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# Genomic Analysis of the Principal Members of Antioxidant Enzymes in Simulated Stresses Response and Postharvest Physiological Deterioration in Cassava

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## Abstract

ROS act as an signaling molecule in the biological growth and development process. The homeostasis of ROS must be kept by different antioxidant defense mechanisms. Currently, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) as major antioxidant enzymes are not well understood in cassava (*Manihot esculenta*). In this research, 7 SODs, 6 CATs, and 6 APXs were identified from the cassava genome by hidden Markov models, which was supported by gene structure, protein motifs, and phylogenetic relationship analyses. *SOD, CAT,* and *APX* genes expressed differentially in different tissues of cassava, of which most *SODs* showed high expression levels. The comprehensive expression profiles revealed the participation of *SOD*, *CAT*, *APX* genes during postharvest physiological deterioration (PPD) of storage root and in response to osmotic stress and ABA as well as *Xanthomonas axonopodis* infection. Together, this study increases our understanding of cassava *SOD*, *CAT*, *APX* genes feature and their potential function during PPD process and in response to biotic and abiotic stresses in cassava, laying a solid foundation for further gene function analysis in cassava.

**Keywords** Cassava · Genome wide · SODs/CATs/APXs · Stresses · PPD

## Abbreviations

| | |
|---|---|
| ABA | Abscisic acid |
| APX | Ascorbate peroxidase |
| CAT | Catalase |
| FEC | Friable embryogenic calli |
| FR | Fibrous root |
| $H_2O_2$ | Hydrogen peroxide |
| HP | Hour postharvest |
| MT | Melationin |
| OES | Organized embryogenic structure |
| PPD | Postharvest physiological deterioration |
| RAM | Root apical meristem |
| ROS | Reactive oxygen species |

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SAM Shoot apical meristem
SOD Superoxide dismutase
SR Storage root
Xam *Xanthomonas axonopodis* Pv. Manihotis

## Introduction

Reactive oxygen species (ROS), such as hydroxyl radical, singlet oxygen, superoxide anion, and hydrogen peroxide ($H_2O_2$), are a group of products that are produced during plant growth and development (Choudhury et al. 2017). However, excessive ROS would cause damage to cell membranes and organelles (Mittler et al. 2011). The dynamic balance must be kept between the production and scavenging of ROS. ROS acts as an important signaling molecule in the biological growth and development process (Mittler et al. 2011). In order to maintain the homeostasis of ROS content, especially in response to biotic and abiotic stresses, plants have evolved complex antioxidant system for resisting oxidation, which includes antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), etc. (Hasanuzzaman et al. 2020). In these enzymes, SOD is the first line of defense against oxidative damage by converting $O_2^-$ into $H_2O_2$, which is then converted into oxygen and nontoxic water by APX or CAT (Abreu and Cabelli 2010a). SOD exists in almost everywhere of cellular and complete the water-water cycle in chloroplasts. CAT is mainly in peroxisomes. APX exists in cytosol, chloroplasts, mitochondria and peroxisomes to complete ascorbate–glutathione cycle (Mittler 2002).

As the first step to scavenge ROS, $O^{2-}$ was converted to $H_2O_2$, which was further converted to oxygen and nontoxic water (Isabel A. Abreu and Cabelli 2010b). SOD can be divided into three types in terms of different binding metal cofactors, namely Cu/Zn-SOD, Fe-SOD, and Mn-SOD (Zelko et al. 2002). Numerous researches showed that SOD could improve the tolerance of plants to different abiotic/biotic stresses, such as drought, salinity, and pathogen infection (Han et al. 2019; Jianhui Wu et al. 2016). The member of family has been identified in various plant species, including *Arabidopsis thaliana* (7) (Kliebenstein et al. 1998), *Oryza sativa* (7) (Dehury et al. 2013), wheat (26) (Jiang et al. 2019), banana (13) (X. Feng et al. 2015), tomato (8) (K. Feng et al. 2016), cucumber (9) (Zhou et al. 2017), and cotton (18) (Wang et al. 2017).

CATs and APXs are capable of converting $H_2O_2$ into water, however, the catalytic mechanism is different (Mittler 2002). APXs show the strongest affinity with $H_2O_2$ and require ascorbate as an electron donor to complete the catalytic process (Liao et al. 2020). CATs also play an important role in decomposing of $H_2O_2$ and do not need cellular reductants to catalyze the dismutase reaction (Mhamdi et al. 2010; Abreu and Cabelli 2010a). There is a compensating mechanism between CAT and APX (Apel and Hirt 2004). In higher plants, CAT and APX are usually encoded by small multigene families. APX family have been identified in *Oryza sativa* (6), *Arabidopsis thaliana* (8), and *Zea mays* (8) (Ozyigit et al. 2016). CAT family also have been identified in *Arabidopsis thaliana* (3) (Du et al. 2008), *Oryza sativa* (3) (Joo et al. 2014), *Zea mays* (3) (Guan and Scandalios 1995), and *Gossypium hirsutum* (7) (Wang et al. 2019). Numerous researches have revealed that CAT and APX are associated with plant tolerances to different abiotic/biotic stresses. For example, *OsAPx8* overexpression enhanced rice resistance to salt stress (Hong et al. 2007). *AtAPX1* plays an important role in plant response to drought and heat stresses (Koussevitzky et al. 2008; Davletova et al. 2005). Overexpression of *OsCatA* and *OsCatC* in rice increased drought resistance (Joo et al. 2014). AtCAT1 acted as an important scavenger to reduce $H_2O_2$ content during various abiotic stresses (Mhamdi et al. 2010). The amount of $H_2O_2$ increased dramatically when *AtCAT2* was knocked out in *Arabidopsis* (Queval et al. 2007).

