Current Research in Insect Science 5 (2024) 100071

Contents lists available at ScienceDirect

Current Research in Insect Science

journal homepage: www.elsevier.com/locate/cris

Response of cassava (*Manihot esculenta* Crantz) genotypes to natural infestation by scale insect pest *Stictococcus vayssierei* Richard (Hemiptera: Stictococcidae)

Patrice Zemko Ngatsi [a,*], Bekolo Ndongo [a], Zachée Ambang [a], Pierre Eke [b,d], William Norbert Tueguem Kuate [a], Sylvere Landry Lontsi Dida [a], Jude Ndjaga Manga [a], Champlain Djiéto-Lordon [c]

[a] Department of Plant Biology, Laboratory of Biotechnologies and Environment, Phytopathology and Plant Protection Unit, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon
[b] Department of Biochemistry, Laboratory for Phytobiochemistry and Medicinal Plants Studies, Antimicrobial and Biocontrol Agents Unit, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon
[c] Department of Animal Biology and Physiology, Laboratory of Zoology, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon
[d] Department of Crop Production Technology, College of Technology, University of Bamenda, Cameroon

ARTICLE INFO

Keywords:
Manihot esculenta
Scale insect
Growth
Biochemical parameters
Yield

ABSTRACT

Cassava is mostly grown for its starchy roots, which ensure food security. However, it is heavily attacked by the African root and tuber scale (ARTS) *Stictococcus vayssierei* in Central Africa. This pest is a severe constraint to the production of cassava, food and income security for smallholder farmers. Crop resistance development through the selection of varieties with resistant traits against targeted pests is a promising approach to pest control. This study investigated cassava genotypes' response to natural infestation and determined their resistance levels against *S. vayssierei.* Six cassava genotypes (two local and four improved) were planted in a completely randomized block design with four replicates. Agronomic parameters and ARTS density were evaluated at 3, 6, 9 and 12 months after planting (MAP). Biochemical content was determined on the pith and cortex of 12 MAP aged tuberous roots. As a result, the improved Excel variety recorded the highest scale density per plant with 102.83 ± 4.14 ARTS/P at 9 MAP. At 12 MAP, high activity of total cyanide (69.18 ± 0.88 and 69.16 ± 1.44 mg/kg) and phenylalanine ammonia-lyase (0.142 ± 0.020 and 0.145 ± 0.010 ΔA/min/mg) were observed in the cortex of the tuberous roots of the improved varieties TMS 96/0023 and TMS 92/0057 which were colonized by the lowest ARTS density. The local variety (Douma) had a high content of total phenols (44.87 ± 1.15 μg/g) in the pith. It also produced the highest yield (23.8 ± 2.9 t ha-1). Varieties TMS 96/0023, TMS 92/0057 and Douma may be the most suitable varieties for the control of ARTS stress.

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial shrub plant native to South America which is cultivated for its leaves and starchy tuberous roots (Chikoti et al., 2015; Sartiami et al., 2015). It contributes to global food security and livelihood improvement as it is an important source of carbohydrates for more than 800 million humans across the African, American, and Asian continents (Liu et al., 2011). It is further used as livestock feed and as raw material for processing industries (Jose et al., 2008). Thanks to its extreme hardiness and low-cost production, the crop represents a valuable resource for socioeconomic development, especially in low-income populations (Nweke et al., 2002; FAO 2013). Its starch-rich roots (up to 90 %) and ability to withstand heat and drought stress make cassava a crop that meets the critical present and future climate change challenges (Montagnac et al., 2009).

However, many abiotic and biotic stresses contribute to yield losses (Nassar and Ortiz 2007). Abiotic stress refers to salinity, drought, heat, and cold, while biotic stress refers to the action of diseases and pests. One of the most damaging pests of cassava in the semi-humid forest regions of the Congo Basin is the African Root and Tuber Scale (ARTS)

* Corresponding author.
E-mail address: ngatsipatrice@gmail.com (P.Z. Ngatsi).

https://doi.org/10.1016/j.cris.2024.100071
Received 30 June 2023; Received in revised form 3 January 2024; Accepted 4 January 2024
Available online 14 January 2024

Stictococcus vayssierei Richard (Dejean and Matile-Ferrero 1996; Ambe et al., 1999; Ngeve 2003; Tata-Hangy et al., 2006). *Stictococcus vayssierei,* is a cryptic polyphagous pest that feeds by sucking sap from plants. Scale insects have mouthparts in the form of stylets that allow them to pierce and suck liquids from the tissues or vascular cells of the plant's roots. Damage caused by scale insects is not limited to specific tissues near the feeding sites but affects the entire plant (Kot et al., 2015). According to Doumtsop et al. (2020), males do not feed due to rudimentary mouthparts, and females feed by sucking sap on all underground plant parts, such as stem portions, tuberous and feeder roots, and cassava cuttings. Cuttings and early bulking stages of cassava are susceptible to the high density of scale insect infestation (Ambe et al., 1999). A study by Bani et al. (2003) reported that 300 scale insects were counted on a 25 cm long cutting. Besides, the scale insect causes loss of vigor, stunted stems and weakening of the plant resulting in leaf fall, wilting, tip dieback and eventually death of cassava plants (Ngeve 2003; Williams et al., 2010). Plants that survived from the infestation yielded small with deformed storage roots, often covered with scales, and therefore unattractive to purchasers. This pest is responsible for significant yield losses of cassava under severe infestation, ranging from 60 to 100 % (Hanna et al., 2004; Lema et al., 2004; Tata-Hangy et al., 2006). Evidence to date suggests that with climate change, scale insects *S. vayssierei* are continuing to spread to new areas (Doumtsop et al., 2020).

To defend themselves, plants have developed various defense mechanisms throughout evolution to counteract the harmful effects of pathogens and pests. These include structural defense (cuticles, resins, etc.) and biochemical defense involving secondary metabolism compounds that would act directly on the parasite (antimicrobial) or indirectly through resistant structures (lignification, callose formation) and activation of non-enzymatic (phenols, flavonoids, carotenoids, glutathione, anthocyanins) and enzymatic (catalase, peroxidases, polyphenol-oxidases or PR proteins) antioxidant responses (Gill and Tuteja 2010; Verma et al., 2016). Oxidative stress, associated to reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), and hydroxyl radical (OH), is a common phenomenon in defense responses of plants to both biotic and abiotic stresses (Hasanuzzaman et al., 2019; Kmieć et al., 2022). ROS production can act as a necessary factor controlling stress perception and inducing plant defense responses against insect feeding (Ali et al., 2005; Golan et al., 2013). For example, peroxidases and catalase contribute to the reduced accumulation of ROS and detoxification of oxidation products, thereby allowing ROS to play crucial functions in signal transduction (Gulsen et al., 2010; Mai et al., 2013). Furthermore, Guaiacol peroxidase (GPX) is a member of a large multigenic heme-containing enzyme family that controls ROS generation when plants are challenged with various stressors (War et al., 2012). Phenylalanine ammonia-lyase (PAL) activates the phenylpropanoid and catalyzes the formation of phenolic compounds (Wen et al., 2005). According to Gulsen et al. (2010); Mai et al. (2013), elevated activities of these enzymes may increase the ability of plants to tolerate insect feeding. Also, phenols play a major role in host plant resistance (HPR) against pest insects and act as a defensive mechanism not only against herbivores, but also against microorganisms and competing plants. Plant phenols constitute one of the most common and widespread groups of defensive compounds (Sharma et al., 2009; Usha-Rani and Jyothsna 2010). Cyanogenic glycosides are a group of amino acids' derived compounds that can offer plant defense against insects due to their bitter taste and release of toxic HCN upon tissue disruption. They can act as feeding cum oviposition deterrents and also phagostimulants (Zagrobelny et al., 2004). However, the speed of reaction and the defense mechanism effectiveness to infestation vary according to the genome from one species to another (Gatehouse 2002). Thus, the selection of resistant varieties and release to farmers appears to be a promising option against *S. vayssierei* attacks. Therefore, the production of biochemical compounds by plants can be involved in their resistance against insect pest. In the development of IPM strategies, research and development of sustainable agricultural pest management techniques that are environmentally friendly and economically accessible to farmers must be successfully adopted. The hypothesis tested in this work was that cassava varieties subjected to scale insect stress under natural conditions react by activating several biosynthetic pathways, the intensity of which varies according to the species and or variety. In this context, this study aimed at investigating the response of six cassava genotypes to natural infestation and determining their resistance levels against *S. vayssierei.*

