Resistant Cassava Cultivars Inhibit The Papaya Mealybug *Paracoccus Marginatus* Population Based On Their Interaction: From Physiological and Biochemical Perspectives

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Abstract

Dangerous *Paracoccus marginatus* papaya mealybugs cause considerable threats and challenges to cassava production and processing. The deployment of resistant cultivars offers effective, economical and eco-friendly management strategies for pest management. We utilized *P. marginatus* mortality, development and reproduction to evaluate the resistance of fifteen cassava cultivars and conducted physiological and biochemical analyses when *P. marginatus* was fed on two resistant cultivars (Myanmar, C1115) and three susceptible cultivars (BRA900, Bread, SC205). Significantly lower digestive (amylase, sucrase, lipase), detoxification (glutathione-S-transferase and carboxylesterase) and antioxidant, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO), enzyme activities were observed in *P. marginatus* feeding on resistant cultivars compared to susceptible cultivars. For resistant cultivars, a significant reduction was found in nutritional components containing free amino acids, nitrogen, soluble sugars and the secondary metabolite malondialdehyde. Additionally, significantly higher enzymatic activity (SOD, CAT, POD and PPO) levels and secondary metabolite quantities (total phenol and tannins) were found in resistant cultivars induced by *P. marginatus* compared with susceptible ones. Additionally, RT-qPCR tests showed that the transcripts of ten genes involved in nutrition, secondary metabolites and antioxidant activities were consistent with their physiology and biochemistry changes. Thus, resistant cultivars prevented *P. marginatus* populations from suffering lower *P. marginatus* damage by elevating secondary metabolite contents and antioxidant activities, reducing nutrition levels and decreasing enzymatic activities. This study will be beneficial in determining the important indexes for developing standard regulations to evaluate *P. marginatus*-resistant cassavas, helping the development of effective strategies for pest management.

Key Message

- We identified and characterized the resistance of fifteen cassava cultivars to *P. marginatus*.
- The activities of digestive, detoxification and antioxidant enzymes of *P. marginatus* were significantly inhibited after they were fed on resistant cultivars.
- Lower contents of nutritional components and malondialdehyde, and higher levels of toxic secondary metabolites together with antioxidant enzyme activities were observed in resistant cassava cultivars infested with *P. marginatus*.
- The resistant cultivars prevented *P. marginatus* populations from suffering lower *P. marginatus* damage by elevating secondary metabolite contents and antioxidant activities, reducing nutrition levels and decreasing enzymatic activities.

Introduction

Cassava (*Manihot esculenta* Crantz), which is known as manioc, is an ancient tropical root and tuber crop that serves as food, feed and industrial production (Parmar et al. 2017). Currently, cassava is widely cultivated in over 100 countries in the tropical and subtropical regions of Africa, America, and Asia (Parmar et al. 2017). More importantly, when cassava is treated as a primary foodstuff with high starch content it fulfils the daily caloric demands of more than 800 million people in developing countries (Parmar et al. 2017). Because of its adaptability to nutrient-poor soil and harsh climatic conditions, easy management and ability to be stored on the ground for long periods of time, cassava is considered a crucial food security crop (Sayre et al. 2011). Nevertheless, some invasive and exotic pests, such as mealybugs, mites and whiteflies, which lead to serious economic damage or yield losses, cause considerable threats and challenges to cassava production worldwide (Bellotti et al. 2012).

Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), was first described in 1992 from samples collected on cassava in Mexico. Papaya mealybug is a polyphagous insect pest with a host range of more than 200 plants, such as *M. esculenta* (cassava), *Carica papaya* (papaya), *Solanum lycopersicum* (tomato) and *Capsicum annuum* (chili pepper) (Finch et al. 2021). Due to human-assisted transport, *P. marginatus* has been exported rapidly around the world over the last 30 years. Papaya mealybug is currently found in many tropical and subtropical regions of America, Africa, and Asia, including Mexico, Central America, Florida of the USA, Ghana, Tanzania, Kenya, Malaysia, Thailand, India and China (Finch et al. 2021). Infestation by *P. marginatus* to different host plants can cause crop losses of 10 to 60%, depending on the crop species (Myrick et al. 2014), and even over 90% in some cases (Macharia et al. 2017). Thus, searching for effective strategies to control *P. marginatus* and aid in the development of agricultural, economic and social benefits is very important.

To avoid damage by insect herbivores, plants have developed many defensive strategies by forming diverse morphological, biochemical and molecular mechanisms to restrict the effects of herbivore attack (War et al. 2012). These defensive strategies of host plant resistance are commonly classified into constitutive and induced defences. Moreover, biochemical traits are regarded as more effective, as they directly affect insect growth, development and survival (Kariyat et al. 2013), through the production and accumulation of plant defensive substances, including plant secondary metabolites, oxidative enzymes, proteinase inhibitors and other defensive proteins (War et al. 2012). Phenols are one of the most common and widespread defensive compounds among plant secondary metabolites and play important roles in defence against insect herbivores and microorganisms and even against competing plants (War et al. 2012). Although phenols are constitutively produced in plants, their concentration is induced by insect herbivore attack, which is found in many plants, such as groundnut *Arachis hypogaea* (War et al. 2014) and cotton *Gossypium hirsutum* (Dixit et al. 2017). Phenols have direct toxic activity to insects or act as antifeedants (War et al. 2013, Dixit et al. 2017). Tannins, as one group of the most abundant plant secondary metabolites, play an important role in plant defence against phytophagous insects. Tannins can interact with insect midgut proteins and digestive enzymes to limit their activities in insect pests and ultimately reduce insect growth and development (Barbehenn and Peter Constabel 2011). In addition, the overproduction and rapid accumulation of harmful reactive oxygen species (ROS) in plants are early defence mechanisms in response to herbivorous insect infestation or other abiotic stresses, leading to plant cell and tissue oxidative damage (Mithöfer et al. 2004). To overcome this oxidative damage, plants have developed complex and effective enzymatic antioxidant defence systems, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO) and ascorbate peroxidases (APX), to eliminate ROS (Mittler et al. 2004). The roles of these antioxidant enzymes in plant resistance to phytophagous insects have been reported in many plant systems, including alfalfa, buffalograss, rice, and tomato (War et al. 2012).