Cassava (*Manihot esculenta*) is an important crop in Latin America and Africa, and regards as staple food for more than 750 million. Cassava shows excellent drought resistance during the growth process (Okogbenin et al. 2013). Nevertheless, the storage roots are very easy to appear postharvest physiological deterioration (PPD) within 72 h after harvest (Hu et al. 2016). ROS content is highly associated with abiotic/biotic stresses response and PPD. For example, MeCu/ZnSOD and MeCAT1 were used to delay postharvest physiological deterioration of cassava tuberous (Zidenga et al. 2012; Vanderschuren et al. 2014; Xu et al. 2013a). *Cu/ZnSOD* and *CAT* were simultaneously overexpressed in cassava also improving the tolerance to drought and cold stresses (Xu et al. 2013b). MeRAV5 functions on controlling $H_2O_2$ content and improving drought stress resistance in cassava through regulating *MePOD* (Yu Yan et al. 2021). Overexpression of *MeCu/ZnSOD* and *MeCAT1* in cassava increased the resistance to *Tetranychus cinnabarinus (*Lu et al. 2017*)*. However, the mechanism underlying cassava response to abiotic and biotic stresses as well as sensitivity to PPD process remains less known. In this research, the comprehensive analysis of the cassava SOD, CAT, APX family and their potential functions associated with stresses response and PPD were discussed. Our findings would lay a solid foundation for genetic improvement of cassava using antioxidant enzymes.

## Results

### Identification and Phylogenetic Analysis of the Cassava Sods, Cats, and Apxs

Genomic analysis identified 7 SOD, 6 CAT, and 6 APX proteins from the cassava genome. The physicochemical properties of the predicted proteins were shown in Table S1. The subcellular

prediction results were shown in the Table S2. Phylogenetic trees were constructed to investigate the phylogenetic relationship of SOD, CAT, APX proteins from *Arabidopsis*, rice, and cassava (Fig. 1). According to the phylogenetic analyses, the SOD, CAT, and APX families could be divided into 3 (subgroup 1–3), 2 (subgroup A, B), and 3 (subgroup 1–3) subgroups, respectively. SOD, CAT, APXs in cassava showed a closer relationship with those in *Arabidopsis* compared with those in rice, which is consistent with plant phylogenetic research.

## Conserved Motifs and Gene Structure Analyses of the Cassava SODs, CATs, and APXs

In order to analyze essential feature of the SOD, CAT, APX families, conserved motifs of these gene families were identified using MEME database and annotated with InterPro database. There were 10 conserved motifs for each gene family (Fig. 2, Table S3). For the SOD family, all the MeSODs contained motif 7. Subgroup 1 and 2 (MeSOD-3, -7, -1, and -2) commonly contained SOD-related motifs 4, 6, 7, and 9. Subgroup 3 (MeSOD-4, -5, and -6) commonly contained SOD-related motifs 1, 2, 3, and 7. For cassava CAT family, all the CATs included motifs 3–10, of which 5 motifs (motifs 3, 5, 6, 7, and 9) related with catalase domain. MeCAT-2, -3, -4 and -6 contained other two catalase-related motifs (motifs 1 and 2). For cassava APX family, all the APXs contained motifs 2, 3, 6, 8, and 9, in which motifs 2 and 3 were related with peroxidase. These results showed that cassava SOD, CAT, APXs contained the typical motifs of the corresponding family.

The exon–intron features of cassava SOD, CAT, APX families were examined by GSDS database (Fig. 3). The number of exons was 6–9, 5–8, and 8–13 in SOD, CAT, and APX families, respectively. For the SOD family, subgroup 1 and 3 were almost 6 exons, except for MeSOD-5 with 7 exons; subgroup 2 were 8-9 exons. For the CAT family, subgroup A was 5 or 8 exons; subgroup B were 8 exons. For the APX family, subgroup 1 and 2 were 9 exons, except for MeAPX-2 with 8 exons; subgroup 3 were 11 or 13 exons. In general, cassava SODs, CATs, APXs in the same subgroup presented similar exon–intron organization.