Materials and methods

Study site and field experiment

The experiment was conducted from March 2018 to March 2019 in Akonolinga (N 03°48.136′ and E 012°15.518′, altitude 671 m), a locality situated in the center region of Cameroon. This locality belongs to the humid forest with bimodal rainfall (1500 to 2000 mm/year) agroecological zone (Moudingo 2007). A cassava field was set up on a fallow land (3 years old) naturally infested by the scale insect *S. vayssierei* and the ant *Anoplolepis tenella* (Santchi), which is intimately associated with ARTS (Dejean and Matile-Ferrero 1996). Six cassava varieties selected based on their agronomic performances were used in the experiment. Four improved varieties were obtained from the International Institute of Tropical Agriculture (IITA) (TMS 96/0023 and TMS 92/0057) and Institute of Agricultural Research for Development (IRAD) of Nkolbisson (8034 and Excel) and two local varieties for their susceptibility to cassava mosaic disease (Douma and Miboutou) based on field observations and locally grown by farmers as a control variety. The experiment was set up in a completely randomized block design (CRBD), with four blocks spaced at 1.5 m. Each block was consisting of six plots (3 m x 4 m) spaced at 1 m. Cassava cuttings (about 20 cm long) were planted obliquely by pushing 2/3 of the cutting into the soil (IITA 2000). The plots each had 24 cuttings at 1 m x 0.8 m spacing. All cuttings used were not infested with ARTS and were not subjected to any pesticide treatment. Weeding was done with a hand hoe at 3 months intervals as from planting. No chemical fertilizers were applied during the crop growth.

Quantifying agronomic parameters

Agronomic parameters including stem collar diameter and fresh shoots weight were evaluated at 3, 6, 9, and 12 months after planting (MAP). The stem collar diameter was measured using a caliper on three labeled plants per plot. The fresh shoots' weight was measured at harvest on three randomly uprooted plants using a precision balance (Pesola). Cassava harvest yield was evaluated at 12 MAP of five plants per plot. The yield was estimated in tons per hectare on fresh tuber weight using the formula from Kamau et al. (2011).

$$\text{Yield}\left(\text{tha}^{-1}\right) = \text{fresh tuber weight } \left(\text{kg}/m^2\right) x 10000\left(m^2/\text{ha}\right) x 1\,(\text{t})/1000(kg).$$

Scale insect density

The scale insects were counted on three randomly sampled and uprooted plants in each plot. All life stages (first-instar nymphs L1, second-instar nymphs L2, adults, dead individuals) of the scale insects were counted on underground organs of the cassava plant using a 10x lens at 3, 6, 9 and 12 months after planting (Ambe et al., 1999; Tindo et al., 2007; Ndengo et al., 2016; Ngatsi et al., 2020).

Biochemical compounds analyses

Cassava root samples (12 months old) of the six cassava varieties on which the scale insects were counted, were collected and immediately brought to the laboratory for determination of total phenols, flavonoids, cyanide (HCN) and some antioxidant enzymes: catalase (CAT), guaiacol peroxidase (GPX) and phenylalanine ammonia-lyase (PAL). Two (2)

harvested tubers were washed, cut transversely to the middle (0.5–1 cm) and on the cross section obtained, the pith and cortex were stored at (−80 °C) until analysis. The experiments were carried out in triplicates. The assessment of biochemical compounds was made at 12 MAP because some work shows that at 12, 15 and 18 MAP, cassava tubers are generally at full maturity than at 3 MAP (Ayanru and Sharma, 1984–1985; Akinwale and Oguntona 2012).

Extraction of phenolic compounds

The extraction of phenolic compounds was carried out using the Luthria and Pastor-Corrales (2006) protocol modified by Mujica et al. (2009). To 1 g (pith and cortex level) of each sample of ground cassava varieties in a mortar, 10 mL of 80 % acidified methanol was mixed with 0.1 % HCl and centrifuged at 1800 rpm for 5 min. The methanol extracts obtained were used to determine the phenols and flavonoid contents.

Determination of total phenol contents

Total phenol contents (TPC) assay was performed according to the slightly modified protocol of Singleton and Rossi (1965). One (1) mL of the 10 % Folin-Ciocalteu reagent was added to 0.2 mL of methanolic extract. The mixture was homogenized and incubated for 5 min. Then, 0.8 mL of 20 % Na_2CO_3 was added and incubated for 1 hour. Absorbance was measured at 725 nm spectrophotometer (TECAN INFINITE M200) against a blank. Calibration was performed using a chlorogenic acid solution (10 µg/mL). The content was expressed in µg chlorogenic acid equivalent/g of extract (µg ChlAE/g) using a standard curve (Y = 0.005x, R^2 = 0.9981).

Determination of total flavonoid contents

The total flavonoid content (TFC) of plant extract was estimated using the method described by Kramling and Singleton (1969). It is based on the precipitation by formaldehyde of the flavonoids present in the extracts and their quantification by the difference between the quantity of total phenols and the non-flavonoid phenols remaining in the extract. To 4 mL methanolic extract, 2 mL HCl diluted at 50 % and 2 mL formaldehyde respectively were added and incubated for 24 h. The filtrate collected representing non-flavonoid phenolic compounds were determined by the Singleton and Rossi (1965) method, and the flavonoid content was determined according to the following formula:

$$\text{Total Flavonoid} = \text{Total phenols} - \text{Non flavonoid phenols}$$

Determination of total cyanide (HCN) contents

To determine total cyanide (HCN) content, the method described by Bradbury et al. (1999) with some modifications was used. One thousand (1000 mg) of cassava root tuber sample (pith and cortex level) was crushed and introduced into plastic bottles. Afterward; 10 mL phosphate buffer (0.1 M pH 7) was added. A picrate paper was introduced into the bottles, to which 5 mL of Na_2CO_3 was added. The bottles were closed and incubated for 16 h at 30 °C. The picrate paper was removed and eluted with 5 mL of water, then incubated for 30 min and the absorbance was measured at 510 nm spectrophotometer (TECAN INFINITE M200) against a blank. The following equation was used to determine the total content of the cyanide released:

$$\text{HCN}(\text{mg}/\text{kg}) = 396 \times \text{absorbance} \times 100/\text{g}$$

Extraction and estimation of total soluble proteins

The tuber section of the varieties (1 g of pith and cortex) was ground in a mortar containing 5 mL of 0.1 M sodium phosphate buffer at pH 7 placed on ice. After centrifugation at 5000 rpm for 10 min , the recovered supernatant formed the crude enzyme extract according to the protocol described by Tarafdar and Marschner (1994).

The total soluble protein contents of the extracts were estimated according to the protocol of Bradford's (1976). To 100 µL of the previously obtained extract, 0.5 mL phosphate buffer, 400 µL distilled water and 2 mL of the brilliant blue Coomassie G250 reagent were added, for a final volume of 3 mL with BSA (Bovin Serum Albumin). The values obtained after 595 nm spectrophotometer (TECAN INFINITE M200) assay were compared to the values expressed by a calibration curve (Y = 1.205x, R^2 = 0.9045) against a blank. Protein contents are expressed in µg/g of fresh weight.

Enzymatic activities

Catalase activity (CAT: EC 1.11.1.6) was measured by the decrease in optical density at 240 nm due to H_2O_2 consumption, according to Cakmak and Horst (1991) method. The decrease in absorbance was recorded for 3 min. The reaction mixture contains 100 µL phosphate buffer (0.1 M, pH 7) and 2 µL of 0.3 % H_2O_2, and 30 µL of enzyme extract. Catalase activity is expressed in (ΔA 240/min/mg of protein).

Guaiacol peroxidase activity (GPX: EC 1.11.1.7) was measured using a reaction medium containing 1425 µL of phosphate guaiacol buffer, 50 µL of enzyme extract, 25 µL H_2O_2 at 0.3 %. The evolution of absorbance at 470 nm was measured for 1 min due to the polymerization of guaiacol to tetra guaiacol in the presence of H_2O_2 against a blank (Hiner et al., 2002). GPX activity is expressed in (ΔA 470/min/mg of protein).

Phenylalanine ammonia-lyase activity (PAL: EC 4.3.1.5) was evaluated according to the Whetten and Sederoff (1992) protocol. The reaction medium consists of 50 µL phosphate buffer (0.1 M, pH 7), 50 µL phenylalanine, and 50 µL enzyme extract and incubated for 30 min. The reaction was stopped by adding 0.5 mL of 5 M HCl and the absorbance was read at 290 nm against a blank. PAL activity is expressed in (ΔA 290/min/mg of protein).

