutants having PPD tolerance might have inactivated at least one gene putatively involved in the occurrence of PPD symptoms in cassava roots. However, since irradiation mutagenesis is a random process (Morante et al. 2010), the generated mutants from the same mother plants may have many mutants with various PPD tolerance levels, such as GJ-11 and GJ-8.

Evaluating association among PPD responses and other agronomic traits might help select PPD tolerance among cassava germplasm or breeding materials. This research also evaluated the correlation between four agronomic traits and PPD response. Subsequently, a multivariate stepwise regression analysis was also assessed to determine the model of the agronomic trait contribution to the PPD responses (Luna et al. 2020). However, our finding did not show the different regression models for either PPD tolerant, medium tolerant, or sensitive cassava genotypes. As reported in the previous studies (Sanchez et al. 2013; Luna et al. 2020) and our study, dry matter content was highly correlated with PPD symptoms. Maximum root diameter was also significantly correlated to PPD.

This study proved that PPD is a complex phenomenon and is probably associated with genetics and environmental factors. Particular attention should be directed toward dry matter content and maximum root diameter traits when evaluating for the PPD symptoms. A comprehensive biochemical and molecular analysis of cassava roots should provide excellent data for understanding the mechanism associated with the PPD. We have developed standard procedures for identifying genotype responses to PPD and showed that cassava roots harvested as early as eight months after planting and stored as late as five days under the control relative humidity were suitable materials for other studies. Previously, there was no rigid standard for grouping PPD responses in cassava. Therefore, the finding in this research should help support PPD resistance cassava variety development through breeding programs and provide materials for biochemical and molecular analysis associated with the mechanisms of PPD in cassava roots.

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