## Expression Analyses of SOD, CAT, and APX Genes in Different Tissues of Cassava

To detect the expression levels of cassava SOD, CAT, APX genes in different tissues, the expression data set contained 11 cassava tissues was downloaded from public database (Wilson et al. 2017). These 11 tissues included friable embryogenic calli (FEC), organized embryogenic structure (OES), root apical meristem (RAM), fibrous root (FR), storage root (SR), shoot apical meristem (SAM), lateral bud, stem, petiole, midvein, and leaf. As shown in Fig. 4 and Table S4, all *MeSODs* had gene expression based on transcriptomic data, of which four *MeSOD* genes (*MeSOD-2*, *-3*, *-4*, and *-6*) showed high expression level (FPKM value > 20) in the 11 tissues. All *MeAPXs* showed gene expression based on transcriptomic data, except for *MeAPX5*. For the expressed *MeAPXs*, only *MeAPX-4* showed high expression (FPKM value > 20) in the 11 tissues, and *MeAPX-6* showed high expression in stem, petiole, SR, and SAM (FPKM value > 20). For the *MeCATs*, only *MeCAT1* and *MeCAT2* presented gene expression, with high expression levels (FPKM value > 20) in leaf and leaf/midvein, respectively. These results should provide clues for further study of tissue development and function.

