Postharvest physiological deterioration in cassava: potential problems, possible inhibition, and resistant level identification

R S Rahmawati1, D Sukma1, S W Ardie1 and S Sudarsono1*

1 Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, IPB Dramaga Campus, Bogor 16680, Indonesia

*Email: sudarsono_agh@apps.ipb.ac.id

Abstract. Cassava roots can only be utilized quickly because the roots suffer physiological damage in only 1–3 days after harvest because of postharvest physiological deterioration (PPD). Once the cassava roots are physiologically damaged, they cannot either be consumed or marketed. Indonesia is the second-largest cassava producer in Southeast Asia that is most used for food and feeds. Unless there is a solution, the PPD problem may become the main obstacle in cassava production. Therefore, finding solutions to the PPD problem in cassava is necessary, may be approached either by treatment to inhibit PPD occurrences, identification of tolerance accessions among cassava germplasm, and development of PPD tolerance cassava through breeding programs. In our research, the identification method was done by applying various staining methods to detect color changes associated with PPD symptoms during the cassava storage period and then compared to the conventional observation of PPD symptoms. Subsequently, the effective staining approaches are used to evaluate various genotypes' responses in the cassava germplasm collections. Finally, the breeding approaches to develop PPD tolerance cassava varieties are outlined. Hopefully, with these research results, the solution for PPD problems associated with cassava production in Indonesia will be available.

1. Introduction

Cassava (Manihot esculenta) is one of the most important carbohydrate crops globally, especially in Africa and most developing countries, because it is used as a staple food. Cassava can be used to face global climate change because it is widely adapted even on marginal land. Indonesia is the second-largest producer of cassava in Southeast Asia after Thailand [1], with an average production of 23.90 million tons per year [2]. Indonesia is also an exporter of cassava, especially to several other Asian countries, where Indonesia's market share reaches 9.26% of the world's total production [2], so that Indonesia has potential as a big cassava producer in the world trade.

The utilization of cassava is now growing from foods, industrial raw materials, animal feed to biofuels [2][3]. However, the use of cassava is hampering with a short shelf life ranging from 1 to 3 days, after which roots experience physiological damage known as Postharvest Physiological Deterioration (PPD) [4][5][6][7]. PPD causes roots color change to be blue, black, or brown. Moreover, the taste becomes bitter, so they cannot either be consumed or marketed [6][8][9]. Wounding can speed up the process of PPD [5, 8], but mechanical damage is difficult to avoid in the process of harvesting and distribution of
cassava. Besides, in Indonesia, cassava is much more reserved for food consumption than for industry, as it is used for food diversification [2][3]. Therefore, the short shelf life due to PPD is one of Indonesia's main problems in cassava production. Nevertheless, research on PPD in Indonesia is still minimally developed, so many potential problems need to be solved. This paper will review the PPD problem from aspects socio and economic, current progress research to understanding PPD, the development method to identification and inhibition PPD, and our next project research to overcome PPD.

2. Socio and economic impact of PPD

PPD can lead to physical losses because the roots become unfit for consumption. And then, the economic detriment will occur due to the decline in the selling price or even cannot be sold. In Indonesia, the physical losses due to PPD can reach 25% of total production [10], and the rate of crop loss will increase in countries where PPD is the main problem in the production and distribution of cassava [11]. Also, the distance between on-farm and processing or marketing sites will affect the physiological damage, so cassava should be used immediately after harvesting [11]. Indonesia has length supply chain management and less efficient. Therefore PPD can be the main problem in the cassava value chain.

The inhibition of PPD through the assembly of new varieties can provide high economic benefits between countries. The estimated value gains obtained over 20 years by Nigeria are about $US 2.9 million, Uganda $US 280 million, and Ghana can reach $US 885 million by inhibiting PPD up to 20 days after harvest [12]. Unlike Thailand, which needs to inhibit it up to 45 days to get the economic potential of about $US 35 million [13]. Plant breeding is one of the long-term solutions to overcome PPD, although the assembly of PPD resistant varieties requires large resources and takes a long time.

