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BIOCHEMICAL FACTORS ASSOCIATED WITH CASSAVA RESISTANCE TO WHITEFLY INFESTATION

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) an important food security crop, is inflicted by whitefly (*Bemisia tabaci*) worldwide, causing direct damage of up to 80% of yield loss. Although resistance to the pest has been associated with antibiosis, changes that occur in metabolite activity, and their effect on the pest have not been fully elucidated. A study was carried out to evaluate changes in peroxidase, tannin and flavonoid activity in cassava genotypes attacked by *B. tabaci* in order to contribute to knowledge on whitefly resistance in cassava. Five genotypes showing resistance, and three susceptible, were selected based on whitefly count and leaf damage scores, and assayed for peroxidase, tannin and flavonoid activity. There were significant differences among genotypes for leaf damage ($P < 0.01$) of three to six months plants. Genotypes CS1-144, UG 120133 and NAM 130 showed low damage scores (< 2.00); but a high damage score (> 2.5) was incurred by UG 130068. All genotypes showed significant ($P < 0.05$) differences for peroxidase activity, with CS1-144 having a high activity rate three months after planting. The lowest activity was observed in UG 120170, a susceptible genotype. A significant ($P < 0.01$) negative correlation ($r = -0.84$) was observed between peroxidase activity and cassava leaf damage scores, as well as between tannin and damage ($r = -0.57$), indicating that peroxidase and tannin play a part in cassava resistance to *B. tabaci*.

Key Words: Antibiosis, *Bemisia tabaci,* flavonoids, peroxidase, tannins

RÉSUMÉ

Le manioc (*Manihot esculenta* Crantz) qui est une importante culture de sécurité alimentaire, est influencée par la mouche blanche (*Bemisia tabaci*) sur le plan mondial, à travers des dommages directs allant jusqu'à 80% de perte du rendement. Par ailleurs, la résistance à la peste a été associée à l'antibiose, des changements qui apparaissent dans l'activité métabolique, et leur effet sur la peste n'ont pas encore été complètement élucidés. Une étude a été entreprise pour évaluer les changements d'activité en peroxydase, tannin et flavonoïde dans les génotypes du manioc attaqués par *B. tabaci* dans le but de contribuer au savoir sur la résistance du manioc. Cinq génotypes, montrant résistance, et trois susceptibles, étaient sélectionnés sur la base du nombre de la mouche blanche et les sores de dommages et analysés pour l'activité en peroxydase, tannin and flavonoïdes. Il y avait de différences significatives entre les génotypes pour les dommages causés sur les feuilles ($P< 0,01$) des plantes de trois à six mois. Les génotypes CS1-144, UG 120133 et NAM 130 ont montré de faibles scores de dommage (< 2,00); mais

un score élevé de dommage (> 2,5) était reçu par UG 130068. Tous les génotypes ont montré de différences significatives (P < 0,05) pour l'activité de peroxydase, avec CS1-144 ayant un taux d'activité élevé trois mois après la plantation. La faible activité était observée sur UG 120170, un génotype susceptible. Une corrélation significative (P < 0,01) et négative (r = -0,84) était observée entre l'activité de peroxydase et les scores de dommages sur les feuilles de manioc, ainsi que entre tannin et dommage (r = -0,57), indiquant que peroxydase et tannin jouent une part de rôle dans la résistance du manioc au *B. tabaci*.

Mots Clés: Antibiose, *Bemisia tabaci,* flavonoïdes, peroxydase, tannins

INTRODUCTION

The whitefly (*Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is an economically important pest of cassava in Africa (Shu-sheng *et al*., 2012) and worldwide (Cuthbertson and Vänninen, 2015). In Uganda, cassava has been documented as a main host plant of *B. tabaci,* with high prevalence rates on the crop (CABI, 2017). The pest causes significant damage on the plant and yield losses estimated at 79% (Howeler, 2012). *Bemisia tabaci* feeding leads to reduced photosynthetic potential and increased leaf shedding in cassava (CABI, 2017) and is listed in the European Union Plant Health Directive 2000/29/EC as a harmful organism (EPPO, 2014).

Minimising the pest effects remains critical for cassava breeding and improvement programmes (Legg *et al*., 2014). Recent studies have identified some genotypes as resistant to this pest, based on genotypes supporting low numbers of the pest (Seruwagi *et al*., 2005). However, there is limited information on the interactions between the resistant plant and *B. tabaci,* and as such mechanism of resistance in cassava is not well understood (Bohorquez *et al*., 2016). Furthermore, most resistance identification has been based on the number of *B. tabaci* per plant (Ariyo, 2005), which may affect selection, since a high number of whiteflies has been reported on resistant varieties as well (Asare *et al*., 2014).

The ecological relationship between insects and plant tissues is complex, with chemical interactions having been suggested to be involved (Singh, 2009). In this regard, induced plant responses may potentially operate in several capacities, namely accumulation of defensive compounds which influence feeding efficiency, reproductive success, and host plant selection by the insects (Inbar and Gerling, 2008). Bellotti (2003) indicated that cassava resistance to the whitefly was based on antibiosis due to leaf content of a lot of biochemical and anti-nutrient compounds (Achidi *et al*., 2008); free sugars, protein and phenolic compounds (Koubala *et al*., 2015); alkaloids, flavonoids and tannins (Anbuselvi and Balamurugan, 2014). However, the relation of these substances with *B. tabaci* resistance has not yet been studied. There is need to study the different metabolite activity levels during the growth period, especially at peak *B. tabaci* infestation of the plant, which has been reported to be significant from three to six months after planting (Otim *et al*., 2006).

The expression of several defense metabolites is induced within minutes after damage and hours in undamaged leaves, as a systemic response (Hagg *et al*., 2013). In particular, peroxidase has been reported as a defense enzyme, which increases in various plants due to *B. tabaci* attack (Binu and Paliniswami, 2006) and specific accumulation at the site of the attack (Hagg *et al*., 2013; Kerchev *et al*., 2012). The peroxidases (PODs) are expressed to limit cellular spreading of the infestation damage through the establishment of structural barriers (Kerchev *et al*., 2012), or the generation of highly toxic environments by massively producing reactive oxygen species (ROS), which include H_2O_2, O_2, OH - (Hagg *et al*., 2013). Over-expression of class III (EC 1.11.1.7) peroxidases are reported to be key as antioxidants and as stress

response proteins in several metabolic responses in the plant defense system (Almagro *et al*., 2009).