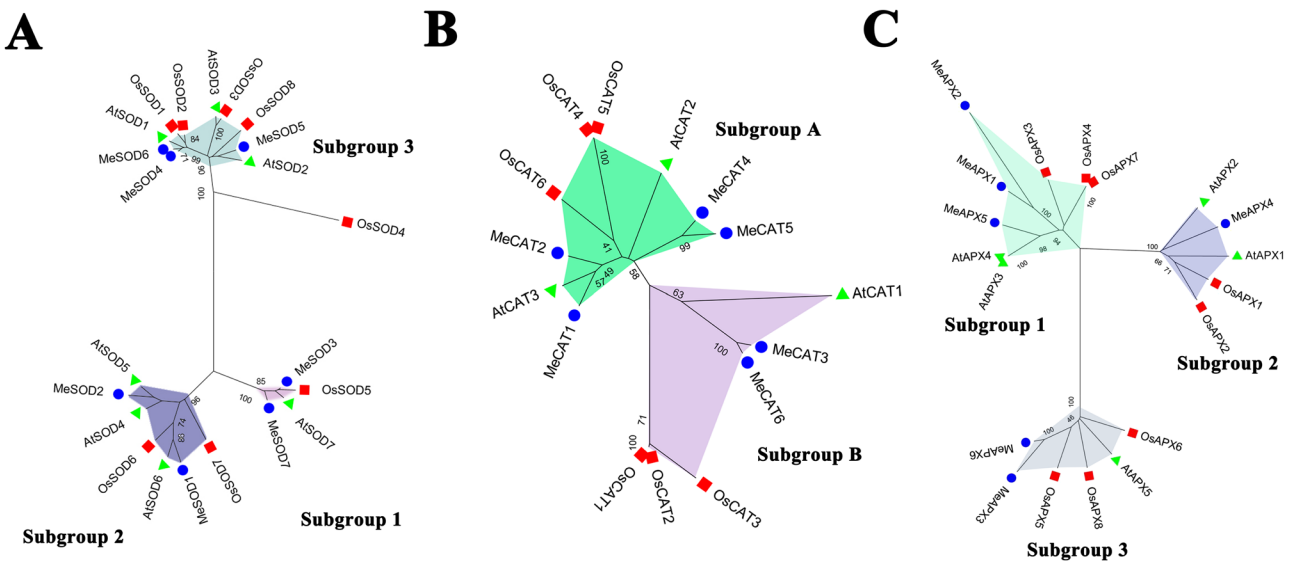


**Fig. 1** Phylogenetic analysis of SOD **A**, CAT **B**, and APX **C** from cassava, *Arabidopsis*, and rice

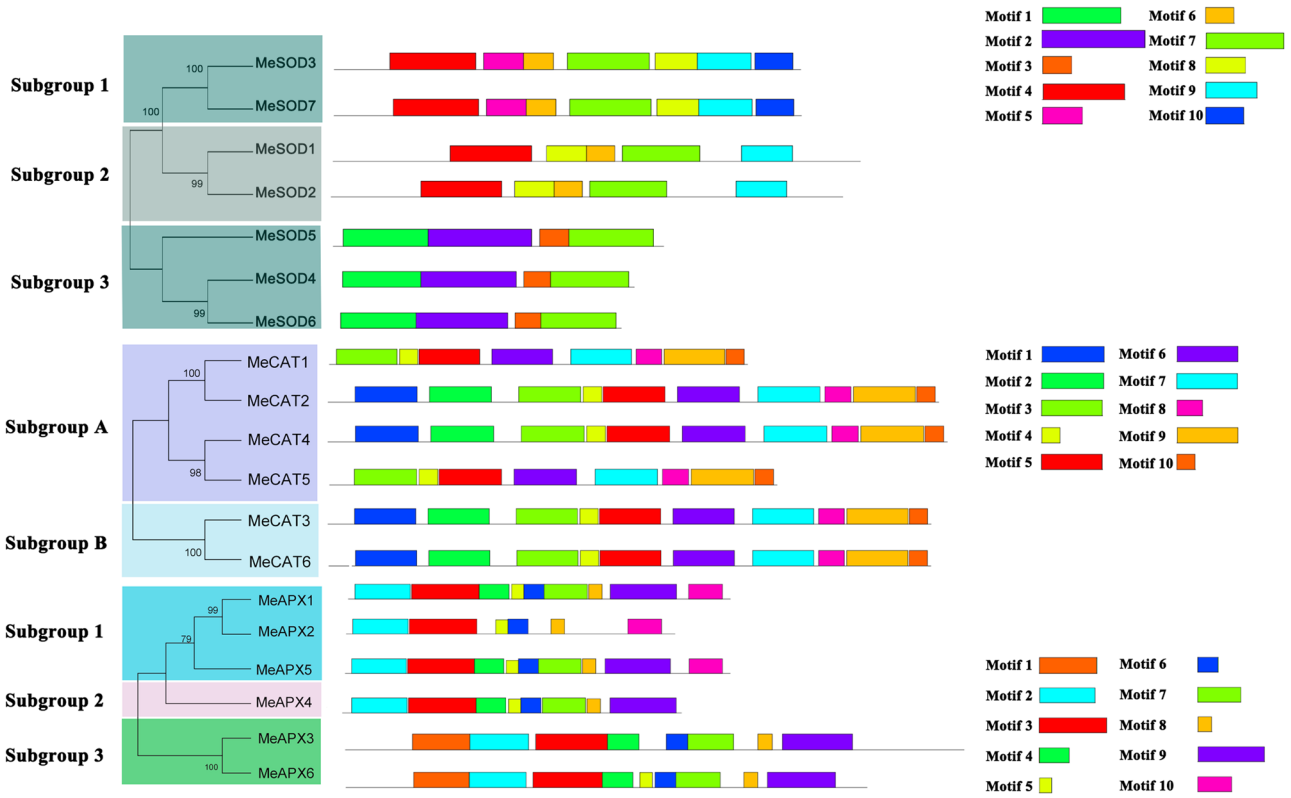


**Fig. 2** The motif analyses of MeSOD, CAT, APX in cassava on the basis of their phylogenetic relationship

## Expression of SOD, CAT, and APX Genes in Response to ABA, MT, and PEG Treatments

Expression of cassava SOD, CAT, APX genes were examined after abscisic acid (ABA), melationin (MT), and PEG treatments (Fig. 5, Table S5). For ABA treatment, *MeAPX-1*, *MeCAT5*, *MeSOD1*, and *MeSOD7* showed significant upregulation, whereas *MeSOD2* showed significant downregulation. For MT treatment, *MeAPX1* showed significant induction, whereas *MeSOD2* showed significant repression. For PEG treatment, *MeAPX1*, *MeCAT5*, and *MeSOD7* showed significant upregulation, whereas *MeAPX3*, *MeCAT2*, and *MeSOD2* showed significant downregulation. Interestingly, *MeAPX1* showed common upregulation after ABA, MT, and PEG treatments, whereas *MeSOD2* showed common downregulation upon the three treatments.

## Expression Analyses of SOD, CAT, and APX Genes During PPD Process

PPD seriously reduces the industrial value of cassava. Previous physiological and biochemical analyses showed that production of ROS is the first step during PPD development. Reduction of ROS is benefit for delay of PPD. The expression of *SOD, CAT,* and *APX* genes were examined during PPD in cassava storage roots after harvest (Fig. 6, Table S6). Compared with 0 h postharvest (HP), *MeAPX-2*, *-4* (log2 based fold change > 1), but *MeAPX-1*, *MeCAT-1*, *-2*, *MeSOD-2*, *-3* showed repression (log2 based fold change < -1) at 6 HP. *MeAPX-2*, *-4*, *-5*, *-6*, *MeCAT-4*, *-6*, and *MeSOD-4*, *-6* showed upregulation (log2 based fold change > 1), whereas *MeCAT-1*, *-2*, and *MeSOD-2* showed downregulation at 12 HP (log2 based fold change < -1). *MeAPX-5*, *-6*, *MeCAT-6*, and *MeSOD-3* showed induction (log2 based fold change > 1), while *MeAPX-1*, *MeCAT-1* and *MeCAT-2* showed repression at 48 HP (log2 based fold change < -1). Of these genes, *MeCAT-1*, *-2* showed repression during 6–48 HP. These results suggested that *MeSOD, CAT, APX* genes are involved in PPD process, and more member are induced than repressed at 12 and 48 HP.