Statistical analyses

Data are represented as means ± standard deviation of a minimum of three repetitions (n = 3). Data collected for the various parameters studied were subjected to one-way analysis of variance (ANOVA) using software R version 4.1.2 (R Development Core Team 2022). The difference between the various means was compared using the Tukey's HSD test at *0.05* levels when the normality test (Shapiro-Wilk test, $P > 0.05$) and homogeneity (Levene's test, $P > 0.05$) of variance were verified. The relationship between ARTS density and agronomic and biochemical parameters at 12 MAP was performed using multiple linear regression and principal component analysis (PCA). Multiple linear regression of each parameter was run for the effect of agronomic parameters, biochemical compounds in pith and cortex on *S. vayssierei* density:

$$Y_i = B_o + B_1X_1 + B_2X_2 + B_3X_3 + \ldots + B_nX_n + \varepsilon_i$$

where: Y_i = ith response variable, B_o is the intercept, B_1 is the regression coefficient for factor X_1, B_n is the regression coefficient for the X_n th factor and εi is the error term.

The best regression selected is the one whose multicollinearity was detected by calculating the variance inflation factor (VIF) for each explanatory variable to remove the variables that are highly correlated with each other. The values of the variables with a VIF lower than 5 (VIF < 5) were retained to build and validate the model and those higher than 5 (VIF > 5) were deleted (Everitt and Skrondal 2010).

Results

Stem diameter

There are significant differences at 6 MAP (F-value=4.700, df=5, P

= 0.00881) and 9 MAP (F-value=4.392, df=5, P = 0.0116) between varieties of stem diameter. The local variety Douma produces the highest stem diameter (2.42 ± 0.18 cm and 2.66 ± 0.16 cm) respectively. At 12 MAP (F-value=4.810, df=5, P = 0.0801), the highest stem diameter is recorded with the local variety Douma (2.94 ± 0.20 cm) and the lowest with Excel (2.52 ± 0.16 cm) followed by Miboutou (2. 57 ± 0.21 cm) varieties (Table 1).

Weight of fresh shoots

For fresh shoots weight there are significant differences at 3, 6, 9 and 12 MAP (Table 2). The weight of fresh shoots were higher in the improved variety 8034 (2.04 ± 0.27 kg) and local variety Douma (203 ± 0.29 kg) at 3 MAP (F-value = 6.457, df = 5, P = 0.00217). At 12 MAP (F-value = 21.634, df = 5, P <0.001), the lowest fresh shoot weight is recorded in improved variety Excel (1.99 ± 0.42 kg).

Yield

A significant difference (F-value = 8.749, df = 5, P = 0.000475, df error = 15) was recorded between varieties concerning yield (). The best fresh tuber yield was observed in the local variety Douma (23.75 ± 1.78 t ha^{-1}) followed by the improved variety TMS 96/0023 (21.83 ± 3.63 t ha^{-1}). Conversely, improved variety Excel showed the lowest yield (14.13 ± 2.41 t ha^{-1}).

ARTS density

All life stages scale insects density per plant (ARTS/P) on cassava varieties are recorded in Table 3. It appears that all varieties were infested by scale insects depending on the cassava varieties. A very highly significant difference was observed at 3 (F-value = 27.337, df = 5, P<0.001), 6 (F-value = 71.625, df = 5, P<0.001), 9 (F-value = 19.671, df = 5, P<0.001) and 12 (F-value = 12.184, df = 5, P<0.001) MAP. Overall (F-value = 73.995, df = 5, P<0.001), the improved Excel variety (90.12±4.97ARTS/P) has the highest average number of scale insects, followed by the local Douma variety (83.32±2.58ARTS/P). The improved varieties TMS 92/0057 (67.63±3.75ARTS/P) and TMS 96/0023 (58.98±2.19ARTS/P) have the lowest average number of scale insects.

Biochemical associated with plant resistance

Biochemical resistance markers in the pith and cortex level of cassava varieties' tuberous roots showed significant variations ($P < 0.05$) for total phenols and flavonoids, HCN, CAT, GPX and PAL contents between improved and local cassava.

Variability of biochemical parameters contents in the pith

In the pith of tuberous cassava roots, the local variety Douma had enough higher total phenols (44.87±1.15 µg/g FW) and total flavonoid (34.75±1.66 µg/g FW) contents, as was the total cyanide content (52.34 ±2.39 mg/kg) than to local variety Miboutou which accumulate the most important concentration with 52.34±2.39 mg/kg. Excel variety recorded the lowest CAT activity of 0.88±0.16 ΔA240/min/mg. However, it is increased in variety 8034 (1.78±0.25 ΔA240/min/mg). Local variety Miboutou (6.96±0.33 ΔA470/min/mg) recorded significantly low concentrations of GPX activity whereas TMS 92/0057 and TMS 96/0023 with values 4.70±0.44 and 4.85±0.36 ΔA470/min/mg) respectively (Table 4). For phenylalanine ammonia-lyase activity and total protein content are highest in the variety TMS 92/0057 with values 0.063±0.010 ΔA290/min/mg and 0.045±0.006 µg/g, respectively.

Table 1
Stem diameter (means ± SD) of cassava varieties planted in a site naturally infested by *Stictococcus vayssierrei* in Akonolinga, center region of Cameroon.

Varieties	3 MAP	6 MAP	9 MAP	12 MAP
8034	1.92 ± 0.41ab	2.20 ± 0.26ab	2.53 ± 0.27ab	2.87 ± 0.42ab
92/0057	2.08 ± 0.23a	2.35 ± 0.22a	2.59 ± 0.36a	2.78 ± 0.41ab
96/0023	1.91 ± 0.34ab	2.25 ± 0.18ab	2.55 ± 0.24a	2.84 ± 0.20ab
Douma	2.16 ± 0.17a	2.42 ± 0.18a	2.66 ± 0.16a	2.94 ± 0.20a
Excel	1.70 ± 0.24b	1.96 ± 0.20b	2.19 ± 0.24b	2.52 ± 0.16b
Miboutou	1.99 ± 0.40ab	2.22 ± 0.22ab	2.41 ± 0.24ab	2.57 ± 0.21b
F-value	5.985	4.700	4.392	4.810
P (>F)	0.00308**	0.00881**	0.0116*	0.00801**

df error= 15; MAP: month after planting. The means followed by the same letter in the column are not significantly different in the Turkey HSD test at ($P < 0.05$).

Table 2
Fresh shoots weight (means ± SD) of cassava varieties planted in a site naturally infested by *Stictococcus vayssierrei* in Akonolinga, center region of Cameroon.

Varieties	3 MAP	6 MAP	9 MAP	12 MAP
8034	2.04 ± 0.27a	2.37 ± 0.27ab	2.63 ± 0.20ab	2.81 ± 0.21b
92/0057	1.67 ± 0.11ab	1.91 ± 0.15bc	2.16 ± 0.16cd	2.32 ± 0.41cd
96/0023	1.77 ± 0.28ab	2.02 ± 0.33bc	2.37 ± 0.32bc	2.60 ± 0.35bc
Douma	2.03 ± 0.29a	2.55 ± 0.30a	2.88 ± 0.38a	3.30 ± 0.43a
Excel	1.42 ± 0.18b	1.61 ± 0.16c	1.81 ± 0.16d	1.99 ± 0.42d
Miboutou	1.82 ± 0.21ab	2.22 ± 0.29ab	2.48 ± 0.30bc	2.68 ± 0.27bc
F-value	6.457	10.482	21.988	21.634
P (>F)	0.00217**	0.000179***	<0.001***	<0.001***

df error= 15; MAP: month after planting. The means followed by the same letter in the column are not significantly different in the Turkey HSD test at ($P < 0.05$).

Table 3
African Root and Tuber Scale (ARTS) density (means ± SD) on six field-screened cassava varieties in Akonolinga, center region of Cameroon.

Varieties	3 MAP	6 MAP	9 MAP	12 MAP	Average
92/0057	51.02 ± 8.82c	92.00 ± 7.94b	70.92 ± 5.92c	56.58 ± 3.42c	67.63 ± 3.75c
96/0023	47.52 ± 3.25c	70.15 ± 3.47d	68.13 ± 4.28c	50.13 ± 5.17c	58.98 ± 2.19d
Excel	78.21 ± 8.28a	113.67 ± 7.63a	102.83 ± 4.14a	65.75 ± 4.58ab	90.12 ± 4.97a
8034	66.17 ± 6.21b	82.25 ± 9.42c	74.39 ± 6.70bc	59.56 ± 4.70b	70.61 ± 2.74c
Douma	72.83 ± 6.21ab	104.54 ± 9.38a	87.21 ± 4.76b	68.49 ± 2.91a	83.32 ± 2.58b
Miboutou	62.33 ± 6.46b	73.71 ± 7.36cd	78.75 ± 5.18bc	70.63 ± 5.57a	71.37 ± 3.92c
F-value	27.337	71.625	19.671	12.184	73.995
P (>F)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

df error=15; MAP: month after planting. The means followed by the same letter in the column are not significantly different in the Turkey HSD test at ($P < 0.05$).