Phenolics have also been documented to serve as defense compounds, through various means such as repelling feeding by *B. tabaci* (Hagg *et al*., 2013). Among the specific phenolics reported are the tannins and flavonoids (Engelberth *et al*., 2007), both of which are the result of condensation of phenyl alanine derived compounds, with malonyl CoA (Hagg *et al*., 2013). Tannins and flavonoids protect the plant against insect pests by influencing the behaviour, growth and development of the insects (Sulistyo and Inyati, 2016), by reducing the nutritive value of plant parts to the insect (War *et al*., 2012; Hagg *et al*., 2013). According to Taggar *et al.* (2012), a negative correlation exists between tannins and flavonols with *B. tabaci* plant population, indicating that increase in the activity of these biochemicals could contribute to the bioprotection of host plants against whiteflies. Identifying the changes in peroxidase, tannin and flavonoid activity in the attacked plant, would elucidate the contribution of these compounds in cassava resistance to the whitefly. The objective of this study was to investigate the role of these metabolites in cassava resistance to *B. tabaci.*

MATERIALS AND METHODS

Study site and plant materials. The study was conducted in four different locations of Uganda, for which information on coordinates, climatic and soil characteristics of the experimental sites is provided in Table 1. A total of fifty cassava genotypes were used in this study.

Screening of cassava genotypes. The 50 cassava genotypes were planted in an augmented design, due to the high number of clones (Federer, 2012). The genotypes were evaluated for whitefly infestation and damage, using natural *B. tabaci* infestation, since the areas chosen were hot spots for whitefly populations, and permit sufficient selection pressure with high damage levels (Legg, 1994).

Population scoring was based on a scale of 1- 6; with 1 = no whitefly stages, 2 = 1 - 200 insects per leaf, 3= 201 - 500 insects per leaf, 4= 501 - 2000 insects per leaf, 5= 2001 - 4000 insects per leaf and 6 = > 4000 insects per leaf (Bellotti, 2002). This was combined with a leaf-damage scale of 1-5; where 1= no apparent symptoms; 2 = mild chlorotic blotches on < 10% of leaves; 3 = moderate chlorotic blotches on 11-30% of leaves; 4 = yellowing or chlorosis on 31-50% of leaves; and 5 = yellowing and deformation of upper leaves, chlorosis on > 50% of leaves and plant stunting (Bellotti, 2002).

Whitefly population counts were recorded monthly on each of three middle row-tagged plants, on the top five fully-expanded apical leaves, from 30 up to 180 days after planting (DAP) (Bellotti, 2002). Adult numbers were counted on three randomly selected sub-set of 10 plants per genotype, starting at 82 DAP and, thereafter, at 30 days for four consecutive periods; damage scores were recorded concurrently (Kawuki *et al*., 2013). Each leaf was held gently and turned upside down to facilitate counting of adult insects during the relatively cooler periods of the day (7.00 am to 10.00 am.) when the pest was less active (Fishpool *et al*., 1995). Nymph counts were taken on the lowest leaf of each of the tagged plants of each genotype; and sooty mold was evaluated on the lateral leaves (Bellotti and Arias, 2001).

Sample collection and preparation. After the plant insect screening, eight cassava genotypes, four resistant (CS1-144, UG 120133, UG 120191 and UG 120257) with NAM 130 as the resistant control and two susceptible (UG 130068 and UG 120170) with NASE 13 as a susceptible control, were planted in April, 2016 in a randomised complete block design (RCBD), with three replications, in Kasese, Kamuli and Namulonge.

The plants were evaluated for whitefly populations, nymph count and damage monthly

TABLE 1. A description of four study sites used for the evaluation of cassava attack by *Bemisia tabaci* in Uganda

Locations	Geographical coordinates —— Longitude (E) ——		Altitude (N) Latitude (masl)	Average annual rainfall (mm)	Average annual temperature (°C)	Soil characteristic	General description
Namulonge (NaCRRI), Central Uganda	32° 37'	0° 32'	Mid-altitude, 1150-1155	Bimodal rainfall, 1000-1450 annually	24 - 30	Acidic Ferralsols, pH below 6.5-to 7.0	Transition forest vegetation' tropical wet and mild dry climate with slight humid conditions (65%)
Kamuli, South Eastern Uganda (Kihige)	33°06'	0°55'	Mid-altitude 1122-1140	Bimodal rainfall 900-135 0 annually	19 - 28	Clay, Ferrisols	Tropical, wet savannah, 53% humidity
Kasese, Southwestern Uganda (Ruimu)	30°05'	0°11'	920-1000	Bimodal rainfall, 800-1600 annually	15 - 32	Clay loamy	Relative productive soil fertility, tropical wet and dry climate
Arua, Northern Uganda (Abi)	30°54'	3°1'	1215	Bimodal rainfall, 1000-1400 annually	21 - 29	Fine, loamy, well drained	Tropical wet and dry climate

Source: Sebuwufu *et al*., 2015

for six months. Samples of cassava leaves and sap were collected biweekly and placed in ice boxes at 4ºC. Extraction of metabolites in both leaf and sap was done using a phosphate buffer (Binu and Paliniswami, 2006); and stored at -20ºC (HORTUS technical services, 2016). The biochemicals were analysed on two sampling times (at 60 and 90 DAP), June 15, and July 23, 2016.

Plant metabolite determination. The top most expanded leaves (4-7) were collected and weighed in the field, according to Binu and Paliniswami (2006); after which the leaves were immediately placed in an ice box. Tapping of sap directly from the leaf was achieved according to Munns *et al*. (2016). The sap from the petiole was collected directly into a 1.5 ml vial, which had 0.5 ml of 0.1 M phosphate buffer to prevent the coagulation of the sap due to the development of P-protein which enhances callose development (Beck, 2002). Collection of sap and leaves was done from 07:30 am-12:00 pm as reported in the sap testing and sampling guide (HORTUS technical services, 2016). Thereafter, the leaves and sap samples were placed in a refridgerator at -20 ºC (HORTUS technical services, 2016).

Peroxidase activity. A 100 mg leaf sample was homogenised with 2 ml of 0.1 M ice cold phosphate buffer at a pH=7.5, with a prechilled mortar and pestle. The contents were then transferred into eppendorf tubes and centrifuged (PrismaR, Edison, New Jersey, USA) at 1,000 rpm for 20 minutes. Thereafter, 0.02 ml of the supernatant were placed into a test tube containing 3 ml of 0.1M potassium phosphate buffer (pH=6.5). The reaction mixture, without the H_2O_2, was measured as a blank and recorded as initial reading. The reaction was initiated by adding 0.8M H_20_2 and the breakdown of the H_2O_2 was monitored for 3 minutes at a 30 second interval, at 24 ºC, by recording absorbances at 470 nm in a spectrophotometer (Biowaveii+, Cambridge, England). The activity of the enzyme was calculated and expressed as a change in absorbance min^{-1} mg^{-1} (Mizobutsi *et al*., 2010).