3. Mechanism underlying PPD

Postharvest Physiological Deterioration (PPD) is physiological damage that occurs very quickly from 1 to 3 days after harvest [4][5][6][7]. Physiological damage is characterized by discoloration of the vascular to blue, black, or brown part, then spread throughout the parenchyma part of the cassava (Figure 1). Also, the taste of roots becomes more bitter and emits a less pleasant aroma [4][6][14]. Understanding the mechanisms of PPD is significant since it is a complex phenomenon. Therefore multi approaches, such as transcriptomic, proteomic, and metabolomic analysis, may be necessary as indicated in (Figure 2).

3.1. Oxidative stress and ROS scavenging mechanism

Transcriptome analysis shows from the beginning (3 – 4 h) to the end (12 - 96 h) of the PPD induction phase, the related genes of reactive oxygen species (ROS) are increasing [5][7][8]. Wounding of the roots causes oxidative stress after 15 minutes [8], observed by the rapid increase in free radical compounds such as singlet oxygen (1O2), superoxide (O-2), and hydrogen peroxide (H2O2), namely 'oxidative burst' [4][8][15][16][17]. The ROS concentration increases because the wound causes electrons from oxygen altered into reactive and toxic structures through Haber-Weiss reactions [7]. The increase in hydrogen peroxide concentration is related to PPD [18], and it can be used as a biomarker of deterioration [19]. Oxidative stress causes root metabolism changes, leading to stress defense mechanisms, programmed cell death, cell wall remodeling, and triggered signal transduction [7][20].
Oxidative stress activates plant defense mechanisms [18]. One of them is by increasing the activity of antioxidant enzymes such as peroxide (POD), superoxide (SOD), catalase (CAT), glutathione peroxidase (GPX), and ascorbate peroxide (APX) to change free radical compounds into non-toxic compounds such as water (H₂O) [4][5][7][8][15][16][19][21]. ROS scavenging mechanism is the main regulator in inhibiting PPD [4][18]. Testing of comparisons between resistant and susceptible genotypes showed differences in enzymatic activity patterns, where CAT activity increased dramatically at the beginning of the PPD process in the resistant genotype since in the susceptible genotype to APX and GPX activity increased sharply at the end of the PPD phase [4][14][15][22][23]. These differences are potentially used as valuable biomarkers to identify PPD resistance levels.

Non-enzymatic antioxidants such as β-carotene and Ascorbic Acid are known to negatively correlated with PPD [14][24][25], presumably due to the ROS scavenging mechanism of both compounds. The minimum content of β-carotene that effectively inhibits PPD ranges from 9.1 μg g⁻¹ [24], visually detectable from yellow roots [24][25]. There is a considerable contrast between the ascorbic acid content in resistant genotypes and susceptible genotypes, indicating the ascorbic acid plays a significant role in the resistance to PPD [19].

3.2. Metabolites change during PPD
The various analysis showed that PPD caused several changes in root metabolic processes. The simplest ones are reduced fresh weight and water content during the storage period, resulting in increased root dry matter content. The rate of root respiration increases during the PPD process, causing the starch decomposition into sugar and decreasing the starch/sugar ratio [25][26]. The degradation of starch into sugar in susceptible genotypes is higher than that of resistant genotypes [25]. Therefore, the starch/sugar ratio can be used as an accurate biomarker for PPD, with a high correlation value (0.98) [26].

Figure 1. PPD appearances from five transverse root slices from the proximal to the distal end ten days after harvest. A) Blue and Brown discoloration; B) Black and brown discoloration.
Figure 2. Flow diagram indicating the complex mechanisms of PPD.

The secondary metabolite compounds increased at the beginning of the PPD process in response to oxidative stress, including coumarin, flavanones, phenol, and flavan-3-ol [8][27]. Hydroxycoumarin compounds are widely analyzed since they are assumed to act as precursors of vascular discoloration. It is reinforced by the association between decreasing hydroxycoumarin content and increasing root storage period [14][19][28]. The wounding of cassava roots exposed them to oxygen, resulting in hydroxycoumarin oxidization and causing blue or black discoloration [14][28]. Scopoletin is the most common hydroxycoumarin compound that changes to an insoluble colored product that indicates peroxidase activity [28]. However, there was no correlation between hydroxycoumarin or scopoletin compound with the PPD resistant level [9][20]. This phenomenon probably due to the reduction of hydroxycoumarin has done before the discoloration symptom fully appears [20].