Variability of biochemical parameters contents in the cortex

In the cortex of tuberous roots (Table 5), the highest levels of phenols and flavonoids totals were produced by the Douma variety (80.54±0.16 and 70.43±9.21 µg/g FW, respectively). The total cyanide (HCN) content is high in TMS 96/0023 variety (69.18±0.88 mg/kg) and the Miboutou variety (30.02±0.37 mg/kg). CAT activity is high in the TMS 96/0023 variety (4.91±0.68 ΔA240/min/mg), followed by TMS 92/0057 variety (4.28±0.38 ΔA240/min/mg). Concerning the activity of guaiacol peroxidase, Excel (22.19±1.12 ΔA470/min/mg) has the highest activity compared to TMS 96/0023 (4.59±0.59 ΔA470/min/mg). For phenylalanine ammonia-lyase, activity is high in TMS 92/0057 variety (0.145±0.01 ΔA290/min/mg) and for total soluble protein, Excel variety has recorded (0.046±0.004 µg/g) more concentration than in TMS 96/0023 variety (0.020±0.003 µg/g).

Table 4
Variation of biochemical markers in the pith of cassava varieties planted in a site naturally infested by *Stictococcus vayssierrei* in Akonolinga, center region of Cameroon.

Varieties	TPC	TFC	Proteins	HCN	CAT	GPX	PAL
96/0023	36.00 ± 0.35b	30.58 ± 0.31b	0.036 ± 0.004a	44.44 ± 1.32b	1.08 ± 0.13bc	4.85 ± 0.36c	0.040 ± 0.003bc
Excel	27.00 ± 2.02c	20.37 ± 0.68d	0.038 ± 0.005a	15.30 ± 1.64d	0.88 ± 0.16c	5.08 ± 0.21bc	0.032 ± 0.005c
92/0057	29.20 ± 0.69c	24.24 ± 0.74c	0.045 ± 0.006a	21.75 ± 0.33c	1.30 ± 0.15b	4.70 ± 0.44c	0.063 ± 0.010a
8034	34.07 ± 0.12b	24.31 ± 2.16c	0.037 ± 0.003a	17.37 ± 0.55d	1.78 ± 0.25a	6.02 ± 0.38ab	0.033 ± 0.009c
Miboutou	13.67 ± 2.05d	8.11 ± 0.33e	0.024 ± 0.002b	52.34 ± 2.39a	1.47 ± 0.03ab	4.92 ± 0.62bc	0.055 ± 0.004ab
Douma	44.87 ± 1.15a	34.75 ± 1.66a	0.039 ± 0.003a	9.59 ± 0.47e	1.23 ± 0.08bc	6.96 ± 0.33bc	0.037 ± 0.006c
F-value	191.3	176.8	8.964	501.7	13.37	14.01	11.09
P (>F)	<0.001***	<0.001***	0.000965***	<0.001***	0.000148***	0.000117***	0.000362***

df error=12 ; TPC: Total phenols content, TFC: Total flavonoids content, and Proteins (–µg/g fresh weight), HCN: total cyanide (mg/kg) and enzymatic activity (CAT: catalase, GPX: guaiacol peroxidase, PAL: phenylalanine ammonia-lyase -ΔA/min/mg fresh weight). The values followed by the same letter in the column are not significantly different in the Turkey HSD test at (*P < 0.05*).

Table 5
Variation of biochemical markers in the cortex of cassava varieties planted in a site naturally infested by *Stictococcus vayssierrei* in Akonolinga, center region of Cameroon.

Varieties	TPC	TFC	Proteins	HCN	CAT	GPX	PAL
96/0023	41.00 ± 1.91b	35.58 ± 4.07bc	0.020 ± 0.003c	69.18 ± 0.88a	4.91 ± 0.68a	4.59 ± 0.59c	0.142 ± 0.020a
Excel	28.05 ± 3.19bc	21.41 ± 3.98cd	0.046 ± 0.004a	37.35 ± 2.03c	1.67 ± 0.15c	22.19 ± 1.12a	0.066 ± 0.006c
92/0057	43.53 ± 0.85b	38.58 ± 1.01b	0.024 ± 0.002c	69.16 ± 1.44a	4.28 ± 0.38ab	9.41 ± 1.00bc	0.145 ± 0.010a
8034	32.21 ± 2.75bc	22.45 ± 5.00cd	0.037 ± 0.001b	54.07 ± 0.86b	1.86 ± 0.12c	11.77 ± 3.36b	0.076 ± 0.003c
Miboutou	17.72 ± 1.67c	12.17 ± 5.11d	0.040 ± 0.005ab	30.02 ± 0.37d	1.87 ± 0.26c	19.33 ± 3.11a	0.061 ± 0.005c
Douma	80.54 ± 3.73a	70.42 ± 9.21a	0.039 ± 0.001ab	54.88 ± 1.40b	3.65 ± 0.13b	20.80 ± 3.79a	0.115 ± 0.003b
F-value	44.2	44.95	33.61	477.8	48.68	24.06	44.1
P (>F)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

df error=12 ; TPC: Total phenols content, TFC: Total flavonoids content, and Proteins (–µg/g fresh weight), HCN: total cyanide (mg/kg) and enzymatic activity (CAT: catalase, GPX: guaiacol peroxidase, PAL: phenylalanine ammonia-lyase -ΔA/min/mg fresh weight). The values followed by the same letter in the column are not significantly different in the Turkey HSD test at (*P < 0.05*).

Relationship between parameters studied

Multiple linear regression (MLR)

The regression made from biochemical (pith and cortex) and agronomic parameters were selected as independent variable and scale insect density as dependent variable in the multiple regression models (Table 6). The variance inflation factor (VIF) values of the variables included in the model are less than 5. Regarding the biochemical parameter in the cortex, catalase had a significant negative effect and guaiacol peroxidase had a significant positive effect on scale insect density (*P < 0.01*). Phenylalanine ammonia-lyase had a significant positive effect (*P < 0.05*). In pith, total phenols (*P < 0.01*) and total cyanide (*P < 0.05*) had a significant positive effect. Regarding agronomic parameters, fresh shoot weight (*P < 0.01*) has a significant positive effect and stem diameter has a significant negative effect on scale insect density. The variance of scale insect density explained was $R^2 = 0.7858$ for the biochemical parameter in cortex, $R^2 = 0.447$ in pith and $R^2 = 0.3901$ for the agronomic parameters.

Principal component analysis

A principal component analysis (PCA) was performed to screen the best varieties among agronomic parameters, biochemical content in the pith and cortex of six cassava tuberous roots infested by scale insects (Fig. 2). The visualized dispersion of the two main components (PC1 and PC2) explains 72.2 % of the total variation of the system and contains more information and graphically summarized genotypes performance about the ARTS infestation. The improved variety Excel, 8034 and local variety Miboutou, following ARTS infestation produce a higher level of guaiacol peroxidase, protein in the cortex and hydrogen cyanide, catalase in the pith. These varieties have a low fresh tuber yield and represent the first group (Group 1). TMS 96/0023 and TMS 92/0057 varieties (Group 2) produce the higher level of hydrogen cyanide (HCN), catalase (CAT), and phenylalanine ammonia-lyase (PAL) in the cortex, protein and flavonoid in the pith. They are close to the yield. The third group (Group 3) consists to the local variety Douma which produce in the pith and cortex high level of total phenol and flavonoid, guaiacol peroxidase. This variety has the best agronomic parameters and is very close to yield.

Table 6
Multiple linear regression (MLR) analysis between study variables and ARTS density at 12 months after planting.

Variables	Biochemical parameters						Agronomic parameters			
	Biochemical in cortex			Biochemical in pith						
	Estimate	*P* (>|t|)	VIF	Estimate	*P* (>|t|)	VIF	Variables	Estimate	*P* (>|t|)	VIF
Intercept	50.72	<0.001***		83.05	<0.001***		Intercept	88.8821	<0.001***	
CAT	−4.65	0.0013**	2.55	−1.00	0.8261ns	1.01	FSW	14.24	0.0064 **	2.61
GPX	0.85	0.0062**	2.15	–	–	–	yield	−1.26	0.0515.	2.41
PAL	155.74	0.0248*	3.65	–	–	–	SD	−14.66	0.0171 *	1.22
HCN	−0.052	0.7158ns	2.91	0.18	0.0110*	2.11				
TPC	–	–	–	−0.88	0.0059**	2.10				
TFC	–	–	–	–	–	–				
Proteins	–	–	–	–	–	–				
R^2	0.7858			0.447			R^2	0.3901		

ns: no significant; (-) variables which not retained in the model; VIF: variance inflation factor, R^2: coefficient of determination, TPC: Total phenols content, TFC: Total flavonoids content, HCN: total cyanide, CAT: catalase, GPX: guaiacol peroxidase, PAL: phenylalanine ammonia-lyase, FSW: fresh shoots weight, SD: stem diameter.