On the other hand, herbivorous insects have evolved multiple counter-adaptation mechanisms to overcome the plant defensive system, including in morphological, behavioural, biochemical mechanisms (Zhu-Salzman et al. 2005, War et al. 2012, Birnbaum and Abbot 2018). In biochemical traits, digestive enzymes, detoxification enzymes and antioxidant enzymes are considered crucial biochemical enzymes for insect development and reproduction and play important roles in withstanding plant secondary metabolites, allelochemicals or pesticides (Després et al. 2007, Deng et al. 2013). The activities of digestive enzymes such as amylase, sucrase, lipase and proteinase make significant contributions to the adaptive nature of polyphagous herbivory insects facing various food resources (Zhi et al. 2021). Glutathione-S-transferase (GST), carboxylesterase (CarE) and cytochrome P450-mediated mixed-function oxidase (MFO), as important detoxification metabolism enzymes in insects, can bind and metabolize various endogenous substrates or exogenous plant secondary metabolites (Li et al. 2019b). In addition, antioxidant enzymes including SOD, CAT, POD and PPO play an important role in defending against the pro-oxidative effects of plant secondary toxic metabolites in insects by eliminating excess ROS (Deng et al. 2013).

In a previous study, hundreds of cassava genotypes were evaluated by the International Center for Tropical Agriculture (ICTA), and a variety of potential sources of resistance to *Mononychellus tanajoa* (mite), *Aleurotrachelus socialis* (whitefly) and *Frankliniella williamsi* (thrip) were identified (Parsa et al. 2015). Recently, the resistance of multiple cassava cultivars to *Tetranychus cinnabarinus* was confirmed via laboratory and field evaluations, and cassava cultivars BRA900 and C1115 were defined as susceptible and resistant cultivars, respectively (Lu et al. 2017). However, little is known about cassava cultivars in response to *P. marginatus*. In addition, this notorious pest has the characteristics of quick development and high fecundity (Amarasekare et al. 2008), which gives it great potential to adapt to new areas and reach high density in the short term. Hence, it is important and meaningful to identify the resistance of cassava cultivars to *P. marginatus*. Here, we selected fifteen cassava cultivars to evaluate their susceptibility and resistance to *P. marginatus*. To understand why resistant cultivars inhibit the *P. marginatus* population, the activity changes of digestive enzymes, detoxification enzymes and antioxidant enzymes in *P. marginatus* were tested. In addition, the effects on the contents of nutrients and secondary metabolites, antioxidant enzyme activities and mRNA levels were measured in susceptible and resistant cassava cultivars after *P. marginatus* infestation. This study is helpful to understand cassava resistance to *P. marginatus* and will contribute to establishing standard regulations to evaluate *P. marginatus*-resistant cassava germplasms.

Materials And Methods

Cultivation of cassava cultivars

Fifteen cassava cultivars, BRA900, Bread, Nuomi, ZM9066, Swiss T7, SC205, GR5, SC6068, J1301, DG hongwei, SC11, SC8002, SC8, Myanmar and C1115, were supplied by the National Cassava Germplasm Nursery of China, Chinese Academy of Tropical Agricultural Sciences (CATAS). The cassava cultivars BRA900 and C1115 functioned as susceptible and resistant cultivars, respectively (Lu et al. 2017). All cassava cultivars were cultivated in pots (33 cm in diameter, 25 cm in height) with 5 kg of well-mixed soil (soil: peat: perlite = 1:1:1) and were grown in an insect-free greenhouse at $28 \pm 1^\circ\text{C}$ with a 14:10 h (light/dark) photoperiod. Three months later, these plants were used to test the effects of *P. marginatus* on development and reproduction or for enzyme activity assays (details below).

Insect rearing

A laboratory colony of *P. marginatus* was maintained on the back of susceptible BRA900 cultivar leaves at the Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences under the following conditions: temperature $28 \pm 1^\circ\text{C}$, $75 \pm 5\%$ relative humidity (RH), and a 14:10 h (L/D) photoperiod in a temperature-controlled growth room. Similar growth status leaves were used to raise *P. marginatus* from 10-16 leaves below the apical bud of a 3-month-old BRA900 cultivar.

Mortality evaluation of *P. marginatus* feeding on BRA900 and C1115

To examine the optimal timepoints of *P. marginatus* in enzyme activities after feeding different cassava cultivars, cassava cultivars BRA900 and C1115 were used to evaluate the adaptation of *P. marginatus*. Thirty 1-day-old female adults were inoculated on the back of cassava leaf from 3-month-old plants, and the leaf margin was surrounded with a water-saturated blotting paper strip to prevent the papaya mealybug from escaping and to keep the leaf fresh. In addition, the cassava leaf was replaced every two days. The survival was analysed every day within 10 days.

The effects on mortality, development and reproduction of *P. marginatus* in different cassava cultivars

To evaluate the influence of 15 cassava cultivars on the mortality, development and reproduction of *P. marginatus*, thirty 1-day-old female adults were fed on the backs of leaves of different cassava cultivars. The mortality of female adults was assessed on days 1 and 4 based on adaptability analysis. In addition, the female adults were removed 24 h after inoculation, and the eggs remained near. The developmental duration of eggs, 1st to 3rd instar nymphs and female adults of F_0 *P. marginatus* was investigated every 12 h. In each developmental stage, at least 20 tested insects were observed per cassava cultivar. Furthermore, the mean eggs per female and egg hatchability of F_1 *P. marginatus* were recorded. Each cassava cultivar contained three biological replications.

***P. marginatus* collection for enzyme solution**

Based on the abovementioned cassava cultivar identification, the influences of diverse enzyme activities in *P. marginatus* were measured from the following cassava cultivars: BRA900, Bread, SC205, Myanmar and C1115. Among them, BRA900 and C1115 were used as the mealybug-susceptible control and mealybug-resistant control, respectively. For each cassava cultivar, several 1-day-old female adults were placed on the back of the leaf, and 30 surviving mealybugs were collected for enzyme extraction after one day and four days. The samples were ground in liquid nitrogen and thoroughly homogenized in distilled water before undergoing centrifuging at 7,000 rpm for 15 min at 4°C . The supernatant was collected and frozen at -80°C until enzyme activity assay. Each sample included three independent biological replicates.

Measurement of digestive enzyme activity in *P. marginatus*

Amylase activity was determined following a method previously described (Vatanparast and Hosseini-naveh 2010) with slight modification. In brief, 20 μL enzyme extract and 100 μL phosphate buffer (0.2 M, pH = 5.8) and 50 μL of 2% soluble starch were incubated for 30 min at 35°C . Then, the mixed solution was terminated by the addition of 250 μL dinitrosalicylic acid (DNS) and heating in boiling water for 5 min. After cooling, the absorbance of the reaction mixture was detected at 550 nm using a microplate reader. In the blanks, phosphate buffer was used instead of enzyme extract. Maltose was used to generate the standard curve. All assays were conducted in triplicate.

Sucrase activity was measured following a previous report with some minor modifications (Karley et al. 2005). Enzyme extract (20 μL) was mixed with 100 μL phosphate buffer (0.2 M, pH = 6.5) and 50 μL 1% sucrose solution for 10 min at 35°C . The termination

and absorbance measurement of the reaction were performed as described above. The glucose was used to generate the standard curve. The measurement was repeated three times.