Flavonoid absorbance. A leaf sample (50 mg) was homogenised with 2 ml of 0.1 M ice cold phosphate buffer at a pH =7.5. Fifty microlitre of the leaf extract was made up to 1 ml with methanol and 4 ml of distilled H_20; followed by 0.3 ml of 10% (w/v) aluminium chloride (AlCl3) solution after 5 minutes of incubation at 40 ºC, and then the mixture allowed to stand for 6 minutes (Li *et al*., 2013). Thereafter, 2 ml of 1 M NaOH solution was added and brought to a final volume of mixture of 10 ml with double distilled water. Then, the mixture was allowed to stand for 15 minutes at 24 ºC and absorbance measured at 510 nm, using UV-visible spectrophotometer (Biowaveii+, Cambridge, England) (Shatishkumar and Basker, 2014).

Tannin absorbance. A 100 mg leaf sample was placed into a 2 ml eppendorf tube, where 0.5 ml of 5% ascorbic acetone solution was added to dissolve leaf precipitate, and the set-up placed on an orbital shaker for 20 minutes. After which, 0.5 ml of petroleum ether containing 1% acetic acid, was added to remove pigments. The set-up was then left on the bench until it all evaporated, before 0.3 ml of distilled water was added and centrifuged for 10 minutes at 1,000 rpm. This was followed by placing the solution in a 50 ml conical flask and added 2.4 ml of 5% hydrochloric acid (HCL)-butanol solution.

The solution in the flask was run through a 240 mm filter paper (WHA1001240-ALDRICH, Missouri, USA). Then 0.5 ml of the filtrate was made up to 1 ml with distilled water and 0.5 ml of folin ciocalteau reagent was added; followed with 2.5 ml of 20% sodium carbonate solution and mixed. A total of 0.1 ml of the mixture was then incubated at 80 °C for 1 hour and 20 minutes and the samples were cooled to 24 °C and spectrophotometric readings were taken at 550

nm (Harborne, 1998). A similar procedure was followed for the sap samples.

Data analyses. The data collected were analysed using analysis of variance (ANOVA) in Breeding View software. The linear Equation used was:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + E_{ijk}$$

Where:

Y = response in terms of whitefly count, nymph count, damage, sooty mold, peroxidase, tannin and flavonoid of the jth replication of genotype "i" in environment "k"; m = overall mean of the responses; G_i = genotype effect (μ_i-μ): the mean response of genotype i minus the overall mean; E_j = environment effect (μ_j- μ): the mean response of environment j minus the overall mean; GEij = interaction effect (μ_{ij}- μ_i - μ_j + μ): the mean response for genotype i in environment j minus the mean response for genotype i, minus the mean response for environment j, plus the overall mean; and Eijk = difference of the response in the jth replication (often the jth plot) from the mean response for genotype i in environment k.

This was based on the model:

Response = M + G + E + GE + e :

Where:

M = overall mean response for all genotypes in all environments, G = genotype main effect, E = environment main effect, GE = Genotype by environment interactions, and e = unexplained effects (Mcdermott and Coe, 2012).

To identify the genotype performance in the locations, the genotype by genotype by environment (GGE) model was used:

Average response – M – E = G +GE

This was based on descriptions according to Mcdermott and Coe (2012) and used the Equation:

$$Yij = \mu + Ej + \Sigma^{K}_{k} = 1\lambda k\gamma ik\delta jk + Eij$$

Where:

Y = response variable of genotype (i) in environment (j), μ the overall mean of the responses, k = the environmental effects, i = genotype effect and j the replications.

The genotypes were considered as fixed; while the environments were random variables in the GGE analysis (da Silva *et al*., 2015). The peroxidase, tannin and flavonoid data were analysed using GenStat software (12th Edition).

RESULTS

Cassava genotypes attacked by *Bemisia tabaci*. The results for whitefly count, nymph count and cassava leaf damage are presented in Table 2. The genotypes showed significant (P<0.05) differences for counts in the different locations, at 30 to 90 DAP, (P<0.01) at 120 and 150 DAP, and (P<0.001) at 180 DAP. Significant (P<0.01) differences in genotype by location interactions were observed for whitefly count at 30 DAP, (P<0.05) at 60 DAP and (P<0.01) at 90 to 180 DAP. There were significant (P<0.05) differences among genotypes for nymph count at 60 DAP, (P<0.01) at 90 to 150 DAP and (P<0.001) at 180 DAP. The genotype by location interaction significantly (P<0.01) influenced the nymph count from 60 to 120 DAP and (P<0.05) at 150 and 180 DAP. There were significant (P<0.01) differences among genotypes for leaf damage at 90 to 180 DAP, and (P<0.01) genotype by location interactions at 90 to 180 DAP.

Genotype by environment interactions. There was varying performances of genotypes across locations and the 50 genotypes were

TABLE 2. Mean squares for whitefly count, nymph count and cassava leaf damage on fifty cassava genotypes across four locations (Arua, Kamuli, Kasese and Namulonge) in Uganda

Sources of variation	d.f.	WC						NC						LD					
DAP		30	60	90	120	150	180	30	60	90	120	150	180	30	60	90	120	150	180
Genotype (G)	49	2.0*	2.0*	1.8*	4.5**	5.4**	7.5***	6.3^{ns}	3.0*	4.9**	6.6**	7.7**	8.2***	0.1^{ns}	0.1^{ns}	0.3**	0.2**	0.3**	0.3**
Locations (L)	3	105.2***	165.5***	9.5**	48.7**	49.7***	31.7***	59.7**	75.6***	24.8***	51.5***	8.0**	7.1**	1.0**	1.0**	0.6**	0.8**	0.9**	1.4**
G x L	143	2.1**	1.0*	1.6**	1.7**	1.2**	0.4**	6.1^{ns}	3.2**	2.8**	2.7**	2.3*	1.4*	0.1^{ns}	0.1^{ns}	0.1**	0.6**	0.4**	0.5**
Residual	4	0.3	0.8	0.3	4.7	0.2	0.1	3.3	0.1	0.1	3.9	0.3	0.3	0.1	0.1	0.01	0.2**	0.1	0.1
cv (%)		29.4	39.9	24.8	27.1	15.9	7.7	17.8	7.3	9.3	21.2	14.3	17.4	13.9	17.8	7.9	28.8	20.2	24.1

***, **, * = significant at P<0.001; 0.01 and 0.05, respectively. ns = non-significant at P> 0.05; DAP = days after planting; WC = whitefly count, NC = Nymph count, LD = Leaf damage

ranked based on cumulative leaf damage scores across the four environments (Table 3). The resistant genotypes, UG 120257, UG 120133, CS 1-144, UG 120191 and NAM 130, showed lower damage scores (<2) to whitefly damage; while the susceptible, NASE 13, UG 130068 and UG 120170 showed high levels of leaf damage across the environments.

Biochemical compounds. The results for peroxidase, tannin and flavonoid absorbances among the genotypes are shown in Table 4. The mean squares for peroxidase showed that in the first minute of running the spectrophotometer, there were significant (P<0.05) differences among genotypes; after which the genotypes responded similarly for the next two minutes. Also, there were significant (P<0.001) differences at different sampling times (60 and 90 DAP), among genotypes across three minutes for both tannin and flavonoid. There were significant (P<0.05) GXE interactions affecting peroxidase activity at 60 seconds as well as at 150 seconds and (P<0.01) at 90, 120 and 180 seconds. The genotypes did not show any significant differences for tannins and flavonoids.