## Expression Profiles of SOD, CAT, and APX Genes in Response to *Xanthomonas Axonopodis* pv. Manihotis (*Xam*) Infection

Cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. Manihotis (*Xam)* infection have most severe threats to cassava production. Expression of *MeSOD, CAT , APX* genes upon infection with pathogenic (TALE1Xam) and non-pathogenic (ORST4) *Xam* strains were investigated

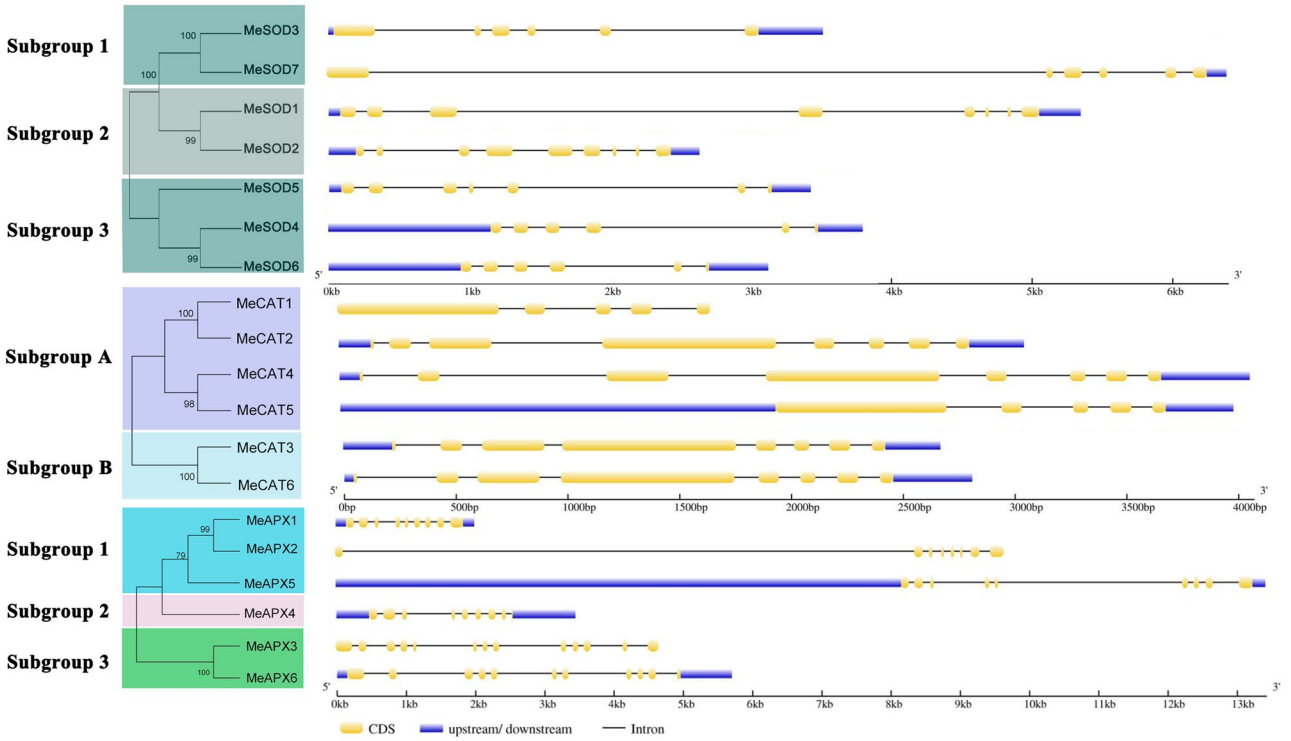


**Fig. 3** The exon–intron organization analyses of *MeSOD, CAT, APX* genes in cassava according to the phylogenetic relationship

using available transcriptomic data (Fig. 7, Table S7). At 0 d after infection, only *MeAPX-2* was suppressed (log2 based fold change < -1). At 5 d after infection, *MeAPX-2* and *MeCAT-1*, *-5* was suppressed. At 7 d after infection, *MeAPX-2* and *MeCAT-1* was suppressed, whereas *MeSOD-4, -6* was induced (log2 based fold change > 1). In these genes, *MeAPX-2* always showed suppression during the infection process. These results showed that more genes were suppressed than the genes induced in response to *Xam* infection, which is coincided with improving ROS content to resist infection.

