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Gene Action of Cassava Resistance Metabolites to Whitefly (*Bemisia tabaci)*

N. Mwila[1*], T. L. Odong[1], E. Nuwamanya[2], A. Badji[1], S. Agbahoungba[1], P. A. Ibanda[1], F. Kumi[1], M. Mwala[3], P. Sohati[3], S. Kyamanywa[1] and P. R. Rubaihayo[1]

[1]Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P.O.Box 7062, Kampala, Uganda.
[2]National Crops Resources Research Institute, Namulonge, P.O.Box 7084, Kampala, Uganda.
[3]Department of Plant Science, School of Agricultural Sciences, University of Zambia, P.O.Box 32367, Lusaka, Zambia.

Authors' contributions

Author NM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first and final draft of the entire manuscript. Author TLO assisted the author with statistical methods used and reviewed the manuscript. Author AB read and reviewed manuscript especially on the gene action. Author EN assisted the author in the protocol set up, read and reviewed the manuscript. Authors SA, FK, PAI, MM and PS read and reviewed the manuscript. Author SK supervised the author throughout the study, read and reviewed the manuscript especially from an entomological point of view. Author PRR supervised the author throughout the study, read, reviewed and assisted the author shape the manuscript from a plant breeders perspective.

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ABSTRACT

Several metabolites are linked to cassava resistance to whitefly. There is limited information however, on the mode of gene action of the metabolites associated with cassava resistance to whitefly (*Bemisia tabaci)*. The objective of the study was to determine the combining abilities and

**Corresponding author: E-mail: mwilanatasha@yahoo.co.uk;*

mode of gene action of salicylic acid, antioxidative capacity, total phenolic content, flavonoid, tannin, peroxidase and protein of selected cassava genotypes. Ten genotypes were crossed in half diallel and the parental and 45 S1 progenies evaluated for nymph count, whitefly count, leaf damage and sooty mold at Namulonge in season two of 2016. In season one and two of 2017, the parental genotypes and their corresponding forty-five S1 progenies were evaluated in randomized complete blocks with two replications for *Bemisia tabaci* population and leaf damage and leaf metabolite content assayed. The results indicated highly significant (P<.001) differences among genotypes for general combining ability (GCA) to antioxidative capacity; (P<.05) for total phenolic content, peroxidase and protein and (P<.01) for salicylic acid, tannin and flavonoid. The specific combining ability (SCA)showed significant (P<.001) differences for salicylic acid; (P<.01) for antioxidative capacity and total phenolic content. The additive variance was significant (P< .05) for flavonoid, protein; (P<.01) for antioxidative capacity, total phenolic content, tannin and peroxidase. The dominance variance had high significance (P<.001) for salicylic acid; (P<.05) for flavonoid and (P<.01) for antioxidative capacity and total phenolic content. Although additive gene action was higher than non-additive, both were influencing most metabolites indicating complexity of inheritance. A critical evaluation is necessary when exploiting metabolite related traits in breeding for resistance to *Bemisia tabaci*.

Keywords: General combining ability; specific combining ability; salicylic acid; antioxidative capacity; total phenolic content; flavonoid; tannin; peroxidase; protein; heritability.

1. INTRODUCTION

Whitefly is one most significant insect on cassava [1] causing direct damage [2] through plant injury [3], acts as a vector of African cassava mosaic disease (ACMD)[4] and cassava brown streak disease (CBSD) [5]. Direct and indirect damage reduces crop root yield up to 80% to 100% [6] depending on the duration and severity of attack [7]. In addition, root and leaf quality is affected, as regards metabolite, nutrient content and aesthetic value, lowering household income of farmers by about 50% annually [8].