The results of mean trend of changes among the genotypes in peroxidase absorbances over a three minute period at 60 and 90 DAP are presented in Figure 1. There was lower peroxidase absorbance exhibited among the genotypes at 60 DAP (Fig. 1A) compared to 90 DAP (Fig. 1B). The resistant genotypes, CS 1-144 and NAM 130, showed the highest peroxidase absorbance at 90 DAP (2.17 and 1.98) at 180 seconds (Fig. 1B), compared to all the genotypes evaluated at both sampling times. The susceptible genotypes, UG 130068 and UG 120170, at 90 DAP showed the least peroxidase absorbance values of 1.02 and 0.8, respectively, at 180 seconds compared to the rest of the genotypes (Fig. 1B). Further, at 60 DAP, UG 120170 was among genotypes that showed lower absorbance value of 0.7 compared to resistant CS 1-144 and NAM 130 which had a value of 1.31 at 180 seconds.

TABLE 3. Ranking of fifty cassava cultivar superiority based on cumulative leaf damage across four locations (Arua, Kamuli, Kasese and Namulonge) in Uganda

Genotype	Rank	Mean	Genotype	Rank	Mean
NAM 130	1	0.62	UG120135	12	2.37
UG120133	2	1.00	UG120227	12	2.37
UG120257	2	1.00	UG120267	12	2.37
CS1-144	3	1.25	UG130089	12	2.37
UG120174	4	1.37	UG130004	13	2.39
UG120293	5	1.56	UG130083	14	2.41
UG120191	6	1.66	UG120183	15	2.43
UG120124	7	1.68	UG120295	15	2.43
CS1-75	8	1.75	UG120202	16	2.47
TP 294	8	1.75	TME 204	17	2.50
UG120161	8	1.75	UG120001	17	2.50
UG120286	8	1.75	UG120071	17	2.50
UG120291	8	1.75	UG120190	17	2.50
UG130085	8	1.75	UG120198	17	2.50
UG120160	9	2.00	UG130018	17	2.50
UG120024	10	2.25	UG130078	17	2.50
UG120063	10	2.25	UG120072	18	2.56
UG120220	10	2.25	UG120170	18	2.56
UG120193	11	2.31	UG120189	18	2.56
UG120251	11	2.31	UG120210	18	2.56
UG130008	11	2.31	UG130038	18	2.56
UG120050	12	2.37	UG130075	18	2.56
UG120109	12	2.37	UG130029	19	2.62
UG120127	12	2.37	UG130068	19	2.62
			UG130066	20	2.67
			NASE 13	21	2.87

*The rank having the same number indicates genotypes that are not significantly different and have identical mean damage values

The results of the genotypes mean peroxidase, tannin and flavonoid response at 60 and 90 DAP are presented in Figure 2. The resistant CS 1-144 showed the highest peroxidase (0.86); followed by NAM 130 with a value of 0.57 at 90 DAP. Meanwhile, the susceptible UG 120170 and 130068 had the lower values of peroxidase at 90 DAP of 0.28 and 0.33, respectively, compared to 60 DAP with values of 0.35 and 0.36, respectively.

Although all genotypes had low flavonoid scores at 90 DAP, resistant NAM 130 and UG 120133 showed decreased response from 60 DAP to 90 DAP from 0.63 to 0.04 and from 0.63 to 0.042, respectively (Fig. 2). The susceptible UG 130068 had the lowest flavonoid score at 90 DAP of 0.02, compared to all the other genotypes. Resistant UG 120257 and NAM 130 genotypes had the lowest tannin at 60 DAP compared to all the genotypes with absorbances of 0.06 and 0.07, respectively; which increased to 0.28 and 0.276, respectively, at 90 DAP. Further, resistant UG 120191 at 90 DAP had the highest reading of tannin of score 0.47, compared to all the eight genotypes, as well as at 60 DAP with a score of 0.12. The susceptible UG 120170 and NASE 13 had the lowest values of tannin at 90 DAP of 0.11 and 0.13 respectively. Peroxidase and tannin showed a

TABLE 4. Summary of mean squares for peroxidase, tannin and flavonoid on eight cassava genotypes at two sampling times (60 and 90 DAP) at Kamuli, Kasese and Namulonge in Uganda

Source of variaton	df	Peroxidase							Tannins	Flavonoids
		initial	0 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec		
ST	1	0.123***	0.144***	0.427***	3.231***	6.437***	6.891**	7.295***	11.255***	5.586***	0.407***
Location (L)	2	0.242*	0.437*	0.662*	1.545*	2.090^{ns}	2.357^{ns}	1.937^{ns}	1.144^{ns}	0.044^{ns}	0.131^{ns}
Genotype (G)	7	0.024*	0.078*	0.605*	1.359*	1.804^{ns}	1.521^{ns}	1.657^{ns}	1.188^{ns}	0.075^{ns}	0.134^{ns}
GXL	14	0.015^{ns}	0.041^{ns}	0.209^{ns}	0.590*	0.963**	1.120**	1.045*	1.078**	0.023^{ns}	0.054^{ns}
Error	96	0.005	0.007	0.034	0.058	0.224	0.057	0.058	0.069	0.019	0.008
CV(%)		33.4	29.1	36.2	32	28.1	22.2	20.5	21.5	36.7	30.8

***, **, * significant at P<0.001; 0.01 and 0.05 respectively and ns: non-significant at P > 0.05; Abs=Spectrophotometric absorbance readings from initial to 180 seconds; ST=sampling time; DAP=days after planting

corresponding increasing trend among all the genotypes; while flavonoid showed a decrease at 90 DAP.

Leaf damage and biochemical activity. Peroxidase activity per minute at 60 DAP and 90 DAP is shown in Table 5. Resistant genotypes, NAM 130 and CS 1-144, showed increased peroxidase activity at 60 DAP in the first minute from 0.42 and 0.38 U min^{-1} mg^{-1}, respectively; to 0.98 and 1.35 min^{-1} mg^{-1}, respectively, at 90 DAP compared to all the other genotypes. Susceptible genotype, UG 120170; showed low activity at 60 DAP of 0.15 U min^{-1} mg^{-1} with a low increase in activity to 90 DAP at a rate of 0.18 U min^{-1} g^{-1} at 0-60 seconds.