Lipase activity was determined as previously described (Choi et al. 2003) with a minor modification. The standard reaction mixture (pH = 7.5) was composed of 40 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 10 mM 2,3-dimercapto-1-propanol tributyrates (DMPTB), 0.5 M ethylenediaminetetraacetic acid, 10% Triton X-100, and 1 M Tris-Cl. A volume of 20 µL enzyme sample was added to 180 µL of this mixture in 96-well microtiter plates and incubated at 37°C for 30 min. The absorbance variation was measured at 405 nm. We used a blank that had no DMPTB, and triglycerides were used to generate the standard curve. Three independent biological replicates were performed for each reaction.

Measurement of detoxification enzyme activity in *P. marginatus*

Carboxylesterase (CarE) activity was determined as previously reported (Asperen 1962) with some modifications. Briefly, 75 µL enzyme sample was added to 100 µL substrate with 1×10^{-4} M eserine and 3×10^{-4} M α -naphthyl acetate (α -NA) in phosphate buffer (0.04 M, pH = 7.0). After incubating for 10 min, 25 µL of the fast blue conjugate dye was added to the reaction mixture for 10 min at 30°C. The assay was carried out in 96-well microtiter plates, and the absorbance was measured at 600 nm using a microplate reader. In the control, phosphate buffer was used instead of enzyme sample. α -NA was used to generate the standard curve. All assays were conducted in triplicate.

Glutathione S-transferase (GST) activity was determined following a method previously described (Habig et al. 1974) with some modifications. Briefly, 1-chloro 2–4-dinitrobenzene (CDNB) was used as the substrate. A 200 µL mixture of 0.6 mM CDNB and 6 mM reduced glutathione (GSH) was pipetted into 96-well microtiter plates and was incubated at 37°C for 20 min. Then, 80 µL of enzyme sample was added to the reaction mixture, and the absorbance at 340 nm was recorded every 30 s in 30 min. In the control, the same volume of phosphate buffer (0.04 M, pH = 7.0) was used instead of enzyme sample. The GSH was used to generate the standard curve. Three independent biological replicates were evaluated for each reaction.

Mixed-function oxidase (MFO) activity was measured using a modified method (Zhang et al. 2016). In brief, an aliquot of 100 µL enzyme sample was added to 50 µL substrate p-nitroanisole (1 mM) and was incubated at 37°C for 5 min. Thereafter, the mixture was mixed with 50 µL nicotinamide adenine dinucleotide phosphate (NADPH, 1 mM), and the absorbance was estimated at 450 nm. The reaction mixture without the enzyme extract was treated as a blank control. p-Nitrophenol was used to generate the standard curve. The measurement was repeated three times.

Measurement of the antioxidant enzyme activity in *P. marginatus*

Activities of SOD, CAT, POD and PPO were assayed using the UV–Vis UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan) according to a previous description (Lu et al. 2016) with minor modifications. The absorbance for SOD, CAT, POD and PPO assay was estimated at 325, 415, 470 and 398 nm. All experiments were performed in triplicate.

Cassava sample collection and the determination of antioxidant enzyme activity

To evaluate the physiological and biochemical responses of five cassava cultivars (BRA900, Bread, SC205, Myanmar and C1115), thirty 1-day-old female adults of *P. marginatus* were placed on the back of leaves from 3-month-old plants. After inoculation for 0, 1 and 4 d, the adults were removed, and 100 mg of damaged leaves without stems were collected at different time points. The plant samples were ground into powder with a mortar and pestle and thoroughly triturated in extract solution before centrifugation at 7,000 rpm for 15 min at 4°C. Each cassava cultivar contained three biological replications. Additionally, the antioxidant enzyme activities of SOD, CAT, POD and PPO in cassava plants were measured as described above.

Determination of the nutrient substance in *M. esculenta*

In each cassava powder, 100 mg was extracted with 10 mL 80% ethanol and was incubated in a water bath at 80°C for 20 min. Then, the extraction was centrifuged at 5,000 *g* for 15 min, and the supernatant was used to determine the content of soluble sugar (SS) and free amino acids (FAA) after reacting with anthrone and ninhydrin, respectively (Yemm and Willis 1954, Yemm et al. 1955, Cao et al. 2008). The absorbance was estimated at 620 and 570 nm, and glucose and alanine were used as standards. The analysis of free proline (FP) content was carried out following a previous method with some modifications (Gibon et al. 2000)

(Karalija and Selović 2018). In brief, 100 µL of each supernatant was mixed with 1 mL of reaction mixture (1% ninhydrin, 60% acetic acid, and 20% ethanol) and heated at 95°C for 20 min. After cooling, the chromogen that formed was extracted with 3 mL toluene and separated at 3,000 g for 5 min. The supernatant was used to estimate the absorbance at 520 nm, and L-proline was used as a standard. The soluble nitrogen (N) content of each sample was determined using Coomassie brilliant blue at a wavelength of 595 nm. All experiments were performed with three biological replicates.

Determination of the secondary metabolites in *M. esculenta*

Total phenol content was determined following the spectrophotometric method previously described (Maisetta et al. 2019) with some modifications. In short, 1 mL of each cassava sample was mixed with Folin-Ciocalteu reagent (0.25 M) and was incubated at 25°C for 3 min. Then, a 1 h incubation in the dark at 25°C with 1 mL Na₂CO₃ (10%, w/v) solution followed. The absorbance was read at 760 nm, and gallic acid was used as a standard to perform the calibration. The method used to determine tannin content was similar to the method of total phenol content described above. For the determination of malondialdehyde (MDA), a mixture of 1 mL of each cassava sample and 2 mL 0.25% thiobarbituric acid (TBA, w/v) in 10% trichloroacetic acid (TCA, w/v) was boiled for 15 min, quickly cooled on ice, and then centrifuged at 10,000 g for 20 min. The supernatant was used to estimate the absorbance at 600, 532 and 450 nm following a formula previously described (Wei et al. 2013). All the experiments were performed in triplicate.