Fig. 1, Table 6.

Discussion

Plants generally respond to insect attacks by altering the composition and physical properties from cell walls to secondary metabolite biosynthesis to defend themselves (Golan et al., 2013). Thus, the African Root and Tuber Scale (ARTS) inserts its long stylet into the cortex, periderm and root storage parenchyma cells to suck plant sap, where small areas of brownish discoloration subsequently are observed on its parts. *Stictococcus vayssierei* feed on underground parts of the stem and mother cuttings, concentrating mainly around nodes (Ambe et al., 1999). By taking the food resource, scale insects have a direct impact on food production in agriculture, causing loss of weight and quality. Thus, the plant, thus subjected to the stress caused by the pest according to the genotype, produces secondary metabolites to defend itself.

The experiment was conducted in a fallow naturally infested by scale insects that were difficult to provide control. The results of varietal screening in the field naturally infested by ARTS show that the stem diameter of cassava varieties did not differ significantly at 3 MAP ($P > 0.05$) underfeeding of scale insects. But at 6, 9 and 12 MAP, there is a significant difference between varieties. The same trend was observed with the fresh shoot weight irrespective of the sampling date. Overall, the local variety Douma has a high production of above-ground biomass and stem diameter compared to the improved varieties. This could be explained by the low susceptibility of the local variety to ARTS attacks. Indeed, Prüter and Zebitz (1991) showed that aphid attacks caused a reduction in root dry weight, shoot dry weight, leaf area and average relative growth rate. The yields obtained in these experiments (14–21 t ha^{-1}) were nevertheless low compared to the expected yield (25–30 t ha^{-1}) from the experimental stations (where fertilization, pest and disease control were respected). According to Udealor and Asiegbu (2006), the best yields are obtained when the improved cassava genotypes respect appropriate cultural practices, freshness, shape, vigor, vitality and potential genetic material of the plantation, absence of weeds, pests, pathogens, etc. Cassava varieties were infested differently by scale insects. The improved variety Excel and the local variety Douma had the highest average number of scale insects than the improved variety TMS 96/0023. This could be explained by the fact that the selected varieties do not have the same genetic heritage and therefore the efficiency of the defense mechanisms used by the plants to defend themselves may influence the attachment behavior of scale insect pests. Identification, biochemical and molecular characterization of tolerance or resistance genes could be used to maintain yield losses caused by scales insects on improved varieties.

The pitting of scale insects on the underground parts of cassava can activate the plant's defense mechanisms (Mai et al., 2013). According to War et al. (2012), plants to counter and or compensate for insect attack

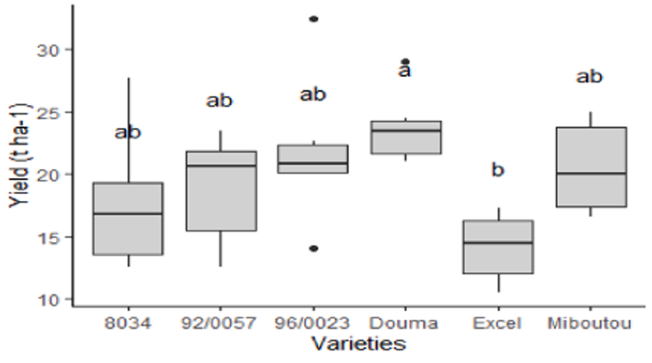

Fig. 1. Yield (t ha^{-1}) at 12 months after planting of cassava varieties planted in a site naturally infested by *Stictococcus vayssierrei* in Akonolinga, center region of Cameroon.

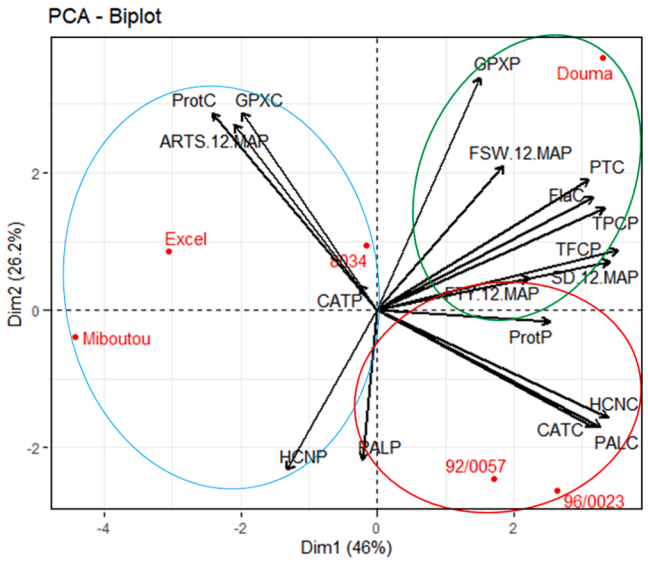

Fig. 2. Principal component analysis (PCA) integrating the agronomic parameters, biochemical parameters content in pith and cortex of six cassava varieties infested by scale insects at 12 months after planting. SD: stem diameter, ARTS: African Root and tuber Scale; FSW: fresh shoot weight, HCNP: total cyanide in pith, HCNC: total cyanide in cortex, CATP: catalase in pith, CATC: catalase in cortex, GPXP: guaiacol peroxidase in pith, GPXC: guaiacol peroxidase in cortex, PALP: phenylalanine ammonia-lyase in pith, PALC: phenylalanine ammonia-lyase in cortex, TPCP; total phenols in pith, PTC: total phenols in cortex, TFLP: total flavonoids in pith, FLC: total flavonoids in cortex, ProtP: proteins in pith; ProtC: proteins in cortex.

effects respond through various morphological, biochemical and molecular mechanisms. The biochemical mechanisms of defense against insects are varied and dynamic. Indeed, defensive compounds are produced either constitutively or in response to plant damage and affect the feeding, growth and survival of insect pests. Several factors such as cassava variety, growing conditions, soil and climate can influence the content of biochemical compounds in tuberous cassava roots. The results show a significant production of biochemical parameters that increase with the size of the scale insect population in the different screened cassava varieties. The content of phenolic compounds (phenols and flavonoids) in cassava tuberous roots is high in the improved cassava variety 8034, TMS 96/0023, TMS 92/0057 and the local variety Douma at the pith level, followed by the improved varieties TMS 92/0057 and TMS 96/0023 at the cortex level. According to Calatayud et al. (1994), phenols can significantly influence the attachment mechanisms of mealybugs on the plant, they are generally extracellular and precursors of many parietal polymers such as lignin, cutin or suberin (Goodmann 1986). Phenols also have an antioxidant role by counteracting prooxidants produced during stress (Dixon et al., 1995). Flavonoids are involved in many interactions between plants during biotic and abiotic stresses (Hutzler et al., 1998). They interact with different enzymes to protect plants against insect pests by influencing insect behavior, growth and development (Simmonds 2003; Treutter 2006). Determination of total cyanide content shows that it is more abundant in the cortex than in the pith. It is also observed that in the pith, the local variety Douma produced high total cyanide content (52.34 mg/kg), whereas, in the cortex, the improved varieties TMS 92/0057 and TMS 96/0023 have higher levels. Cyanogenic glycosides are the most studied group of chemical defenses, and are compounds present in all parts of the plant. They are one of the biochemical characteristics of cassava, and enable the appearance of a toxic molecule in a kind of plant protection system against insect pests (Gleadow and Møller, 2014). Riis et al. (1995) reported negative effects of cyanogenic glycosides against the generalist burrowing bug *Cyrtomenus bergi* (Hemiptera: Cydnidae), which feeds on

tuberous cassava roots. They demonstrate that intracellular penetration of the stylet in the root parenchyma during feeding results in the accumulation of linamarin (1) in the hemolymph, causing greater nymphal mortality, particularly during the early instars. In contrast, Calatayud (2011) reports in a study of the *Phenacoccus manihoti*/manioc interaction that cyanogenesis does not appear to be involved in cassava defense probably because the penetration processes of mealybug stylets induce little tissue damage, making it unlikely to be triggered. Besides, scale insects have an efficient system of excretion and/or detoxification of hydrocyanic acid (HCN) that may be released in their digestive tract (Catalayud et al. 1997; Catalayud et al. 1994). The high content of phenolic compounds (total phenols and flavonoids) and total hydrogen cyanide in the cortex of the improved varieties TMS 96/0023 and TMS 92/0057 could justify the low number of scale insects infesting these varieties. According to Helmi and Mohamed (2016), the elevation of phenols can be explained as a mechanism of defense that acts as a barrier to insect feeding and are directly toxic to insects.