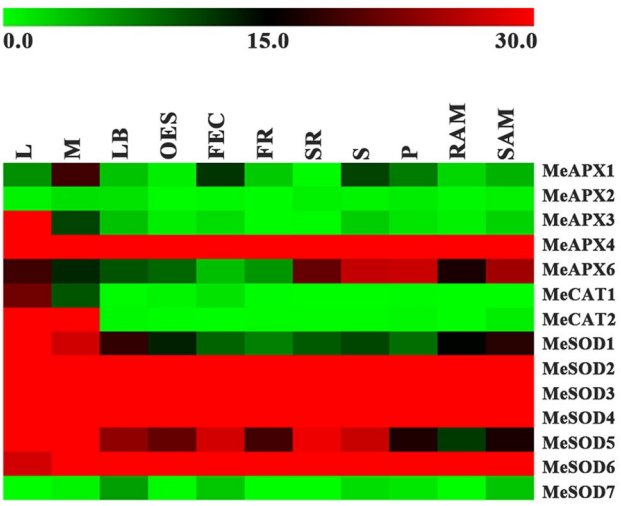


**Fig. 4** Expression data of *MeSOD, CAT, APX* genes in various tissues

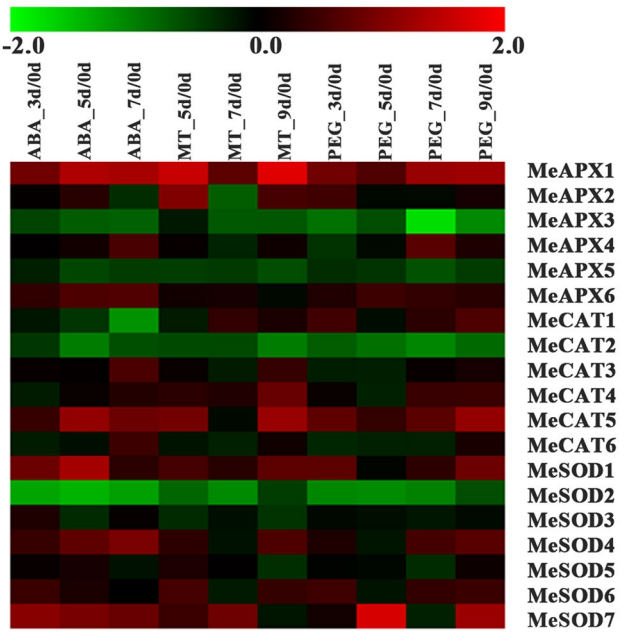


**Fig. 5** The expression data ($log_2$-based values) of the *MeSOD, CAT, APX* genes after various abiotic stress treatments

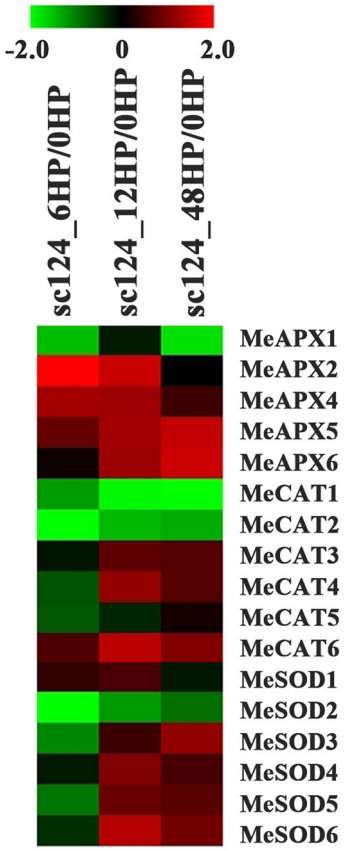


**Fig. 6** The expression data ($log_2$-based values) of the *MeSOD, CAT, APX* genes during PPD process

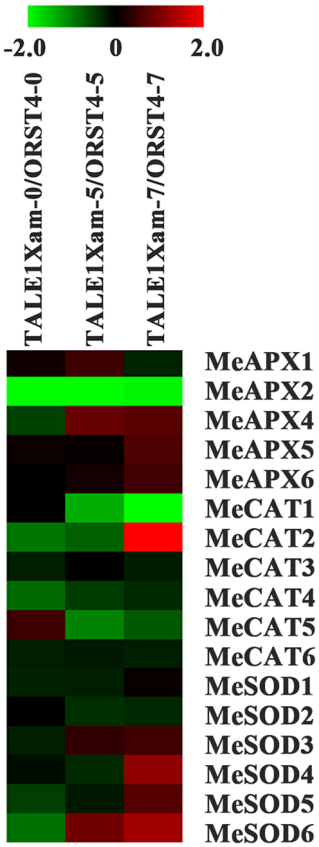


**Fig. 7** The expression data ($log_2$-based values) of the *MeSOD, CAT, APX* genes in response to *Xam* infection

## Discussion

Cassava is an important crop in subtropical and tropical regions, which is considered as a staple food for more than 750 million people around the world (Okogbenin et al. 2013). Due to its high starch production, cassava is also regarded as a raw material for food processing industry. However, the PPD restricts cassava to apply in large-scale industrial production (Hu et al. 2016). Numerous studies have shown that large amount of ROS appears in the early stage of PPD. Lower ROS accumulation results in delayed PPD development by manipulating ROS-scavenging-related genes or exogenous application of chemicals (Vanderschuren et al. 2014; Zidenga et al. 2012). SOD, CAT, APX genes are directly associated with controlling ROS content and also related with abiotic and biotic stresses (Hasanuzzaman et al. 2020). Hence, it is necessary to analyze the principal members of antioxidant enzymes in cassava. The POD family in cassava were identified by our previous study (C. Wu and Ding 2019). In this study, 7 SODs, 6 CATs, and 6 APXs were identified from the cassava genome and were divided into 3, 2, and 3 subgroups based on their phylogenetic relationship (Fig. 1). The number of SOD, CAT, APX genes in cassava was similar with that identified in other species, such as *Arabidopsis* and rice (Dehury et al. 2013; Kliebenstein et al. 1998; Ozyigit et al. 2016; Joo et al. 2014; Du et al. 2008). The phylogenetic classification of SOD, CAT, APX genes in cassava was consistent with that in rice, *Arabidopsis*, and cotton (Dehury et al. 2013; Kliebenstein et al. 1998; Ozyigit et al. 2016; Joo et al. 2014; Du et al. 2008; Wang et al. 2019, 2016). The results of phylogenetic classification were also confirmed by conserved motif and gene structure analysis. Cassava SOD, CAT, APXs contained the typical motif of SOD domain, catalase domain, peroxidase domain, respectively (Table S3), which were also identified in other plant species. The analysis of phylogenetic relationship, conserved motifs, and gene structure showed that the identification and grouping of SOD, CAT, APX genes in cassava were reliable. The subcellular localization of MeSOD, CAT, APX genes was predicted (Table S2). The same subgroup generally presented similar subcellular localization. Subgroup 1 and 2 of MeSODs localized in mitochondrion, and subgroup 3 located in cytoplasm. However, the subcellular localization of MeAPXs in different subgroup