RNA extraction and quantitative realtime PCR (qPCR)

Total RNA was extracted from each damaged leaf using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), the integrity was verified by 1% agarose gel electrophoresis, and the quantity was evaluated using a Nanodrop spectrophotometer 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Next, 1 µg RNA was converted to first strand complementary DNA using an RT EasyMix Kit with gDNA Eraser (Tolo Bio, Shanghai, China) in accordance with the manufacturer's instructions. Five nutrient synthesis genes (nitrate reductase, *NR*; glutamine synthetase, *GS*; sucrose synthetase, *Susy*; pyrrolin-5-carboxylic acid synthetase, *P5CS*; ornithine aminotransferase, *OAT*), two tannin synthesis genes (leucoanthocyanidin reductase, *LAR*; anthocyanidin reductase, *ANR*) and three antioxidant enzyme encoding related genes (*POD*, *CAT*, *Cu/ZnSOD*) were used to assess the changes in transcription levels in *M. esculenta* damaged by *P. marginatus*. Ten specific genes for RT-qPCR were designed using Primer-BLAST software (<https://www.ncbi.nlm.nih.gov/tools/primerblast>) and are shown in Table S1. An ABI StepOnePlus Real-Time PCR System (PerkinElmer Applied Biosystems, CA, USA) was used to analyse the tested gene transcripts with SYBR qPCR Master mix (Tolo Bio). The RT-qPCR program was as follows: denaturation at 95°C for 1 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Melting curve analysis (60–95°C) was used to validate the specificity of each primer. Cassava *Actin* (KM583807.1) was used as a reference, and the relative quantification was calculated based on the comparative 2^{-ΔΔCt} method (Livak and Schmittgen 2001). Three biological replicates and three technical replicates were employed to detect gene expression levels.

Statistical analyses

All data were statistically analysed using GraphPad Prism software 8.0 (GraphPad Software, San Diego, CA, USA) and Statistical software SPSS 26.0 (SPSS Inc., Chicago, IL, USA). For the *P. marginatus* experiment survival evaluation, the log-rank (Mantel–Cox) test was used to analyse the significant difference. The mortality, development and reproduction of *P. marginatus* experiments, various enzymatic activities, the contents of nutrition compositions and secondary metabolites among different cassavas, and gene transcript levels were compared by one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) multiple comparison test. Additionally, the changes in nutrition compositions, secondary metabolites and antioxidant enzyme activities within cassava were analysed using Student's *t*-test. All data are shown as the means ± standard errors (SE), and a *P* value less than 0.05 was considered statistically significant.

Results

Mortality analysis of *P. marginatus* responding to BRA900 and C1115

There was a significant difference in the variation in mortality of female adults between the susceptible cultivar BRA900 and resistant cultivar C1115 (Fig. 1). From the first day, the difference became more obvious. The mortality of female adults feeding on C1115 increased from 13.33 to 100% on days 1-10. However, the changes in mortality of the BRA900 group maintained a slow increasing trend within 10 days. Significantly, the mortality of the C1115 group increased to 50%, with higher variation on the fourth

day compared to that of the BRA900 group. Therefore, these results suggest that the first and fourth days were important timepoints for *P. marginatus* responding to susceptible and resistant cassava cultivars.

Influence of *P. marginatus* on mortality, development and reproduction in different cassava cultivars

To investigate the influence of 15 cassava cultivars on *P. marginatus*, the mortality, development and reproduction of *P. marginatus* were evaluated. The results showed that the average mortality of female adults (F_0) was significantly different ($P < 0.05$) among the 15 cassava cultivars (Fig. 2A). Based on the mortality assay at 1 and 4 d, the resistance of different cassava cultivars responding to *P. marginatus* was ranked as follows: BRA900 (negative control), Bread, SC205, ZM9066, Swiss T7, Nuomi, SC6068, GR5 < J1301, DG hongwei, SC11, SC8002 < SC8, Myanmar and C1115 (positive control). Furthermore, the developmental duration of *P. marginatus* feeding on different cassava cultivars was evaluated, including eggs, 1st-3rd nymphs and female adults. Significant differences in developmental duration were observed in the egg, 1st and 3rd nymph stages, but no significant changes in the 2nd nymph stage were seen in 15 cassava cultivars (Fig. 2B). Overall, the whole developmental period (egg to adult) of *P. marginatus* feeding on resistant cassava cultivars was significantly longer than the duration in susceptible cultivars ($P < 0.05$). In addition, there were significant differences in the total eggs laid per female (F_1 , $P < 0.05$) after feeding on different cassava cultivars (Fig. 2C). The average number of eggs per female in eight cassava cultivars (BRA900, Bread, SC205, ZM9066, Swiss T7, Nuomi, SC6068 and GR5) was 367.6, which was significantly higher than that in the six resistant cassava cultivars, including J1301, DG hongwei, SC11, SC8002, SC8 and Myanmar (average 147.7 eggs) and was significantly higher than that in the positive control C1115 (86 eggs). The average egg hatchability of eight cassava cultivars was 98.4%, which was significantly higher than the hatchability of the remaining seven cassava cultivars (47.1%) (Fig. 2D).

Activity of digestive enzymes in *P. marginatus* feeding on cassava plants

To illustrate what physiological responses occurred in *P. marginatus* feeding on susceptible and resistant cassava plants, the activity of digestive enzymes of *P. marginatus* was first measured. As shown in Fig. 3A, the amylase activity of *P. marginatus* feeding on Myanmar and C1115 for 1 and 4 d was 22.37 and 21.34 and 24.67 and 20.49 U/L, respectively, which was significantly lower than that in BRA900 (1 d, 36.23 U/L; 4 d, 34.21 U/L), Bread (1 d, 36.34 U/L; 4 d, 32.62 U/L) and SC205 (1 d, 34.32 U/L; 4 d, 32.26 U/L) ($P < 0.05$). In addition, the average sucrase activity of *P. marginatus* feeding on resistant plants was 61.65 and 56.18 U/L for 1 and 4 d, respectively, which was significantly lower than that in three susceptible plants (1 d, 72.52 U/L; 4 d, 72.03 U/L) ($P < 0.05$; Fig. 3B). Moreover, there were significant differences in the lipase activity of *P. marginatus* after feeding on susceptible (1 d, 10.94 U/L; 4 d, 11.05 U/L) and resistant plants (1 d, 8.03 U/L; 4 d, 8.67 U/L) ($P < 0.05$; Fig. 3C). Our results showed that resistant cassava plants can significantly suppress digestive enzyme activity in *P. marginatus* compared with susceptible plants.

Activity of detoxification enzymes in *P. marginatus* feeding on cassava plants

To investigate the changes in detoxification enzyme activity in *P. marginatus* after feeding on five cassava plants, the activity of CarE, GST and MFO was detected using the spectrophotometric method. As shown in Fig. 4A, CarE activity in *P. marginatus* showed no significant difference between the susceptible and resistant plants at 1 d. However, the average CarE activity of *P. marginatus* in Myanmar and C1115 was 1.95 $\mu\text{mol/min}$ and significantly decreased by 33.45% in comparison with that of three susceptible cassavas (average 2.93 $\mu\text{mol/min}$) at 4 d. As expected, the GST activity measurement of *P. marginatus* in resistant cassavas (1 d, 1.83 mmol/min; 4 d, 1.36 mmol/min) presented a significant reduction compared with that of susceptible cassavas (1 d, 2.45 mmol/min; 4 d, 2.29 mmol/min), decreasing by 25.20 and 40.70% at 1 and 4 d, respectively (Fig. 4B). In addition, the MFO activity of *P. marginatus* in resistant cassavas was significantly increased in contrast to that in susceptible cassavas, increasing by 16.12 and 36.99% at 1 and 4 d, respectively (Fig. 4C).