In this study, CAT, GPX and PAL enzyme activities were found to differ significantly in both improved and local varieties and with scale insects density infesting these varieties. Catalase (CAT) and guaiacol peroxidase (GPX) is used to catalyze and reduce toxic intermediates products of oxygen metabolism, which prevents plant cell damage (Gill and Tuteja 2010). Catalase is essential for detoxification under stress. It releases H_2O_2 generated during mitochondrial electron transport and beta-oxidation of fatty acid (Gill and Tuteja 2010; Kot et al., 2015). Results show that catalase activity in cassava roots pith level of improved variety 8034 was increased by scale insects infestation than improved variety Excel which had high scale insects numbers. At the cortex level, catalase activity was high in the improved varieties TMS 96/0023 and TMS 92M0057. This shows that catalase activity probably increases or decreases depending on the variety and density of scale insect populations present in cassava varieties. Ferry et al. (2011) reported an increase catalase activity in wheat following an infestation of *Sitobion avenae*. Increased CAT activity in plants increases cell wall resistance (Chen et al., 1993). Kaur et al. (2014) observed a decrease catalase activity in leaves, seeds and pod wall of *Cajanus cajan* infested by *Helicoverpa armigera*. In the case of guaiacol peroxidase, an inverse situation to that of catalase activity was observed at the cortex level. The higher catalase activity recorded in the improved varieties TMS 96/0023 and TMS 92/0057 was related to the lower guaiacol peroxidase activity. Several studies have demonstrated an increase in GPX activity in cultivars subjected to biotic or abiotic stress (Zhang and Kirkham 1996; Uarrota et al., 2016). GPX may be involved in lowering stress-induced ROS, for example, converting hydrogen peroxide to water. According to Chaman et al. (2003), Golan et al. (2013), the combined effect of guaiacol peroxidase and catalase reduces ROS action in insect-infested plants. Phenylalanine ammonia-lyase from its product which is cinnamic acid plays a role in plant defense mechanisms through the synthesis of salicylic acid and other phenolic compounds such as lignins involved in the reinforcement of pectocellulose walls (Mauch--Mani and Slusarenko 1996; Shirasu et al. 1997; Chaman et al., 2003; Manga et al., 2016). Our results show that PAL activity is high in varieties TMS 96/0023 and TMS 92/0057 at the cortex. This could probably be confirmed by the low number of scale insects observed on these cassava varieties in the field. A high level of PAL can enhance the plant's ability to tolerate scale insects. Indeed, Dogbo et al. (2008) showed that PAL activity in cassava leaves tissues stimulated by salicylic acid was 4 to 14 times higher.

The linear regression model performed from the biochemical data in the cortex, pith and agronomic to predict scale insect density shows that not all variables were used (VIF > 5). The linear regression model performed from the biochemical data variables in the cortex (CAT and HCN) and in the pith (TPC and CAT) have a negative linear relationship with the scale insect density. This could be explained by the fact that cassava genotypes (TMS 96/0023 and TMS 92/0057, 8034) with a high content of CAT and HCN in the cortex and high content of TPC and CAT in the pith will have a low scale insect density. As for the agronomic data, the variables (SD and yield) have a negative linear relationship with scale insect density. It is clear that high scale insect density reduces SD and yield. The response of varieties to the stress caused by *S. vayssierei* is also recorded at the growth level. Indeed, as shown by principal component analysis (PCA), the secondary metabolites produced by the local variety Douma, in addition to being highly infested by scale insects, has a high content of phenols and flavonoids in the pith, in contrast to the improved varieties TMS 96/0023 and TMS 92/0057, which have a high content of HCN, PAL and catalase in the cortex but with a low infestation of scale insects. Hemm et al. (2004), show that PAL is a key enzyme in the synthesis of several defense-related secondary compounds such as phenols and lignin. These results could be explained by the fact that biochemical compounds other than the one studied are involved in the plant's defense against *S. vayssierei* (Helmi and Mohamed 2016). On the other hand, even if plants' secondary metabolites do not affect scale insect numbers, they could act on insect metabolism. This depressive action on scale insect metabolism could explain the tolerance of the local variety Douma and explain the outbreak of scale insects on these varieties as an adaptation and survival mechanism to the metabolic stresses of these insects.

Conclusion

In summary, this study evaluates the response of six cassava varieties (two local and four improved) to natural scale infestation and determines their resistance levels against *S. vayssierei*. The improved varieties TMS 96/0023 and TMS 92/0057 were the least colonized by scale insects and produced more biochemical as well as greater catalase and phenylalanine ammonia-lyase activity than the other varieties in the cortex were ARTS are the most closely attached. The local variety Douma has the highest number of scale insects, high phenolic compounds content (phenolics and flavonoids) and the highest yield. The improved Excel variety records the highest number of scale insects.

CRediT authorship contribution statement

Patrice Zemko Ngatsi: Writing – original draft, Methodology, Writing – review & editing, Formal analysis, Visualization. **Bekolo Ndongo:** Conceptualization, Writing – review & editing, Investigation, Supervision. **Zachée Ambang:** Writing – review & editing, Investigation, Supervision. **Pierre Eke:** Methodology, Writing – review & editing. **William Norbert Tueguem Kuate:** Writing – review & editing. **Sylvere Landry Lontsi Dida:** Writing – review & editing. **Jude Ndjaga Manga:** Methodology, Writing – review & editing. **Champlain Djiéto-Lordon:** Writing – review & editing, Investigation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Supplementary materials like Characteristics of cassava varieties used in this study and Stictococcus vayssierei population photo were provided

Acknowledgments

The authors are sincerely thankful to the Antimicrobial and Biocontrol Agents Unit, Laboratory for Phytobiochemistry and Medicinal Plants Studies, Faculty of Science, University of Yaoundé Ï for biochemical assays. The authors are also sincerely thankful to the

International Institute of Tropical Agriculture (IITA) and the Institute of Agricultural Research for Development (IRAD) for planting material provision. We sincerely thank Dr Godswill NTSOMBOH NTSEFONG for proofreading the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2024.100071.

References

Ali, M.B., Hahn, E.J., Paek, K.Y., 2005. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in Phalaenopsis. Plant Physiol. Biochem. 43, 213–223. https://doi.org/10.1016/j.plaphy.2005.01.007.

Akinwale, M.G., Oguntona, C.R.B., 2012. Effect of age on the nutritional composition of cassava (*Manihot esculenta* Crantz) tuber. Int. J Food Nutr. Sci. 1 (1), 1–6.

Ambe, J.T., Ntonifor, N.N., Awah, E.T., Yanine, J.S., 1999. The effect of planting dates on the incidence and population dynamics of the cassava root scale, *Stictococcus vayssierei*, in Cameroon'. Int. J. Pest Manage 45, 125–130. https://doi.org/10.1080/096708799227914.

Ayanru, D.K.G., Sharma, V.C., 1984. Changes in total cyanide content of tissues from cassava plants infested by mites (*Mononychellus tanajoa*) and mealybugs (*Phenlacoccus manihoti*). Agric. Ecosyst. Environ. 12, 35–46.

Bani, G., Marpangou Divassa, S., Nzemba, M.D., Magema, J.M., 2003. Présence au Congo-Brazzaville de *Stictococcus vayssierei* Richard, ravageur du manioc, (*Manihot esculenta* Crantz) (Hem., Sticococcidae). Bulletin de la Société entomologique de France 108, 529.

Bradbury, M.G., Egan, S.V., Bradbury, J.H., 1999. Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. J. Sci. Food Agric. 79, 593–601. https://doi.org/10.1002/(SICI)1097-0010(19990315)79:4<593::AID-JSFA222>3.0.CO;2-2.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Ann. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cakmak, N., Horst, W.J., 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidases activities in root tips of soybean (*Glycine* max). Plant Physiol. 83, 463–468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.

Calatayud, P.A., 2011. Plant-insect interactions. Qualification to Conduct Research (HDR). University Paris-sud, p. 86.

Calatayud, P.A., Rahbé, Y., Delobel, B., Khuong-Huu, F., Tertuliano, M., Le Ru, B., 1994. Influence of secondary compounds in the phloem sap of cassava on expression of antibiosis towards the mealybug *Phenacoccus manihoti*. Entomol. exp. appl. 72, 47–57. https://doi.org/10.1111/j.1570-7458.1994.tb01801.x.