presented inconsistent, with localization in cytoplasm, chloroplast, or mitochondrion. In rice and Arabidopsis, the member of APX family were also found to be localized in peroxisome (Teixeira et al. 2004; Shigeoka et al. 2002). MeCATs almost localized in the peroxisome. This phenomenon also existed in other species (Mittler 2002).

Reactive oxygen species are signaling molecules to regulate programmed cell death, pathogen defense, and abiotic stress responses. However, it is important to maintain a stable level of ROS, excessive ROS will cause oxidative damage to organelles and cell membranes (Mittler et al. 2004). There were several antioxidant defense mechanisms to maintain homeostasis of ROS content during biotic and abiotic stresses, such as antioxidant enzymes, including SOD, CAT, APX, and POD, etc. (Hasanuzzaman et al. 2020). SOD, CAT, APX genes are associated with stresses responses of plants. *PaSOD* (*Potentilla atrosanguinea*) played a positive role in tolerance to salt stress in *Arabidopsis* (Shafi et al. 2015). Transgenic plums showed salt stress tolerance by overexpression of *SOD* and *APX* genes. APX amount and activity is associated with increasing drought tolerance in soybean (Kausar et al. 2012). AtWNK8 endowed the salt tolerance by improving the activity of CAT (Zhang et al. 2013). Overexpression of *CatA* and *CatC* in rice improved drought stress tolerance (Joo et al. 2014). Increased SOD, CAT, APX activities are associated with gene expression regulation, and lower oxidative damage was perceived in different plants accompanied with higher SOD, CAT, APX activities (Hasanuzzaman et al. 2020). *Cu/Zn-SOD* and *CAT* play a positive role in improving cassava tolerance to drought and cold stresses (Xu et al. 2013b). In this research, *MeAPX1, MeCAT5, MeSOD7, MeAPX3, MeCAT2,* and *MeSOD2* presented significant changes at transcriptional levels after PEG treatment. Of which, *MeAPX1* was also upregulated by ABA and MT treatments, and *MeSOD2* was also downregulated by ABA and MT treatments. Many studies have showed that MT increases drought tolerance by improving the antioxidant capacity to keep ROS homeostasis (Shi et al. 2016). ABA levels is also associated with the formation of ROS in plants (Ye et al. 2011). These results suggested that *MeAPX1* and *MeSOD2* might be commonly involved in osmotic, ABA and MT responses.

Previous researches suggested that reducing ROS accumulation leads to a delayed PPD process. *MeCu/ZnSOD* and *MeCAT1* have an effect on delaying postharvest physiological deterioration of cassava tuberous root (Vanderschuren et al. 2014; Zidenga et al. 2012). SOD, CAT, APX genes are directly associated with controlling ROS content; thus, SOD, CAT, APX genes are directly associated with PPD process. ROS was also as a signal in the post-harvest losses of *N. nucifera* (Dong et al. 2015). PPD resistant cultivars showed higher expression and activity of CAT than PPD susceptible cultivars in cassava (Reilly et al. 2001). In this research, 4 SOD (*MeSOD-2*, *-3*, *-4*, and *-6*), 4 CAT (*MeCAT-1*, *-2*, *-4*, and *-6*), and 5 APX (*MeAPX-1*, *-2*, *-4*, *-5*, and *-6*) presented significant changes at transcriptional levels during PPD process. Of which, the number of induced *SODs, CATs, APXs* are significantly more than the repressed *SODs, CATs, APXs* at 12 and 48 HP. This is consistent with previous studies showing the activation of antioxidant system during PPD process for ROS scavenging (Hu et al. 2016; Vanderschuren et al. 2014). These genes may be regarded as candidate genes for manipulating to delay PPD process.