Activity of antioxidant enzymes in *P. marginatus* feeding on cassava plants

The activities of antioxidant enzymes (SOD, CAT, POD and PPO) of *P. marginatus* were differently affected by inoculation on susceptible and resistant cassavas (Fig. 5). The results showed that significantly lower levels of SOD, CAT, POD and PPO activities were detected in two resistant cassava plants than in three susceptible cassava plants ($P < 0.05$), decreasing by 31.73, 26.61 and 27.16, 27.70 and 33.99, 29.30 and 36.38, 39.33% in 1 and 4 d, respectively. Our results showed that resistant cassava plants can significantly inhibit the antioxidant enzyme activity of *P. marginatus* in comparison with that of susceptible plants.

Content analysis of the nutrition compositions in *M. esculenta*

To determine what physiological responses occurred in susceptible cassavas (BRA900, Bread, SC205) and resistant cassavas (Myanmar, C1115) after being damaged by *P. marginatus*, the contents of SS, FAA, N and free proline were measured, and the results are shown in Fig. 6. As expected, there were no significant differences in the SS, FAA and N contents between susceptible and resistant cassavas before *P. marginatus* damage (0 d). However, the contents of SS, FAA, N and free proline in resistant cassavas were significantly lower than those in susceptible cassavas at 1 and 4 d. Additionally, the contents of SS, FAA and free proline significantly increased in susceptible cassavas after damage, but no significant differences in N were seen in susceptible cassavas. A significant reduction in the contents of FAA, N and SS was observed in resistant cassavas after damage, and no significant difference in free proline was observed.

Content analysis of the secondary metabolites in *M. esculenta*

The total phenol and tannin contents of cassava leaves damaged by *P. marginatus* from susceptible and resistant cultivars are shown in Table 1. The total phenol and tannin contents in resistant cassavas were significantly higher than those in susceptible cassavas at 1 and 4 d. However, the MDA content of resistant cassavas was significantly reduced compared with that of susceptible cassavas. In addition, the tannins showed significantly decreased content in susceptible cassavas following *P. marginatus* infection, and the total phenol and MDA contents were not significantly different. The contents of total phenol and tannins were significantly reduced in resistant cassavas following *P. marginatus* infection, but the MDA content was significantly increased in resistant cassavas.

Table 1
Changes of secondary metabolite in *M. esculenta* damaged by *P. marginatus*

Cultivars	0 d	1 d	4 d	0 d	1 d	4 d	0 d	1 d	4 d
BAR900	1.03 a	1.00 a ^{ns}	0.99 a ^{ns}	65.55 a	60.31 a [*]	59.99 b [*]	0.50 a	0.49 a ^{ns}	0.49 a ^{ns}
Bread	1.05 a	1.04 a ^{ns}	1.04a ^{ns}	65.20 a	53.56 a ^{**}	52.90 a ^{**}	0.52 a	0.52 a ^{ns}	0.51 a ^{ns}
SC205	0.96 a	0.95a ^{ns}	0.95a ^{ns}	67.20 a	56.89 a ^{**}	55.56 a ^{**}	0.51 a	0.50 a ^{ns}	0.51 a ^{ns}
Myanmar	0.67 b	2.34 b ^{**}	2.36 b ^{**}	80.17 b	113.42 b ^{**}	113.75 c ^{**}	0.56 a	0.054 c ^{**}	0.052 c ^{**}
C1115	0.64 b	2.13 b ^{**}	2.11b ^{**}	78.96 b	134.89 c ^{**}	136.52 d ^{**}	0.55 a	0.30 b ^{**}	0.21 b ^{**}

Activity of antioxidant enzymes in mealybug-damaged *M. esculenta*

The results of activities of PPO, POD, CAT and SOD in susceptible and resistant cassavas following *P. marginatus* infection were present in Table 2. Before *P. marginatus* feeding (0 d), the activities of PPO, POD, CAT and SOD were significantly lower in resistant cassavas than in susceptible cassavas. However, after *P. marginatus* infection (1 and 4 d), the activities of these four antioxidant enzymes were significantly higher in resistant cassavas than in susceptible cassavas. In addition, compared with undamaged leaves (0 d), the activities of antioxidant enzymes showed significantly reduced levels of susceptible cassavas in 1 and 4 d mealybug-damaged leaves; however, antioxidant enzyme activities in resistant cassavas had significantly increased levels. Our results indicated that resistant cassava plants can elevate antioxidant enzyme activities to defend against *P. marginatus* infection in comparison with susceptible plants.

Table 2 Changes of antioxidant enzyme activity in *M. esculenta* damaged by *P. marginatus*

	SOD (U/mL)			CAT (U/mg.min)			POD (U/mg.min)			PPO (U/mg.min)		
Cultivars	0 d	1 d	4 d	0 d	1 d	4 d	0 d	1 d	4 d	0 d	1 d	4 d
BAR900	264.96 a	132.07 b **	135.63 b **	19.75 a	17.45 b *	17.53 b ns	56.25 a	49.53 a **	49.24 ab *	10.93 a	8.73 b *	8.49 b *
Bread	263.36 a	165.63 c **	164.60c **	19.70 a	15.28 a **	15.18 a **	52.20 a	47.64 a *	46.38 a **	10.03 a	9.53 b ns	9.29 b ns
SC205	256.02 a	97.88 a **	92.12a **	18.85 a	17.05 b **	15.38 a **	55.13 a	49.62 a *	52.31ab ns	10.06 a	7.55 a *	7.74 a *
Myanmar	161.75 b	203.53 d **	202.46 d **	16.90 b	23.48 c **	24.05 c **	43.47 b	56.29 b **	56.16 b **	9.14 b	14.66 c **	14.86 c **
C1115	157.50 b	235.98e **	238.44 e **	16.58 b	39.18 d **	38.60 d **	43.62 b	68.82 c **	68.47 c **	8.95 b	23.54 d **	23.47 d **