In addition to increased antioxidant activity, cyanide is expected as one of the defense compounds against PPD because its content increases after the root wounding and can regulate ROS homeostasis [19][29]. In regulating ROS homeostasis, cyanide plays a role through cytochrome oxidase in the root respiratory system, where the release of cyanide can increase ROS accumulation [29]. Therefore, overexpression of the AOX gene can inhibit PPD through impaired cytochrome oxidase, blocking the cyanide release after that ROS content decreases during the PPD process [29]. Linamarin is a precursor in cyanogenesis precisely increases at the end of the PPD due to microbial infections [21][25]. However, it can suspect that the bitter taste in the deteriorated root comes from an increase in HCN content during the PPD process.

3.3. Programmed cell death and Ca$^{2+}$ signal transduction
Cassava root wounding induced Ca$^{2+}$ signal transduction and programmed cell death (PCD) [18]. The wound-induced Ca$^{2+}$ signaling [18] is indicated by the increased expression of the calmodulin gene (CaM) as the Ca$^{2+}$ sensors at the beginning of PPD [21][30]. Ca$^{2+}$ signal transduction plays a significant role in the reactive oxygen species (ROS) homeostasis, as ROS also in PCD. Based on the cDNA microarray analysis, two genes associated with PCD (cysteine protease and class IV chitinase) are up-regulated during
the PDD processes [7][21]. Cysteine protease acts as the mediator of signal transduction or the effectors of PCD, induced in the absence of wound stress [7, 21]. Simultaneously, chitinase serves to hydrolyze chitin, which is the essential component of the fungal cell walls [7]. Increase expression of chitinase genes results in reduced microbiological infections associated with a long storage period. In conclusion, wounding induces several processes, such as oxidative burst, which increases the Ca^{2+} influx. Subsequently, the oxidative burst activates the plant cell death (PCD) at the end of the PPD processes.

4. Resistant level identification

Methods to evaluate the cassava roots' resistance levels are crucial stages to understand and overcome PPD problems. The visual PPD resistance level identification is easy to observe, and it is based on root vascular discoloration after a certain storage period. In such cases, the PPD resistance was evaluated based on the average score of three to seven pieces of transverse cassava roots, also known as the percentage of discoloration areas [19][31][32]. However, the results of such visual evaluations are very subjective. Therefore, the more objective scoring methods were developed using image processing software, such as Pixcavator Image Analysis [20], Image J [15][17][29], and the PPD Symptom Score Software MatLab [5][16].

Blue or black discoloration can occur after roots are stored intact for some time. This method takes a relatively long observation time, which can reach 40 days after harvest. Still, it is more appropriate to evaluate the PPD resistant level because it corresponds to the general storage conditions [24]. The discoloration of the storage root can also be induced by wounding to initiate oxidative stress. The standard method set by The International Center for Tropical Agriculture (CIAT) is most widely used to identify PPD resistance. In the CIAT method, the proximal and distal parts slit to leave 15 cm roots, then the distal end is covered with PVC film so that the PPD process occurs from the proximal end. CIAT method can accelerate the instance of PPD but also increase the chance of microbiological infection [17][19][24][29][31][32]. Another wounding method with the root slice methods is to cut roots to a thickness of 5 - 10 mm, and then the root slice is stored in a petri dish. The root slice method forcefully and quickly induces PPD, so the discoloration occurs 12 hours after cutting and reaches the maximum symptom at 72 hours in both resistant and susceptible genotypes [4][6][19]. Therefore, these methods are less suitable for identifying PPD resistance levels but are widely used to analyze gene expression, proteomics, and metabolomics.

Various methods of identification are laborious because it requires a large number of cassava storage roots, that is ten or even fifteen roots per genotype with an economic root size [24][33], a root circumference of at least 10 cm, and mostly uniform [34]. That was to minimize trial errors, as tests performed destructively on different roots at each time point and PPD scores of the same genotype can be very different for each, resulting in a large chance of trial errors [9][24][33]. More accurate results should identify PPD resistance in several different locations or seasons [34]. Furthermore, visual identification generally followed with an analysis of other characters that correlated with PPD resistance as dry matter content, β-carotene, or starch/sugar ratio [15][19][24][26]. A non-destructive sampling method to perform a biochemical and visual analysis of PPD has been developed by taking a sample on the center of the root with a solidified cylinder rather than a sampling hole coated with paraffin wax. It makes it possible to get more accurate results, such as biochemical analysis and visual scoring of PPD because they used the same roots [33].