The analysis of variance results of the whitefly count, leaf damage and sooty mold on the selected eight genotypes are presented in Table 6. The data were recorded at the same time when leaf samples were collected for laboratory assay. The genotypes showed significant (P<0.05) differences for whitefly count at 60 DAP, (P<0.01) at 90 and 120 DAP and (P<0.001) at 150 and 180 DAP. There were also significant differences among genotypes for leaf damage. The genotypes showed significant (P < 0.01) differences at 30 and 60 DAP and (P < 0.001) at 90 to 180 DAP. As regards, sooty mold, genotypes varied significantly (P < 0.05) at 30 and 60 DAP, P <0.01 at 90 DAP and P < 0.001 from 120 to 180 DAP.

The results of mean whitefly count, leaf damage and sooty mold show changes among the genotypes in the three parameters across a six month period (Table 7). CS 1-44 had the least number of average whitefly count (26.73); while susceptible UG 130068 had the highest whitefly count (94.54) across the 180 DAP. CS 1-144 and NAM 130 showed a marked decrease in leaf damage score from 90 to 120 DAP with CS 1-144 reducing from 2.8 to 2.2, respectively, and NAM 130 from 3.0 to 1.3, respectively. Meanwhile, UG 130068 and UG 120170 showed an increase in leaf damage from 90 DAP to 120 DAP with

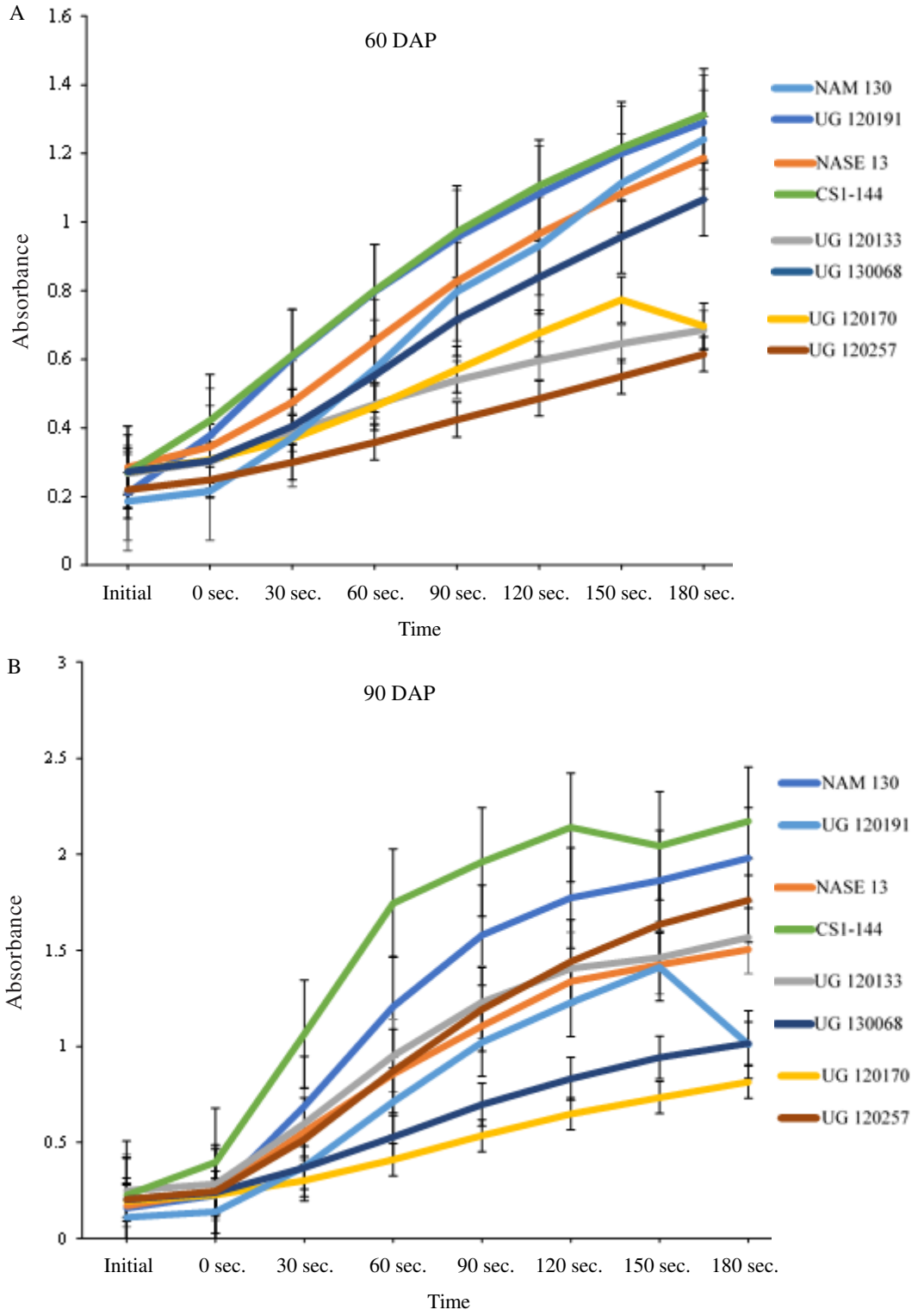

Figure 1. A comparison of the different peroxidase absorbances of eight cassava genotypes at 60 DAP (A) and 90 DAP(B) across a three minute period.

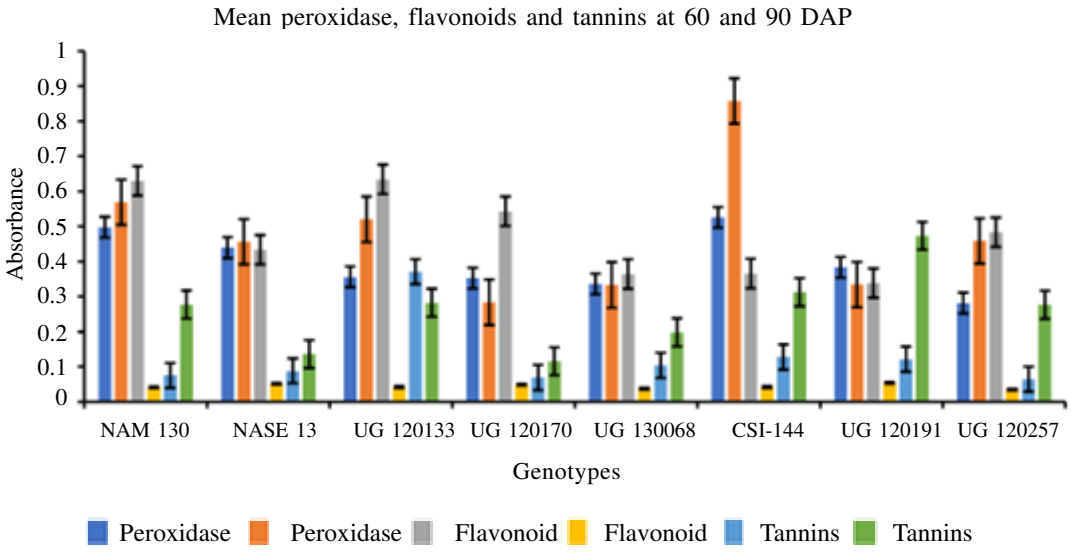

Figure 2. An average peroxidase, tannin and flavonoid response of eight cassava genotypes at 60 DAP (S1) and 90 DAP(S2).

scores from 3.6 to 4.0 and 3.0 to 4.0, respectively.