Expression analyses of defence response genes in *M. esculenta*

To determine the defence response changes of susceptible (BRA900, Bread, SC205) and resistant (Myanmar, C1115) cassavas damaged by *P. marginatus*, RT-qPCR was employed to test the expression levels of ten genes. The transcription levels of *MeNR*, *MeGS*, *MeSusy*, *MeP5CS* and *MeOAT* in three susceptible cassavas were significantly upregulated by *P. marginatus* induction at 1 and 4 d compared to 0 d (undamaged leaves) (Fig. 7A–E). In contrast, the transcripts of *MeNR*, *MeGS* and *MeSusy* in two resistant cassavas were significantly downregulated, but *MeP5CS* and *MeOAT* showed relatively high expression levels in 1 and 4 d damaged leaves compared to undamaged leaves. Nevertheless, the expression variations of these five nutrient synthesis genes induced by *P. marginatus* had more dramatic changes in susceptible cassavas than in resistant cassavas (Fig. 7A–E). In addition, the expression levels of two tannin synthesis genes (*MeLAR*, *MeANR*) and three antioxidant enzyme encoding related genes (*MePOD*, *MeCAT*, *MeCu/ZnSOD*) exhibited significant differences between susceptible and resistant cassavas (Fig. 7F–J). As expected, a significant increase in the transcript of these five genes was observed in resistant cassavas. However, a significant reduction in the transcription of these five genes occurred in susceptible cassavas at 1 and 4 d compared with 0 d.

Discussion

Mortality, developmental duration and fecundity are crucial factors in the adaptation of pests to host plants (Lu et al. 2016, Zhang et al. 2021). Previous studies reported that herbivorous insects respond to host variations not only among different plant species but also among different cultivars of the same plant variety (Iida et al. 2009, Lu et al. 2016). For example, *Bemisia tabaci* Q-biotype eggs were able to successfully develop into adults on five cultivars of *Phaseolus vulgaris* (kidney bean), while B-biotype could only survive on one of five cultivars (Iida et al. 2009). The fecundity, hatchability, developmental duration and lifespan of *T. cinnabarinus* on transgenic cassava lines SC2, SC4 and SC11 were significantly lower than those on wild-type cultivar TMS60444 (Lu et al. 2017). Similarly, there were significant differences in the growth, development and reproduction of papaya mealybugs fed on different cassava cultivars. Based on the adaptability of papaya mealybug to cassava cultivars, fifteen cultivars can be ranked as BRA900, Bread, SC205, ZM9066, Swiss T7, Nuomi, SC6068, GR5 > J1301, DG hongwei, SC11, SC8002 > SC8, Myanmar and C1115. Among these, BRA900, Bread and SC205 can be identified as susceptible to *P. marginatus*, whereas SC8, Myanmar and C1115 were identified as resistant to *P. marginatus*. The development and reproduction of *P. marginatus* feeding on resistant cassava cultivars were significantly restricted in our results, suggesting that mortality, egg hatchability, developmental duration and fecundity can be used as important indexes to evaluate the susceptibility and resistance of different cassava cultivars in response to *P. marginatus*.

The nutrition levels and allelochemicals in plants are closely related to plant suitability and resistance to herbivorous insects (Chen et al. 2009). We first detected the changes in nutritional composition of susceptible and resistant cassava cultivars after *P. marginatus* infestation and found that the contents of FAA, N and SS in resistant cassava cultivars were significantly lower than those in susceptible cassava cultivars post *P. marginatus* infestation. Amino acid nutrition is critical for insect development. For example, the fecundity and growth rate of *Rhopalosiphum padi* are directly proportional to the free amino acid (FAA) levels in its

host plant (Weibull et al. 1990). Thus, a lower FAA content in resistant cassava cultivars limits the development of *P. marginatus*. In contrast, the content of FAA in susceptible cassava cultivars acting as suitable plants can be significantly induced by *P. marginatus* damage. Similar results were also found in Asian citrus psyllid, *Diaphorina citri*, infested Satsuma orange leaves (Malik et al. 2014). Constituents of host plant quality, such as sugar and nitrogen (N), play a determinant role in the development and fecundity of insect herbivores (Awmack and Leather 2002). Our results showed that resistant cassava cultivars inhibit the development and fecundity of *P. marginatus* by reducing N and SS contents, while *P. marginatus* infestation results in a significant increase in the nutritional composition of susceptible cassava. Furthermore, the mRNA transcription level changes of two genes, *MeNR* and *MeGS*, involved in N metabolism and one gene, *MeSusy*, involved in sucrose synthesis were consistent with the changes in nutrient contents. This indicated that the observed reduction in nutrition levels may be due to the decrease in mRNA expression in resistant cassava cultivars. In addition, proline is known to be involved in the adaptation of plants in response to biotic and abiotic stresses, such as insect herbivore attack, pathogen infection and drought (Yang et al. 2011, Qamar et al. 2015, Kang et al. 2018). For example, the accumulation of free proline has been observed in potato induced by *Bactericera cockerelli* (potato psyllid) feeding (Yang et al. 2011). In our study, the free proline content in susceptible and resistant cassava plants was significantly increased after *P. marginatus* attack, and the expression of two genes, *MeP5CS* and *MeOAT*, in relation to proline synthesis also were significantly upregulated in both the susceptible and resistant cassava plants. In a previous report, the accumulation of *AtP5CS2* expression levels and increased proline content were observed in *Arabidopsis thaliana* infected with avirulent pathogens (Fabro et al. 2004). Thus, the transcript and free proline accumulation in *P. marginatus*-damaged cassava indicated that the plant defensive mechanism was triggered.