Visual identification of PPD is still a preference over biochemical because it is easy to do and observe. Consequently, these are still needed development methods, one of which is by looking for related characters that correlate with physiological damage of PPD. Hydroxycoumarin is a secondary metabolite compound that its content increases at the beginning of the PPD process, then decreases with the increasing shelf life of cassava [14][19][28]. The decrease due to hydroxycoumarin compounds oxidizing...
during the storage period, causing root discoloration to be blue or black [14]. Scopoletin is the most abundant among three other compounds (esculin, esculetin, and scopolin) [28]. It is potentially used as a biomarker to evaluate resistance to PPD [14][19]. Also, decreased scopoletin production by inducing the down-regulated gene MeF6'H can lower vascular discoloration symptoms in the transgenic cassava storage root [6].

Scopoletin can also produce fluorescent under UV light, and it easy observed visually as a biomarker. Nevertheless, scopoletin fluorescent observation showed no correlation with the discoloration of roots due to PPD [9][20]. Correlation analysis conducted between fluorescent and PPD visual scoring using the entire root method five days after harvest showed a low correlation coefficient value [20]. Correlation measurement performed between fluorescent at the beginning of storage and PPD scoring at the end of storage. However, the correlation coefficient between fluorescent on day one and PPD visual scoring using the root slice method on day 4 is only worth 0.1183 [9]. Both results indicate that scopoletin is not reliable to use as a visual biomarker. Therefore, it is still important to develop another method to identify the resistant level of PPD.

Another possible to develop a method to identify PPD visually is with the use of staining to show PPD symptoms clearly. PPD is related to programmed cell death (PCD) characterized by increased expression of PCD genes from the beginning still before the PPD process thoroughly occurs [5][7][8]. Oxidative stress occurs during PPD, is closely related to irreversible cell damage so that in the end, the roots will undergo cell death [18]. Therefore, different coloring patterns can be expected in the root surface after immersion with the staining solution of tetrazolium chloride. The living cells will reduce tetrazolium to red formazan deposits, while in dead cells, it does not occur that will have a pale color (less colored) than a living cell. The resistant genotypes assumed that the percentage of red root surface would be large compared to susceptible genotypes. The difference coloring response use to identify the resistance level of PPD for an accurate and easy method.

5. Possible inhibition of PPD

PPD inhibition methods to extend the shelf life of cassava storage roots widely developed, ranging from cultivation, postharvest, plant breeding to genetic transformation. Aside from the effectiveness of inhibiting PPD, the developed methods should also consider the ease and cost of application.

5.1. Cultivation and Postharvest Approaches

In the cultivation process, PPD inhibition methods could easy to do with delaying harvesting and pruning. Both procedures can reduce the starch/sugar ratio but also lead to hesitancy because it can decrease the quality of taste and texture and the number of product roots [26][32][35]. The application of fertilizers containing Ca2+ and Mg2+ has shown PPD inhibition for more than ten days. Fertilization with Ca2+ is more effective than Mg2+ due to the function of Ca2+ in signal transduction and ROS scavenging, which is proved by increased enzymatic antioxidant activity in roots given Ca2+ fertilization [36].

PPD process occurs since roots separate from their crops. Several postharvest treatments to cassava roots are employed to minimize the wounding’s negative effects and prevent direct oxygen contact. Setting the appropriate temperature and relative humidity in the cassava storage rooms is a simple procedure for inhibiting PPD. Stored the cassava roots in a condition with high relative humidity can encourage the formation of periderm layers at the wounding site [37]. It can be easy to do by storing the roots in a wooden box filled with sawdust. This method successfully protected cassava roots from physiological and microbiological damage for up to 28 days after harvest [24]. Low temperature and high RH settings are optimum to inhibit PPD when combined with soaking hot water (54 - 56 o C) for 10 minutes [38]. Edible coatings such as xanthan gum and guar gum are also known to effectively keep moisture water content of
the root due to avoiding contact with the air directly. Moreover, respiration and the deterioration of the cassava root also inhibit [39].