The results of correlation analysis for peroxidase, tannin, flavonoid, whitefly count, leaf damage, and nymph count are presented in Table 8. Whitefly and nymph counts were both significantly ($P<0.05$) positively correlated to leaf damage ($r = 0.788$ and $r = 0.646$), respectively. The sooty mold was significantly ($P<0.01$) correlated to the leaf damage ($r = 0.82$). Peroxidase activity was significantly ($P<0.01$) negatively correlated ($r = -0.84$) to leaf damage and ($r = -0.483$) to sooty mold. The tannin activity was significantly ($P<0.05$) negatively correlated with leaf damage ($r = -0.569$), nymph count ($r = -0.774$) and with whitefly count ($r = -0.442$). Flavonoid activity had a negative association ($r = -0.36$) to leaf damage. Tannin activity was significantly ($P< 0.05$) positively correlated to peroxidase activity ($r = 0.45$).

The results of a partial linear regression analysis to predict the overall contribution of peroxidase, tannin and flavonoid to leaf damage are presented in Table 9. The linear combination of strength measures for peroxidase, tannin and flavonoid were shown to be significant ($P<0.05$). The sample coefficient was $r = 0.85$, indicating that 85% of the damage can be accounted for by the linear combination of the metabolite strength measures. The Equation generated based on the estimates was:

$$Y= 5.264-7.08x+1.331y-5.49z$$

Where:

Y = leaf damage, x = peroxidase, y = flavonoid and z = tannin

Peroxidase and tannin were significantly associated to leaf damage at $t(4)$, -5.53, ($P<0.01$) and $t(4)$, -4.82, ($P<0.01$) respectively. The result of a partial linear regression showed that peroxidase and tannin predicted lower damage by 74 and 55%, respectively with standard errors of 1.28 and 1.14, respectively.

TABLE 5. Peroxidase activity based on the reaction rate of eight cassava genotypes per minute

ST (DAP)	Time	Genotypes							
		NAM 130	NASE 13	UG 120133	UG 120170	UG 120191	CS 1-144	UG 130068	UG 120257
		Reaction rate (U $min^{-1}mg^{-1}$)							
60	0-60 seconds	0.419	0.308	0.166	0.154	0.356	0.379	0.248	0.109
	60-120 seconds	0.286	0.313	0.128	0.214	0.359	0.306	0.287	0.128
	120-180 seconds	0.207	0.221	0.089	0.02	0.31	0.207	0.226	0.129
90	0-60 seconds	0.982	0.612	0.667	0.183	0.572	1.349	0.288	0.625
	60-120 seconds	0.566	0.482	0.455	0.238	0.516	0.395	0.304	0.57
	120-180 seconds	0.208	0.167	0.161	0.166	-0.218	0.032	0.184	0.319
	Mean	0.445	0.351	0.278	0.163	0.316	0.445	0.256	0.313
	LSD (0.05)	0.556	0.299	0.514	0.286	0.294	0.961	0.358	0.483
	CV (%)	16.346	10.725	20.290	17.401	11.281	22.223	13.195	20.489
	Error	0.038	0.011	0.032	0.009	0.010	0.111	0.015	0.028
	Df	30	30	30	30	30	30	30	30

ST = Sampling time at 60 and 90 days after planting (DAP), Time = one minute interval of spectrophotometer readings; Df = Degrees of freedom for the error; U = Change in absorbance units; min = minute;mg = milligramme of sample; LSD = Least significant difference; CV = coefficient of variation

TABLE 6. Mean squares for whitefly count, cassava leaf damage and sooty mold on eight cassava genotypes across three locations (Kamuli, Kasese and Namulonge) in Uganda

Source of variation	d.f.	WC						LD						SM					
DAP		30	60	90	120	150	180	30	60	90	120	150	180	30	60	90	120	150	180
Genotype (G)	7	530.8ns	2267.8*	4692.2**	4155.4**	3614.3***	3150***	1.4**	1.2**	1.2***	3.8***	5.6***	7.5***	0.5*	0.6*	1.2**	2.0***	2.4***	6.1***
Locations (L)	2	1172.8^{ns}	2445.1^{ns}	1487^{ns}	208.9^{ns}	387.6^{ns}	45.1^{ns}	0.1^{ns}	0.2^{ns}	0.01^{ns}	0.00^{ns}	0.05^{ns}	0.39^{ns}	0.77^{ns}	0.08^{ns}	0.42^{ns}	0.92^{ns}	0.20^{ns}	0.02^{ns}
Residual	14	443.9	805.0	946.6	669.0	420.6	147.2	0.3	0.2	0.0	0.0	0.1	0.2	0.1	0.2	0.3	0.3	0.4	0.5
cv (%)		39.3	43.5	42.8	40.1	34.6	25.5	33.4	28.4	7.9	2.8	9.8	18.9	36.2	41.7	32.1	34.2	34.4	44.6

***, **, * significant at P<0.001; 0.01 and 0.05, respectively, and ns = not significant at P> 0.05; MAP = months after planting; W C= whitefly count, LD = Leaf damage, SM = Sooty mold

TABLE 7. Monthly means of whitefly count, cassava leaf damage and sooty mold on eight cassava genotypes across three locations (Kamuli, Kasese and Namulonge) in Uganda

Genotypes	WC (DAP)						LD (DAP)						SM (DAP)					
	30	60	90	120	150	180	30	60	90	120	150	180	30	60	90	120	150	180
CS1-144	54.3	22.5	10.1	5.7	12.3	9.5	1.5	1.1	2.8	1.3	1.7	2.2	1.0	1.0	1.0	1.0	1.2	1.1
NAM130	78.3	67.9	77.7	14.0	25.7	22.2	1.0	1.4	3.0	1.3	1.4	1.3	2.0	1.3	1.3	1.0	2.1	1.0
NASE 13	46.5	34.3	43.2	67.4	98.8	70.8	1.8	1.2	2.4	2.3	2.3	2.3	1.0	1.0	1.1	1.3	1.7	1.6
UG120133	68.3	42.4	72.4	105.1	15.7	14.1	2.0	2.4	1.9	1.8	1.8	2.0	1.0	1.1	1.0	0.9	1.4	0.5
UG120170	45.5	140.4	119.5	158.9	121.9	104.3	2.5	1.8	3.0	4.0	4.6	4.9	1.0	1.0	1.2	1.4	1.5	2.5
UG120191	79.3	68.9	119.5	24.7	24.1	50.9	1.0	1.8	2.8	1.7	1.6	1.5	1.0	1.2	2.0	1.1	1.0	1.4
UG120257	46.5	130.5	19.8	16.4	33.6	40.6	1.0	1.7	1.6	1.5	1.5	1.5	1.0	1.2	1.0	1.0	1.6	1.1
UG130068	101.3	169.7	227.0	99.2	92.1	86.5	3.0	3.2	3.6	4.0	4.4	4.5	1.3	1.0	1.7	1.7	2.4	2.5
LSD (0.05)	36.9	49.7	33	45.3	35.9	21.2	0.9	0.9	0.4	0.1	0.4	0.8	0.5	0.7	0.9	0.9	1.1	1.2