*Xam* caused cassava bacterial blight and led to a substantial loss of production (Y. Yan et al. 2018). *SOD, CAT, APX* genes are directly associated with controlling ROS content that is associated with the resistance to pathogen infection. In this research, *MeSOD-4, -6* were significant induced after pathogen infection. Overexpression of *ZmCAT2* and pepper *APX-like-1* in tobacco enhanced capacity of transgenic lines to remove $H_2O_2$ and resist pathogen (Polidoros et al. 2001; Sarowar et al. 2005). In tomato, the SOD and CAT activity were improved to resist *Fusarium oxysporum* through up-regulating SOD genes (Aamir et al. 2019). In sweet potato, *swAPX1* was strongly induced in the leaves following treatment with *Pectobacterium chrysanthemi*, which might be associated with $H_2O_2$-detoxification and thus helpful for overcoming the oxidative stress induced by biotic stresses (Park et al. 2004). Overexpression of *MeCu/ZnSOD* and *MeCAT1* in cassava enhanced the resistance to *Tetranychus cinnabarinus (*Lu et al. 2017*)*. The results indicate the involvement of cassava antioxidant enzymes in *Xam* infection.

## Conclusions

In conclusion, 7 SODs, 6 CATs, and 6 APXs were identified from cassava, and their basic classifications, conserved motifs, and exon-introns were analyzed. Transcriptional profiles presented the involvement of cassava *SOD, CAT, APX* genes in tissue development, PEG and ABA responses, *Xam* infection and PPD process. Together, this study increases our understanding of cassava *SOD, CAT, APX* genes feature and their potential function in biotic and abiotic stress responses as well as PPD process in cassava, laying a solid foundation for further function characterization of cassava *SOD, CAT, APX* genes and genetic improvement of cassava.

## Methods

### Plant Materials and Treatments

Cassava Arg7 can survive under the severe environment of high-latitude of Argentina, and the SC124 is a widely

cultivated cassava cultivar in China. Arg7 were cultured in the glass house (16/8-h light/dark cycle under 70% relative humidity, 35 °C/20 °C day/night, 200 µmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density). After 90 days, Arg7 small plantlet with consistent growth were irrigated with 100 µM abscisic acid (ABA) for ABA, 20% polyethylene glycol (PEG) 6000 solution for drought, and 100 µM melatonin (MT) solution for MT treatment, respectively. After 0, 3, 5, and 7 d or 9 d treatment, the second unfolded leaves from cassava small plantlet were provided for RNA-seq (three replicates for each sample) (Ding et al. 2019). To detect the expression changes of SOD, CAT, APX genes during PPD, SC124 storage roots from 10 months were sliced into 5 mm thick pieces for RNA-seq according to Hu et al. (three replicates for each sample) (Hu et al. 2016).

### Identification and Phylogenetic Analyses

OsSOD/OsCAT/OsAPX and AtSOD/AtCAT/AtAPX protein sequences were downloaded from RGAP and TAIR databases, respectively. The whole protein and genome sequences of cassava was downloaded from Phytozome database (cassava genome version 6.1). Hidden Markov models (HMM) (http://www.hmmer.org/) were built by known SOD, CAT, APX sequences which through by blast by known HMM (PF00080, PF00081, PF02777, PF00199, PF00141). BLAST analysis was used to confirm MeSOD/MeCAT/MeAPX genes by the sequences of OsSOD/OsCAT/OsAPX and AtSOD/AtCAT/AtAPX. Pfam database and conserved domains database were used to confirm the identified MeSOD/MeCAT/MeAPX genes. The phylogenetic tree was constructed using cassava, *Arabidopsis*, and rice SOD, CAT, APXs using MEGA 5.0 and Clustal X2.0 softwares with the pair-wise deletion option. One thousand bootstrap replicates were used to assess tree reliability.

### Protein Properties and Gene Structure Analyses

Molecular weight and isoelectric points of MeSOD, CAT, APX proteins were predicted by the ExPASy database. The conserved motif of MeSOD, CAT, APX proteins were censored with MEME database. All the motifs structure of SOD, CAT, APX proteins were annotated using InterProScan databases. The Exon/intron organization of cassava SOD, CAT, APX genes were examined by Gene Structure Display Server (GSDS) database.

### *Xam* Infection

Culture and inoculation of *Xam* (pathogenic (TALE1Xam) and non-pathogenic (ORST4) strains) were according to Yan et al. (2018). After *Xam* inoculation, the plants with *Xam* infection were cultured in the glass house. At every indicated timepoint, leaves were harvested for samples, and three biological repeats were performed for analysis. The leaves were gently mixed in 70% ethanol solution for 1 min and washed in sterile distilled water for 1 min; thereafter, the leaves were provided for RNA-seq.

### Transcriptomic Analysis

The samples of storage roots collected for RNA-seq and the specific analysis process according to Hu et al. (2016). RNA samples collected from Arg7 by Plant RNA Purification Reagent kit (Invitrogen, Carlsbad, CA, USA) (Ding et al. 2019). Total RNA was used for library preparation, and sequencing platform was HiSeq 4000. Adapter and low-quality sequences were removed by FASTX-toolkit from the raw reads. Clean sequences were aligned to the cassava genome by Tophat v.2.0.10 (Ding et al. 2019), and transcriptome assemblies were performed with Cufflinks (Ding et al. 2019). Finally, the heat map reflected the FPKM (fragments per kilobase of transcript per million mapped fragments) values was created with MeV 4.9 software.

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## Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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