Inhibition methods widely studied include applying certain chemical compounds associated with PPD processes, such as melatonin, CaCl2, ethanol, and methyl jasmonate (MeJa). The role of those chemical compounds in inhibiting PPD is related to increased ROS scavenging capacity by both enzymatic and non-enzymatic antioxidants, reducing the degradation of cells and root quality [4][22][23][40][41]. Melatonin can be directly administered exogenously or by supplying an inducer for increasing melatonin biosynthesis in cassava roots. The Ca2+ application induced melatonin biosynthesis and exhibited similar PPD inhibition effects. On the other hand, exogenous application of melatonin did not increase Ca2+ concentration [40].

Moreover, MeJa application also increases the endogenous concentration of melatonin [42]. However, MeJa and melatonin effectiveness are proven at the laboratory level using root slice methods. Therefore, their effectiveness at the practical level needs more testings. Because PPD inhibition only lasts in a short period (a few hours), the application of MeJa and melatonin to whole cassava roots may have different results.

5.2. Plant breeding and genetic transformation approaches

Plant breeding is a long-term solution because PPD resistance genetically transfers to the next generation. PPD has a wide range of heritability values between 52% and 94%. Hence, the character of PPD resistance is controlled strongly by genetic [31][34], but environmental variance still needs to be minimized. The first step to be done is to establish genetic diversity in both conventional and non-conventional ways. The cross between the wild species Manihot walkerae, which is PPD resistant, and elite genotypes, was done by CIAT for several years. Evaluation of the backcross genotype showed there was a genotype that was resistant to PPD with an average physiological damage value of 0% to 40 days after storage. These results also indicate that PPD is controlling with a dominant or an additive gene [24].

The assembly of varieties with high carotene content can also be an alternative because the antioxidant activity from carotene can inhibit the development of PPD. It is easy to visual identification with the yellow color of the parenchyma part [42][43]. 15 to 20-fold increase in carotene synthesis with DXS gene co-expression and crtB can inhibit the development of PPD only 0-11% while nontransgenic crops reach 50% in 10 days after harvest. Carotene's antioxidant activity also transfers carbon into carotene to reduce starch content and DMC [43]. The accumulation of starch and DMC with silencing gene MeAPL3 is also known to inhibit PPD in transgenic plants [44].

PPD-resistant genotypes can also produce mutations. Although mutation initiates to correct other characters because mutations are random, PPD resistant genotypes could discover. It occurs in the mutant population for the improvement of starch character. After pre-screening PPD against several mutant genotypes, two genotypes are expected resistant to PPD [24]. A similar conclusion was obtained after a conventional storage test with sawdust, suggesting two IPB mutant genotypes had a longer shelf life than Indonesian commercial varieties [45]. Therefore, further testing and development of the mutant population of IPB cassava important to overcome PPD in Indonesia.

Strategy on genetic transformation could base on the biological process that is strongly related to PPD. The onset of PPD is related to the increased reactive oxygen species so that the overexpression gene related to ROS scavenging is successful in delaying PPD. Up-regulated gene McCu/ZnSOD and MeCAT1 delayed PPD until 10 days [17], while GPX gene overexpression can delay PPD until 6 days after harvest [16]. The overexpression of the antioxidant gene reduced hydrogen peroxide and malondialdehyde having ROS scavenging activities and reduced membrane cell damage. Oxidative burst positively correlated with cyanogenesis. Overexpressing the oxidase gene (AOX) that is insensitive to cyanide can reduce ROS
accumulation, and then PPD is also delayed at least for two weeks. This time is enough for distributing cassava storage root before PPD initiate [29].

In another way, the down-regulated gene, which plays negative control on PPD, such as the precursor of blue or black discoloration, can be applied to delayed PPD. Scopoletin could cause vascular discoloration if they oxidized. Therefore, down-regulation of the MeF6'H gene responsible for scopoletin biosynthesis successfully delayed the PPD symptoms [6]. The transgenic plant still needs to be developed further to know the stability of gene expression. Vegetative cassava propagated through stem cuttings facilitates the plant breeding process, as superior genotypes could directly produce without changing their genetic constitution.

### 6. Conclusion

Indonesia is one of the largest cassava producers globally and has the chance to grow even more with the potential use of cassava in industry and renewable energy. However, the short shelf life of cassava because of PPD may become a limiting factor. Unfortunately, PPD management has not become a priority research in Indonesia. Therefore, future investigations on how to overcome the PPD problem in cassava should be more intensified. The proposed research priorities include identifying quick methods for PPD detection and understanding the PPD processes, and eventually, the development of PPD resistant cassava should be the final objective.

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