WC = whitefly count; LD = Leaf damage; SM = Sooty mold; DAP = days after planting;; LSD = Least significant differences at P < 0.05

TABLE 8. Correlation coefficients of peroxidase, tannin, flavonoid, cassava leaf damage, nymph, sooty mold and whitefly count in the eight cassava genotypes across three locations (Kamuli, Kasese and Namulonge) in Uganda

	Leaf damage	Nymph count	Sooty mold	Whitefly count	Peroxidase	Flavonoid	Tannin
Leaf damage	-						
Nymph count	0.646*	-					
Sooty mold	0.821**	0.121^{ns}	-				
Whitefly count	0.788*	0.829**	0.170*	-			
Peroxidase	-0.840**	-0.360*	-0.483**	-0.216*	-		
Flavonoid	-0.363^{ns}	0.124^{ns}	0.096^{ns}	0.166^{ns}	-0.096^{ns}	-	
Tannin	-0.569*	-0.774**	0.402*	-0.442*	0.447*	-0.413^{ns}	-

** Significant at P<0.01; * Significant at P<0.05 (two-sided test)

TABLE 9. Partial regression analyses estimates for peroxidase, flavonoid and tannin among eight cassava genotypes used in a study on cassava leaf damage at Namulonge, Kamuli and Kasese in Uganda

Parameter	Regression coefficients	Standard error of estimate	t (4)	R^2
Constant	5.264***	0.650	8.10	
Peroxidase	-7.08**	1.28	-5.53	0.74
Flavonoid	1.331^{ns}	0.709	1.88	0.086
Tannin	-5.49**	1.14	-4.82	0.55

Correlation coefficient = 0.853
***, ** significant at P<0.001, and 0.01, dependent variable=leaf damage, predictors=peroxidase, flavonoid and tannin

DISCUSSION

Reaction of cassava genotypes to *Bemisia tabaci*. Cassava genotypes responded differently to whitefly count, nymph count and leaf damage in the four different locations (Table 6). The genotype by location interactions were significantly (P<.01) different from 90 to 180 DAP for all the genotypes for whitefly count and leaf damage (Table 2) indicating an association with the environmental conditions of the four sites (Table 1). Increased temperature is reported to increase *B. tabaci* development (Zeshan *et al*., 2015), as it reduces the mean number of days as the nymph changes to adult (Ali *et al*., 2005). Similar results were reported by Sing'ombe *et al*. (2015), who stated that whitefly count and leaf damage were dependent on varying environmental conditions.

Temperatures in Kamuli and Namulonge were high, ranging from a maximum monthly average of 24.2 to 30.5 ºC during the period of data collection and indicated that high temperatures resulted in increased *B. tabaci* activity and feeding; and showed that feeding was dependent on temperature and time of day, with high temperature increasing plant whitefly population and activity. Anderson *et al*. (2013) also reported that *B. tabaci* activity increased in tomato at high temperature locations. White *et al*. (2013) showed that in the early hours of the day when temperatures were low, whiteflies had low activity, with decreased feeding. Similarly, Otim Nape *et al*. (1997) in Uganda and Asare *et al*. (2014) in Ghana found

differences in whitefly infestation among cassava genotypes at different locations, with temperatures ranging from 26.5 to 32.0 ºC.

A cumulative ranking based on leaf damage (Table 3) was used for selection of genotypes. CS 1-144, UG 120133, UG 120257 and UG 120191 were among genotypes selected as resistant; while UG 130068 and UG 120170 as susceptible. Yaqoob *et al*. (2014), working with several bean genotypes in Pakistan reported a similar ranking of genotypes across various locations.

Biochemical activity. High coefficients of variation of peroxidase among the genotypes (Table 4) indicated the possible response of cultivars to produce biochemical constituents that influence the *B. tabaci* populations and leaf damage. The resistant genotypes, CS1-144, UG 120133 and NAM 130 recorded high peroxidase activity compared to the rest of the genotypes from 0 seconds to 60 seconds (Fig. 1). This suggest that peroxidase conferred resistance to these cultivars. Peroxidase has been documented as a defense response enzyme to insect feeding (War *et al*., 2008), increase in peroxidase activity acting to reduce plant damage (War *et al*., 2008). Almagro *et al*. (2012) reported that in maize, peroxidase resulted in plant and insect peroxidation of lipids, oxidation of proteins and damage to nucleic acids showing that peroxidase on its own acts as a direct genotype defense response, as well as indirectly by maintenance of ROS.

The peroxidase reaction rate at 0 to 60 seconds was significantly increased in resistant genotypes, CS 1-144 and NAM 130, between 60 and 90 DAP; with peroxidase activity of 0.970 and 0.563 U min-1mg-1, respectively, more than susceptible UG 130068 and UG 120170 with rates of 0.040 and 0.029 U min-1mg-1 during the same period (Table 5). This could be attributed to the presence of higher amounts of peroxidase in the genotypes, as initial reaction was high and was observed to generate a steep slope in resistant genotypes (Fig. 1). There were more active sites on the peroxidase for the H_2O_2 to get attached to, thus increasing the reaction rate *vis-à-vis* activity (Table 5). Further, the results were an indication of increased catalysis of H_2O_2 by peroxidase, suggesting that high amounts of peroxidase in the resistant plants were acting as part of the plant defense mechanism against *B. tabaci*. This was in agreement with Kus´nierczyk *et al*. (2008) who reported that the role of peroxidases is to oxidise H_2O_2 to H_2O as an immediate response to prevent the oxidative burst and protect the plant by maintaining required levels of the H_2O_2. Similar results were observed with *B. tabaci* in cowpea, cotton and tomato (Singh *et al*., 2013).

The high absorbances observed at 90 DAP (Fig. 1) in resistant CS 1-144 and NAM 130, suggests the presence of high amounts of active peroxidase, and more H_2O_2 converted per unit time. This implies that peroxidase was actively involved in the protection of the plant. These results are in accordance with Bisswanger (2014) and Singh *et al*. (2013), who considered increase in absorbance to be directly proportional to increased peroxidase activity. In addition, War *et al*. (2012) reported that as the whitefly inserts its stylet between the cells, and into the phloem to feed on the plant, the plant is able to initiate increased production of peroxidases.