Calatayud, P.A., Rouland, C., Le Ru, B., 1997. Influence de la linamarine dans la relation manioc cochenille. Acta Bot. Gallica 144, 427–432. https://doi.org/10.1080/12538078.1997.10515782.

Chaman, M.F., Copaja, S.V., Argandona, V.H., 2003. Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) and resistance of barley to aphid infestation. J. Agric. Food Chem. 51, 2227–2231. https://doi.org/10.1021/jf020953b.

Chen, Z., Silva, H., Klessig, D.F., 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262, 1883–1886. https://doi.org/10.1126/science.8266079.

Chikoti, P.C., Tembo, M., Chisola, M., Ntawuruhungu, P., Ndunguru, J., 2015. Status of cassava mosaic disease and whitefly population in Zambia. Afr. J. Biotechnol. 14 (33), 2539–2546. https://doi.org/10.5897/AJB2015.14757.

Dejean, A., Matile-Ferrero, D., 1996. How a ground-dwelling forest ant species favors the proliferation of an endemic scale insect (Hymenoptera: formicidae; Homoptera: stictococcidae). Sociobiology 28, 183–195. Biology 56:481-501.

Dixon, R.A., Harrison, M.J., Palva, N.L., 1995. The isoflavonoid phytoalexines patway from enzymes to genes to transcription factors. Physiol. Plantarum 93, 385–395. https://doi.org/10.1111/j.1399-3054.1995.tb02243.x.

Dogbo, D.O., Bekro, M.J.A., Bekro, Y.A., Sie, R.S., Gogbeu, S.J., Traore, A., 2008. Influence de l'acide salicylique sur la synthèse de la phénylalanine ammonia-lyase, des polyphénoloxydases et l'accumulation des composes phénoliques chez le manioc (*Manihot esculenta* Crantz). Sci. Nat. 5, 1–13. https://doi.org/10.4314/scinat.v5i1.42147.

Doumtsop, A.R.P.F., Hanna, R., Tindo, M., Tata-Hangy, W.K., Fotso, A.K., Fiaboe, K.K.M., Fomena, A., Kemga, A., Normark, B.B., 2020. Geographic distribution and abundance of the Afrotropical subterranean scale insect *Stictococcus vayssierei* (Hemiptera: stictococcidae), a pest of root and tuber crops in the Congo basin. Bull. Entomol. Res. 110 (2), 293–301. https://doi.org/10.1017/S0007485319000658.

Everitt, B., Skrondal, A., 2010. The Cambridge Dictionary of Statistics. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511779633.

FAO, 2013. Grow and Save Cassava: A Guide to Production and Intensification. Rome. Italy.

Ferry, N., Stavroulakis, S., Guan, W., Davison, G.M., Bell, H.A., Weaver, R.J., Down, R.E., Gatehouse, J.A., Gatehouse, A.M.R., 2011. Molecular interactions between wheat and cereal aphid (*Sitobion avenae*): analysis of changes to the wheat proteome. Proteomics. 11, 1985–2002. https://doi.org/10.1002/pmic.200900801.

Gatehouse, J.A., 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist 156, 145–169. https://doi.org/10.1046/j.1469-8137.2002.00519.x.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016.

Gleadow, R.M., Møller, B.L., 2014. Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. Annu Rev. Plant Biol. 65, 155e185.

Golan, K., Rubinowska, K., Górska-Drabik, E., 2013. Physiological and biochemical responses on fern *Nephrolepis biserrata* (Sw.) Schott. to *Coccus hesperidum* L. infestation. Acta Biologica Cracoviensia Series Botanica 55, 93–98. https://doi.org/10.2478/ABCSB-2013-0007.

Goodman, R.N., 1986. Cell-wall composition and metabolism. Columbia. 500 p. University of Missouri press.

Gulsen, O., Eickhoff, T., Heng-Moss, T., Shearman, R., Baxendale, F., Sarath, G., Lee, D., 2010. Characterization of peroxidase changes in resistant and susceptible *Warmseason turfgrasses* challenged by *Blissus occiduus*. Arthropod. Plant Interact. 4, 45–55. https://doi.org/10.1007/s11829-010-9086-3.

Hanna, R., Tindo, M., Wijnans, L., Goergen, G., Tata-Hangy, K., Lema, K., Toko, M., Ngeve, J.M., Dixon, A., Gockowski, J., 2004. The African root and tuber scale problem in Central Africa: the nature of the problem and the search for control options. In: Book of Abstracts of the 9th Triennial Symposium of the International Society for Tropical Root Crops-Africa Branch, 31 October–5 November 2004, Mombasa, Kenya, p. 57.

Hasanuzzaman, M., Bhuyan, M., Anee, T.I., Parvin, K., Nahar, K., Mahmud, J.A., Fujita, M., 2019. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 8, 384. https://doi.org/10.3390/antiox8090384.

Helmi, A., Mohamed, H.I., 2016. Biochemical and ultrastructural changes of some tomato cultivars after infestation with *Aphis gossypii* glover (Hemiptera: aphididae) at Qalyubiyah, Egypt. Gesunde Pflanzen 68, 41–50. https://doi.org/10.1007/s10343-016-0361-9.

Hemm, M.R., Rider, S.D., Ogas, J., Murry, D.J., Chapple, C., 2004. Light induces phenylpropanoid metabolism in Arabidopsis roots. Plant J. 38, 765–778. https://doi.org/10.1111/j.1365-313X.2004.02089.x.

Hiner, A., Ruiz, J., Lopez, J.N., Arnao, M.B., Raven, E.I., Canovas, F.G., Acosta, M.M., 2002. Kinetic study of the inactivation of Ascorbate-peroxidase by hydrogen peroxide. Biochem. J. 348, 321–328.

Hutzler, P., Fischbach, R., Heller, W., Jungblut, T.P., Reuber, S., Schmitz, R., Veit, M., Weissenbock, G., Schnitzler, J.P., 1998. Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. J. Exp. Bot. 4, 953–965. https://doi.org/10.1093/jxb/49.323.953.

IITA, 2000. How to Start a Cassava field, a Guide to IPM Practice for Extension Workers. IITA, Ibadan, Nigeria, p. 24.

Jose, A., Makeshkumar, T., Edison, S., 2008. Host range of Sri Lankan cassava Mosaic Virus. J Root Crops 34, 21–25.

Kamau, J., Melis, R., Laing, M., Derera, J., Shanahan, P., Ngugi, C.K.E., 2011. Farmers 'participatory selection for early Bulking Cassava genotypes in semi-arid Eastern Kenya. J. Plant Breed Crop. Sci. 3, 44–52.

Kaur, R., Gupta, A.K., Taggar, G.K., 2014. Rôle of catalase, H_2O_2 and phenolics in resistance of Pigeon pea towards *Helicoverpa armigera* (Hubner). Acta Physiol. Plant 36, 1513–1527. https://doi.org/10.1007/s11738-014-1528-6.

Kmieć, K., Kot, I., Rubinowska, K., Górska-Drabik, E., Golan, K., Sytykiewicz, H., 2022. The variation of selected physiological parameters in elm leaves (*Ulmus glabra* Huds.) infested by gall inducing aphids. Plants 11, 244. https://doi.org/10.3390/plants11030244.

Kot, I., Kmieć, K., Górska-Drabik, E., Golan, K., Rubinowska, Łagowska B, 2015. The effect of mealybug *Pseudococcus longispinus* (Targioni: tozzetti) infestation of different density on physiological responses of *Phalaenopsis* × hybridum 'Innocence. Bull. Entomol. Res. 105, 373–380. https://doi.org/10.1017/S000748531500022X.

Kramling, T., Singleton, V., 1969. An estimate of the non-flavonoid phenols in wines. Am. J. Enol. Vitic. 20, 86–92.

Lema, K.M., Tata-Hangy, K., Bidiaka, M., 2004. Management of African root and tuber scale using improved cassava genotypes and mineral fertilizers. Afr. Crop. Sci. J. 12, 217–221. https://doi.org/10.4314/acsj.v12i3.27881.

Liu, J., Zheng, Q., Ma, Q., Gadidasu, K.K., Zhang, P., 2011. Cassava genetic transformation and its application in breeding. J. Integr. Plant Biol. 53, 552–569. https://doi.org/10.1111/j.1744-7909.2011.01048.x.

Luthria, D., Pastor-Corrales, M., 2006. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. J. Food Compos. Anal. 19, 205–211. https://doi.org/10.1016/j.jfca.2005.09.003.

Mai, V.C., Bednarski, W., Borowiak-Sobkowiak, B., Wilkaniec, B., Samardakiewicz, S., Morkunas, I., 2013. Oxidative stress in pea seedling leaves in response to *Acyrthosiphon pisum* infestation. Phytochemistry 93, 49–62. https://doi.org/10.1016/j.phytochem.2013.02.011.