To counter insect herbivore feeding, plants have developed a series of defence strategies, including direct and indirect, constitutive and induced, chemical and physical defences, and secondary metabolites, which are a critical group that accelerates plant defence against insect attack (War et al. 2012, Singh et al. 2021). Among the secondary metabolites, plant phenols are one of the most important compounds against insect pests (War et al. 2012). For instance, wild-type sorghum *Sorghum bicolor* with 3-deoxyanthocynadin (flavonoids, phenolic compounds) resulted in significantly higher mortality and weakened corn leaf aphid *R. maidis* population growth in comparison with null mutants devoid of them (Kariyat et al. 2019). Our results showed that a significantly higher total phenol content was found in resistant cassava plants following *P. marginatus* infection than in susceptible cassava plants. This suggested that the increased content of total phenol in resistant cassava negatively affected growth, development and fecundity in *P. marginatus*. Similar results were reported in a previous study, which found a higher level of phenolic compounds in resistant winter triticale responding to *Sitobion avenae* and *Oulema melanopus* infestation than in susceptible winter triticale (Czerniewicz et al. 2017). Many similar examples in connection with phenolic compounds defence against herbivorous insects were documented by Singh et al. (Singh et al. 2021). Qualitative and quantitative changes in phenols and enhancement of the activities of oxidative enzymes against herbivorous insects are general phenomena (Maffei et al. 2007, War et al. 2011, Czerniewicz et al. 2017). For example, higher levels of various antioxidant enzyme activities and contents of secondary metabolites, including phenols, were found in insect-resistant groundnut genotypes pretreated with jasmonic acid and then damaged by *Helicoverpa armigera* compared with susceptible genotypes (War et al. 2014). In this study, the transcripts (*MeCu/ZnSOD*, *MeCAT* and *MePOD*) and activities of antioxidant enzymes (SOD, CAT, POD and PPO) in resistant cassava were significantly elevated following *P. marginatus* infection compared with susceptible cassava. Similarly, significant upregulation of mRNA levels together with activities of SOD, CAT, POD and PPO was also observed in mite-resistant cassavas in response to *T. cinnabarinus* and *T. urticae* feeding but not susceptible (Liang et al. 2017, Lu et al. 2017). These results indicated that phenols are involved in the cyclic reduction of ROS, which in turn activates a set of reactions, resulting in the activation of antioxidant enzymes to defend against insect pest attack (Maffei et al. 2007). On the other hand, the activities of antioxidant enzymes were determined in *P. marginatus*. The results showed that the activities of SOD, CAT, POD and PPO in *P. marginatus* feeding on resistant cassava were significantly lower than those in *P. marginatus* feeding on susceptible cassava. The reduction in the activities of antioxidant enzymes in insect herbivores can contribute to host plant resistance. For example, previous studies reported that the downregulation of endogenous protective enzyme activities significantly inhibited the survival, development and production of *Eotetranychus sexmaculatus* (Lu et al. 2016) and *T. cinnabarinus* (Lu et al. 2017) after feeding on resistant rubber trees and cassava, which may reduce the self-protection capacity of pests and contribute to plant resistance to pests. The activity analysis of antioxidant enzymes in plants and pests aids in understanding their interaction, and our findings showed that resistant cassava plants can elevate the total phenol content as well as antioxidant enzyme activities to defend *P. marginatus*. Conversely, *P. marginatus* can suppress the mRNA transcripts and enzyme activities of antioxidant enzymes in susceptible cassava plants for

growth and development. Our results suggested the potential importance of upregulated total phenol content and antioxidant enzyme activities as key indexes to judge cassava resistance to *P. marginatus*.

Plant tannins, polyphenolic plant secondary metabolites, are involved in plant defence against insect herbivores by deterrence and/or toxicity (Barbehenn and Peter Constabel 2011). Two enzymes, leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), play a crucial role in proanthocyanidin (PA, also called condensed tannin) biosynthesis. Previous research reported that overexpression of the exogenous genes *MrLAR* and *MrANR* (*Malus crabapple* Royalty) in *Nicotiana tabacum* facilitated the accumulation of PAs, but transient silencing of their expression in crabapple led to decreased PAs (Li et al. 2019a). In the present study, resistant cassavas presented significantly higher tannin contents in response to *P. marginatus* damage than susceptible cassavas. Our results also showed that the transcript levels of *MeLAR* and *MeANR* were higher in resistant cassava induced by *P. marginatus* than in susceptible cassavas. This indicated that the observed accumulation of tannins may be because of the upregulation of mRNA transcript levels in resistant cassavas. In addition, the induction of tannins in plant defence against insect damage and their influence on insect management have been studied in various plants, such as *Pinus sylvestris* (Roitto et al. 2009) and *A. hypogaea* (War et al. 2014). Plant tannins are protease inhibitors that bind to digestive enzymes to form a complex, precipitate proteins nonspecifically, and reduce the digestion and utilization of plant nutritive value to insect herbivores (Barbehenn and Peter Constabel 2011). During the growth and development of insects, digestive enzymes play an important role in the digestion and absorption of nutrients. We found that the activities of amylase, lipase and sucrase were significantly inhibited in *P. marginatus* after they were fed on resistant cassavas, which, due to the accumulation of tannins in resistant cassavas, caused the inhibition of digestive enzymes in *P. marginatus*. Similarly, a significant reduction in the activities of amylase and lipase was observed in *Lymantria dispar* larvae under the secondary metabolite carvacrol (terpene compound) treatment (Chen et al. 2021). In addition, digestive enzyme (amylase and trypsin) activities were reduced in both nymphs and adults of *Frankliniella occidentalis* when they were transferred from the more preferred host kidney bean to a less-preferred host broad bean, potentially leading to reduced fitness of *F. occidentalis* (Zhi et al. 2021). These results imply that different plant species or different cultivars of the same plant species with different plant secondary metabolite contents could affect insect fitness by changing its digestive capacity.

Malondialdehyde (MDA) content is considered a key parameter of oxidative damage in the plant cell membrane in response to biotic and abiotic stress, and many studies have used MDA content to evaluate the extent of plant defence against insect herbivore attack (Wei et al. 2007, Hao et al. 2011, War et al. 2011, War et al. 2014). In addition, ROS may induce lipid peroxidation in the membrane and result in the formation of lipid peroxidation products such as MDA. MDA can react with lipids, nucleic acids, glucose and proteins, leading to damage to various cellular constituents and membrane lipid structures (Wei et al. 2007). In this study, our findings revealed that susceptible cassavas showed higher MDA content than resistant cassavas after *P. marginatus* infestation. Similar results were also found in different alfalfa varieties in which susceptible varieties always presented higher MDA contents than resistant varieties when they were pierced and sucked by *Aphis medicaginis* (Wei et al. 2007). Higher amounts of MDA in susceptible groundnut in response to *Spodoptera litura* infestation have been well documented in a previous study (War et al. 2011). These results indicated that the cellular membrane systems in susceptible plants suffer much more serious damage than those in resistant plants.

Insects detoxify plant toxic secondary metabolites through many detoxification enzymes, such as GST, CarE and P450 (Li et al. 2019b, Cai et al. 2021). For example, *S. avenae* developed increased activities of GST and CarE to defend against the plant secondary metabolite gramine, and they were positively correlated with the concentration of dietary gramine (Cai et al. 2009). A previous study suggested that tannic acid elevated GST, CarE and P450 activities in *Hyphantria cunea* larvae in a concentration-dependent and time-dependent manner (Yuan et al. 2020). However, it was shown that tannic acid and quercetin could decrease the activity of GST in two moth species in a concentration-dependent manner (Tang et al. 2014). In this study, we found that the CarE and GST activities of *P. marginatus* were significantly inhibited in resistant cassavas, suggesting that the increase of plant secondary metabolites such as phenols and tannins in resistant cassavas resulted in the reduction of detoxification enzyme activities (CarE and GST) of *P. marginatus*. Similar to the response of *L. dispar* larvae to carvacrol, the activities of CarE, GST and acetylcholinesterase (AChE) were significantly decreased (Chen et al. 2021). In addition, significant upregulation of MFO activity of *P. marginatus* was observed in resistant cassavas compared with susceptible cassavas, implying that *P. marginatus* may have developed different physiological adaptations to plant secondary metabolites.