Leaf damage and biochemical activity. The genotypes varied in peroxidase activity (Table 5) and leaf damage scores (Table 6), as the resistant CS1-144 and NAM 130 showed increased peroxidase activity at 90 DAP (Fig. 2); while the leaf damage scores reduced at 120 DAP (Table 7). However, in susceptible genotypes, UG 130068 and UG 120170, the leaf damage scores increased from 90 DAP to 120 DAP, and kept increasing thereafter (Table 7) with reducing peroxidase activity from 60 DAP to 90 DAP (Fig. 2). This suggest that peroxidase played a key role in resistance to *B. tabaci* damage. The results indicate that peroxidase was closely linked to feeding damage; high peroxidase activity could lead

to reduced leaf damage. Significant *B. tabaci* feeding action was suggested to begin earlier than three months, as it takes time for the feeding response to be observed on the leaves (Hagg *et al*., 2013). The peroxidase increase came days after insect attack, based on the defense response system according to Hagg *et al*. (2013). The biochemicals were spread in the plant at fluxes reported by Sofo *et al*. (2015), could lead to the observed reduction in damage scores after 120 DAP.

Peroxidase activity was reported to increase (Hagg *et al*., 2013) as plants detected damage incurred by recognising insect elicitors, as well as the plant signals that are released when there is insertion or disturbance of the plant membrane potential (Vm). The reduced leaf damage observed in the resistant genotypes, could also be due to the decreased nutritive value of the plant parts that was reported to be increased by peroxidase activity (War *et al*., 2011a). Furthermore, increased formation of lignin, hinders the stylet penetration of the pest in the cell wall (Whetten and Sederoff, 1995).

The tannin in the genotypes increased with decrease in flavonoid (Fig. 2) with decreased damage. Flavonoid was reduced by tannin as they are produced in the same pathway in the defense system, and have a similar mode of action, involving scavenging free radicals, including ROS (Treutter, 2006). On the other hand, peroxidase activity, increased with increased tannin in the resistant genotypes (NAM 130, CS 1-144 and UG 120257) at 90 DAP (Fig. 2), suggesting that both peroxidase and tannin are involved in increasing resistance to *B. tabaci*. The results confirm those of Taggar *et al*. (2012) and Taggar *et al*. (2014) who found that *B. tabaci* feeding in black gram cultivars, increased peroxidase activity and tannin content in the genotypes. Production of phenoxy and other oxidative radicals by the PODs, acting together with phenols such as tannins, have been reported to directly deter the feeding by insects and/or produce toxins that reduce the plant digestibility, which leads to nutrient deficiency in insects with drastic effects on their growth and development (Chen *et al*., 2009).

The positive significant ($P<0.05$) correlation between peroxidase and tannin ($r = 0.447$) (Table 8) could be due to the synergism of the metabolites in defense responses. Similarly, Duffey and Stout (1996) reported that in tomato, phenolics and oxidative enzymes act together, to affect insects during ingestion, digestion and metabolism. The tannin activity showed a significant negative correlation with the leaf damage, but were less effective than peroxidase; while flavonoid activity, although showed negative association to damage, there was no significant correlation to damage (Table 8). The tannin may decrease damage as they are bitter polyphenols that deter insect infestation (War *et al*., 2012). They also reduce the nutritive availability by non-specific protein precipitation and chelating of metal ions (Hagg *et al*., 2013).

The significant positive correlation between whitefly number and damage and between leaf damage and sooty mold (Table 8) was observed in susceptible genotypes (UG 130068 and UG 120170) with high values; while resistant CS1-144, NAM 130 and UG 120257 showed the reverse. The high whitefly feeding was also shown by the sooty mold increase in susceptible genotypes (NASE 13, UG 120170 and UG 130068) from 90 to 180 DAP (Table 7), indicating that there were increased defense responses such as peroxidase and tannin and supported the report of War *et al*. (2012). Similar results were reported by Omongo *et al*. (2012), who showed increased feeding damage observed on various cassava land races at 83 days after planting. Howeler (2012) reported that most indirect damage including foliage feeding on cassava was observed between 90 to 180 DAP. The variations in levels of cassava leaf damage among the resistant genotypes harboring a similar number of whiteflies as shown by NAM 130 and UG 120133 (Table 7), suggests that there were inherent biochemical plant factors responsible

for such differences. This indicates that with high peroxidase the the leaf damage was shown to decrease.

A similar association was reported in several studies (Omongo *et al*., 2012; Shah *et al*., 2015; Sing'ombe *et al*., 2015), indicating that increased plant whitefly population results in increased feeding and increased in defense response reactions in resistant genotypes (Gerling, 1990; War *et al*., 2012).

The significant ($P<0.01$) negative correlation ($r = 0.840$) between cassava leaf damage and peroxidase activity (Table 8), and the high coefficient of determination (R^2 = 74%) (Table 9), suggest that peroxidase has a high influence on resistance of cassava cultivars to *B. tabaci* damage. Peroxidases have been reported to make plants phenotypically flexible as they are associated with lignin formation, and could decrease the chances of the attacking insects to adapt to the induced chemical changes (Hagg *et al*., 2013). Further, oxidation of phenols and H_2O_2, catalysed by peroxidase (POD), is a potential defense mechanism in plants against insects, in addition quinones formed by oxidation bind covalently to leaf proteins, and inhibit protein digestion in insects as reported by War *et al*. (2011b).

The regression coefficients ($R^2 = 0.74$) show that high peroxidase activity (B=-7.08) contributes more to leaf damage protection compared to tannin(R^2=0.55) (B=-5.49) and flavonoid (R^2=0.086) (B=1.331) (Table 9). This was observed in resistant genotypes, NAM 130 and CS 1-144, with reduced damage scores from 90 to 180 DAP (Table 7); showing high peroxidase activity (Fig. 2) compared to susceptible UG 130068, during the same period. This indicates that flavonoid response was reduced due to more production of peroxidase. The results indicated that flavonoids compete directly with other metabolites that lead to lignin biosynthesis and confirm the findings of Singh *et al*. (2010). Since all these metabolites are required for oxidation (Boerjan *et al*., 2003), there would be competition in determining which resources would provide most important effects in which case there would be increased peroxidase than flavonoid as according to Moore *et al*. (2014).

CONCLUSION

Cassava resistant genotypes such as CS1-144 and NAM 130 show a close relationship of high peroxidase activity with low leaf damage, indicating that peroxidase plays a significant role in the genotypes resistance to *B. tabaci*. High peroxidase and tannin are closely associated to low whitefly and damage indicating the two biochemicals protected the plant against *B. tabaci*. The variations observed in peroxidase and tannin activity among the resistant genotypes indicate that these substances increase resistance in cassava. Peroxidases and tannins may be characterised further in cassava to determine which genotypic response is triggered by these metabolites in order to optimise peroxidase and tannin content in cassava genotypes and could be useful for marker assisted selection.

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