Manga, N.J., Effa, O.P., Ondobo, M.L., Djoko, K.J.C., Djocgoue, P.F., 2016. Heritability of the tolerance to *Phytophthora megakarya* Bras.and Grif.of *Theobroma cacao* L. in terms of their necrosis length, phenolic contents and activity of enzymes. Int. J. Biosci. 8, 249–261.

Mauch-Mani, B., Slsarenko, A., 1996. Production of salicylic acid precursors is a major function of phenylalanine ammonia-Lyase in the resistance of Arabidopsis to Peronospora parasitica. Plant Cell 8, 203–212. https://doi.org/10.1105/tpc.8.2.203.

Montagnac, J.A., Davis, C.R., Tanumihardjo, S.A., 2009. Nutritional value of cassava for use as a staple food and recent advances for improvement. Compr. Rev. Food Sci. Food Saf. 8 (3), 181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x.

Moudingo, E.J., 2007. Situation of the Forests in Cameroon. Cameroon Wild life Society Conservation, p. 24.
Mujica, M.V., Granito, M., Soto, N., 2009. Importance of the extraction method in the quantification of total phenolic compounds in *Phaseolus vulgaris* L. Interciencia 34, 650–654.
Nassar, N.M.A., Ortiz, R., 2007. Cassava improvement: challenges and impacts. J. Agric. Sci. 145, 163–171. https://doi.org/10.1017/S0021859606006575.
Ndengo, N.E., Ki-Munseki, A.L., Hanna, R., Ngbolua, K., 2016. Screening cassava (*Manihot esculenta* CRANTZ) genotypes for resistance to African root and tuber scale (*Stictococcus vayssierei* Richard) in different Agroecological zones of Beni (North Kivu, DR Congo). Int. J. Innov. Appl. Stud. 16, 247–259.
Ngatsi, Z.P., Ndongo, B., Kutnjem, D., Djiéto-Lordon, C., 2020. Crop genotype and stem cutting portion affect infestation pressure of the cassava root scale *Stictococcus vayssierei* Richard (Hemiptera: stictococcidea) in a rainforest in Cameroon. Phytoparasitica 48, 629–641. https://doi.org/10.1007/s12600-020-00823-6.
Ngeve, J.M., 2003. The cassava root mealybug (*Stictococcus vayssierei* Richard) (Homoptera: stictococcidae): a threat to cassava production and utilization in Cameroon. Int. J. Pest. Manag. 49, 327–333. https://doi.org/10.1080/09670870310001603900.
Nweke, F.I., Spencer, D.S., Lynam, J.K., 2002. The Cassava Transformation: Africa's Best-Kept Secret. Michigan State University Press, East Lansing, Michigan USA.
Prüter, C., Zebitz, C.P.W., 1991. Effects of *Aphis fabae* and Urumyces viciae-fabaeon the growth of a susceptible and an aphid resistant cultivar of *Vicia faba*. Ann. Appl. Biol. 119, 215–226. https://doi.org/10.1111/j.1744-7348.1991.tb04860.x.
R Development Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.
Riis, L., Bellotti, A.C., Vargas, H.O., 1995. The response of polyphagous pest (Cyrtomenus bergi Froeschner) to cassava cultivars with variable HCN content in root parenchyma and peel. In: International Scientific Meeting (2, 1984, Bogor, Indonesia). The Cassava Biotechnology Network: Proceedings, 2. Centro Internacional de Agricultura Tropical (CIAT), Cali, CO, pp. 501–509 vWorking document no. 150.
Sartiami, D., Watson, G.W., Roff, M.M.N., Hanifah, M.Y., Idris, A.B., 2015. First record of cassava mealybug, *Phenacoccus manihoti* (Hemiptera: pseudococcidae), in Malaysia. Zootaxa 3957 (2), 235–238. https://doi.org/10.11646/zootaxa.3957.2.8.
Sharma, H.C., Sujana, G., Rao, D.M., 2009. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. Arthropod-Plant Interact 3, 151–161. https://doi.org/10.1007/s11829-009-9068-5.
Shirasu, K., Nakajima, V.K., Dixon, R.A., Lamb, C., 1997. Salicylic acid potentiates an agonist dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell 9, 261–270. https://doi.org/10.1105/tpc.9.2.261.
Simmonds, M.S.J., 2003. Flavonoid-insect interactions: recent advances in our knowledge. Phytochemistry 64, 21–30. https://doi.org/10.1016/S0031-9422(03)00293-0. PMID: 12946403.
Singleton, V., Rossi, J., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Tarafdar, J., Marschner, H., 1994. Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil. Biol. Biochem. 26, 387–395. https://doi.org/10.1016/0038-0717(94)90288-7.
Tata-Hangy, K., Hanna, R., Toko, M., Lema, K.M., Solo, M., 2006. Changes in population abundance of the African root and tuber scale *Stictococcus vayssierei* Richard (Homoptera; Stictococcidae) on cassava in the bas-fleuve district in the Democratic Republic of Congo. In: Mahungu, N.M., Manyong, V.M. (Eds.), Advances in Root and Tuber Crops Technologies for Sustainable Food Security, Improved Nutrition, Wealth and Environmental Conservation in Africa. Proceedings of 9th ISTRC-AB Symposium Mombassa, Kenya, pp. 574–582.
Tindo, M., Hanna, R., Wijnans, L., Nolte, C., Goergen, G., Nguekam, A., Ngeve, J.M., 2007. Pre-planting removal of host-plant residues to reduce African root and tuber scale infestations in cassava fields in Cameroon. In: Proceedings of the 9th ISTRC-AB Symposium (pp. 633-639), 1-5 November, Mombasa, Kenya.
Treutter, D., 2006. Significance of flavonoids in plant resistance: a review. Environ. Chem. Lett. 4, 147–157. https://doi.org/10.1007/s10311-006-0068-8.
Uarrota, V.G., Moresco, R., Schmidt, E.C., Bouzon, Z.L., Nunes da Costa, E., Neubert de Oliveira, E., Peruch, L.A.M., Rocha, M., Maraschin, M., 2016. The role of ascorbate peroxidase, guaiacol peroxidase, and polysaccharides in cassava *(Manihot esculenta Crantz)* roots under postharvest physiological deterioration. Food Chem. 197, 737–746. https://doi.org/10.1016/j.foodchem.2015.11.025.
Udealor, A., Asiegbu, J.E., 2006. Effects of cassava genotype and vegetable cowpea populations on the component crop yield and system productivity on cassava/vegetable cowpea intercropping systems. Niger Agric. J. 37, 74–80. https://doi.org/10.4314/NAJ.V37I1.3232.
Usha Rani, P., Jyothsna, Y., 2010. Biochemical and enzymatic changes in rice as a mechanism of defense. Acta Physiol. Plant 32, 695–701. https://doi.org/10.1007/s11738-009-0449-2.
Verma, V., Ravindran, P., Kumar, P.P., 2016. Plant hormone-mediated regulation of stress responses. BMC. Plant Biol. 16, 86. https://doi.org/10.1186/s12870-016-0771-y.
War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C., 2012. Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. 7, 1306–1320. https://doi.org/10.4161/psb.21663.
Wen, P.F., Chen, J.Y., Kong, W.F., Pan, Q.H., Wan, S.B., Huang, W.D., 2005. Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. Plant Sci. 169, 928–934. https://doi.org/10.1016/j.plantsci.2005.06.011.
Whetten, R.W., Sederoff, R.R., 1992. Phenylalanine ammonia lyase from loblolly pine; Purification of the enzyme and isolation of complementary DNA clones. Plant Physiol. 98, 380–386. https://doi.org/10.1104/pp.98.1.380.
Williams, D.J., Matile-Ferrero, D., Miller, D.R., 2010. A study of some species of the genus *Stictococcus Cockerell* (Hemiptera: sternorryncha: coccoidea: stictococcidae), and a discussion on *Stictococcus vayssierei* Richard a species injurious cassava in Equatorial Africa with a description of a new species from Nigeria. ZootaxaZootaxa 2527, 1–27. https://doi.org/10.5281/zenodo.196462.
Zagrobelny, M., Bak, S., Rasmussen, A.V., Jorgensen, B., Naumann, C.M., Moller, B.L., 2004. Cyanogenic glycosides and plant-insect interactions. Phytochemistry 65, 293–306. https://doi.org/10.1016/j.phytochem.2003.10.016.
Zhang, J., Kirkham, M.B., 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. New. Phytol. 132, 361–373. https://doi.org/10.1111/j.1469-8137.1996.tb01856.x.