Conclusion

Resistance analysis of fifteen cassava cultivars from CATAS was performed and characterized by the evaluation of the survival, development and reproduction of *P. marginatus*. The mechanisms of cassava resistance to *P. marginatus* in physiological and biochemical responses were evaluated from the perspectives of plant and insect and are summarized in Fig. 8. Our results revealed that higher levels of toxic secondary metabolites (total phenol and tannins) together with antioxidant enzyme activities (CAT, SOD, POD and PPO) and lower contents of nutrients (SS, N, FAA and proline) and MDA were observed in resistant cassava cultivars infested with *P. marginatus*. In addition, the mRNA transcription level variations of these related genes were consistent with the changes in nutrition, secondary metabolites and enzymatic activities. The activities of digestive enzymes (amylase, sucrase and lipase), detoxification enzymes (CarE and GST) and antioxidant enzymes of *P. marginatus* were significantly inhibited after they were fed on resistant cultivars. Therefore, the resistant cassavas suffered lower pest damage and led to poor survival as well as prolonged development duration and less reproduction of *P. marginatus* than in susceptible cassavas. This study provided an example at the molecular, enzymatic activity and metabolite levels to analyse the physiological and biochemical occurrences of cassava and *P. marginatus*, which will contribute to a better understanding of the mechanism of plant resistance to insect herbivores. These data suggest that these important indexes can be used to evaluate the resistance levels of different cassava cultivars to *P. marginatus* and help in the development of effective strategies for controlling *P. marginatus* populations.

Declarations

Author Contributions

QC conceived and designed this project and revised this manuscript. XQL and XL performed the experiments and wrote the manuscript. YL, CLW, XLX, QC, YW, XWY, YQ and JS contributed to sample collection and helped with data analysis. All authors contributed to the article and approved the submitted version.

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Statements and Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethical approval This research does not contain any experiments using any animal species that requires ethical approval.

Consent to participate Consent was given by all participants included in the study.

Consent for publication All authors consent to the publication of this manuscript in Journal of Pest Science.

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Figures

Figure 1

Survival of *P. marginatus* after feeding on BAR900 and C1115 cultivars. Thirty 1-day-old female adults were included in each treatment, and their survival was evaluated. The difference in survival curves was analysed using the log-rank (Mantel–Cox) test (** $P < 0.001$).

Figure 2

Effects of different cassava cultivars on the mortality, development and reproduction of *P. marginatus*. A, Mortality assay of F0 female adults feeding on 15 cassava cultivars on days 1 and 4. Thirty individuals were used in each treatment. B, Developmental duration (egg to female adult) of *P. marginatus* (F1) feeding on 15 cassava cultivars. The life cycle of *P. marginatus* was described by our previous studies (Liang et al. 2021). C and D, Determination of the total number of eggs laid per female and hatchability of *P. marginatus*. Twenty individuals were used for each cassava cultivar. Different letters on the standard error (SE) bars represent statistically significant differences among different treatments using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test ($P < 0.05$).

Figure 3

Changes in digestive enzyme activities in *P. marginatus* feeding on susceptible and resistant cassavas. A, Amylase activity; B, Sucrase activity; C, Lipase activity. Thirty papaya mealybugs were used for each treatment. All the data shown are the means of enzyme activities $\pm$ SE ($n = 3$), and different letters on the bar indicate a significant difference in ANOVA (HSD, $P < 0.05$).

Figure 4

Changes in detoxification enzyme activities in *P. marginatus* feeding on susceptible and resistant cassavas. A, Carboxylesterase (CarE) activity; B, Glutathione S-transferase (GST) activity; C, Mixed-function oxidase (MFO) activity. Thirty papaya mealybugs were used for each treatment. The vertical bars represent the SE of the mean over three biological replicates. Different letters above the bar represent the significant differences in detoxification enzyme activities among different cassavas determined by one-way ANOVA (HSD, $P < 0.05$).

Figure 5

Changes in antioxidant enzyme activities in *P. marginatus* feeding on susceptible and resistant cassavas. A, Superoxide dismutase (SOD) activity; B, Catalase (CAT) activity; C, Peroxidase (POD) activity; D, Polyphenol oxidase (PPO) activity. Thirty papaya mealybugs were used for each treatment. The vertical bars are SE of the mean ($n = 3$). Different letters indicate statistically significant differences among different cassavas using one-way ANOVA (HSD, $P < 0.05$).

Figure 6

Changes in nutritional composition in *M. esculenta* damaged by *P. marginatus*. A, Soluble sugar (SS); B, Nitrogen (N); C, Free amino acid (FAA); D, Free proline. The vertical bars indicate the SE of the mean over three biological replicates. Different letters represent significant differences in nutrient contents among different cassava cultivars during the same time using one-way ANOVA (HSD, $P < 0.05$). In addition, leaves without mealybug infestation at 0 d were set as the control group. The asterisk indicates significant differences with a cassava among different times using Student's t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, no significant differences).

Figure 7

Changes in transcripts of ten genes in susceptible and resistant cassava cultivars infested with *P. marginatus*. A-J, Nitrate reductase gene, NR; glutamine synthetase gene, GS; sucrose synthetase gene, Susy; pyrrolin-5-carboxylic acid synthetase gene, P5CS; ornithine aminotransferase gene, OAT; leucoanthocyanidin reductase gene, LAR; anthocyanidin reductase gene, ANR; catalase gene, CAT; superoxide dismutase gene, Cu/ZnSOD; peroxidase gene, POD. The data are the mean $\pm$ SE of three biological replicates. Cassava Actin (KM583807.1) was used as a reference gene, and the relative transcripts were analysed using the comparative 2- $\Delta\Delta$ Ct method. Lowercase letters above the bars represent significant differences in expression levels among different times using one-way ANOVA (HSD, $P < 0.05$).

Figure 8

Summary of the induced responses in cassavas and *P. marginatus*. Resistant cassavas are unsuitable for *P. marginatus* adaptation because plants enhance the levels of toxic secondary metabolites as well as the activities of antioxidant enzymes and weaken nutritional compositions in response to *P. marginatus* infestation. Thus, unsuitable plants inhibit the activities of digestive enzymes, detoxification enzymes and antioxidant enzymes and ultimately result in poor population levels of *P. marginatus* and vice versa in susceptible (suitable) cassavas. The red arrow represents upregulation, and the green arrow indicates downregulation.

Supplementary Files

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