Host plant resistance (HPR) in cassava to *B. tabaci* is seen as one of the best available strategies for controlling *B. tabaci* populations [9]. It is suggested that plant resistance to *B. tabaci* is a complex trait, in which several metabolites [10] and genes [11,12] are involved. Metabolites such as salicylic acid (SA), peroxidases and phenolics, [13] have been associated with plant resistance to *B. tabaci* in tomato [10], cotton [14], and cassava [15]. Salicylic acid is reported as a plant defence response to *B. tabaci* [16] with metabolites such as peroxidases and phenolics associated to the SA pathway [17]. Peroxidases are expressed to limit cellular spreading of insect infestation damage through the establishment of structural barriers or the generation of highly toxic environments by producing reactive oxygen species (ROS) of which among them include (H_2O_2, O_2, OH^-) [18]. The ROS inactivate protein or limit tissue protein so as to disturb insect feeding [19]. Phenolic content changes result in increased antioxidative power [20] via protein denaturation [21] and lead to decreased nutrient availability [22] of plant parts to the insect [13]. This occurs through chelating transition metal ions, direct scavenging of molecular species of active oxygen, trapping the lipid alkoxyl radical [23] and lipid peroxidation [24]. As a result, tannins and flavonoids have been reported to protect plants against whitefly by influencing the behavior and growth of the insects [25].

Gene action of some metabolites such as phenolics and flavonoids has been reported in Arabidopsis thaliana [11], soybean [26], amino acids and phenolics in tomato [4]. However, there is limited information about the gene action and mode of inheritance of the metabolites associated with cassava resistance to *B. tabaci*. It is documented that there are several metabolites mediating insect resistance [27] as such, several pathways with different gene action implicated have been reported with some metabolites controlled by non-additive gene action [11]. Further, multiple metabolite resistance associated quantitative trait loci (QTL) were reported by van den elsen et al. [4] in *Solanum pennellii*, indicating a presence of metabolomic diversity of independent metabolites in different plant species with varying inheritance patterns [28]. In cassava, broad-sense heritability was reported as low for amylose content and it's associated proteins, and high for cyanogenic compounds [29]. In *Theobroma cacao* broad sense heritability was high for amino acids which are associated to biosynthesis of phenols and salicylic acid [30].

The frequency of genes for specific desirable traits has been reported to progressively increase [31] through population improvement and recurrent selection in cassava [32]. The success of population breeding largely depends on the choice of parents, but cassava parental line selection have traditionally focused on the parents' performance, with little use of general combining ability (GCA)effects as a criterion [33]. Knowledge on the nature of the combining ability and resulting genetic effects with resistance to *B. tabaci* has paramount significance in the selection process for identification and exploiting desirable cassava genotypes as well as estimation of genetic gains useful to improve desired traits [34]. The evaluation of genetic effect and inheritance patterns of the metabolites is vital in understanding resistance of cassava to *B. tabaci* and enhance the determination of metabolite mediated resistance genetic advance from selection.

The objective of the present study was to assess the nature and magnitude of gene action controlling the inheritance of resistance related metabolites to *B. tabaci* in cassava.

2. MATERIALS AND METHODS

2.1 Study Site and Plant Materials

The study was conducted at Namulonge which is a whitefly hot spot area [35]. Namulonge is located in Central Uganda (32° 37'N 0° 32'E), at altitude 1150 masl [36], receives rainfall of about 1200mm/year and belongs to lake victoria crescent and mbale farmlands AEZ.

An evaluation of the 450 genotypes planted in an augmented design was made for nymph count, whitefly count, leaf damage and sooty mold [37] in season two (July-December) of 2016. Thereafter a selection of five clones (resistant), five (susceptible) and the corresponding S1 progenys' (45) (Table 1) was made based on family size and different resistance levels to whitefly infestation. There were 10 crosses missing.

2.2 Experimental Design

The genotypes (parental and S1 genotypes) were planted in an augmented design with randomized complete blocks (RCBD) with three replications in the first and second season (February-July and August, 2017 – January, 2018). Planting was done using 1-2 cuttings per hole, on 23rd February and 15th August, 2017. Each plot measured a total of 277m^2, consisting of 55 single row plots of 5 plants/row, 1m apart with inter row spacing of 1m and 1m between the replications. Regular weeding of the fields till maturity was done with a hand hoe [7].

2.3 Data Collection

All plant data were collected from three tagged plants randomly selected per row per plot. The following data were collected on the top five fully expanded apical leaves; nymph count, whitefly count [37] and a leaf damage score [2] at 4 months of plant age as this is the peak [38] during the six months peak period of whitefly infestation [39].

Total phenolic content (TPC), salicylic acid (SA), peroxidase, tannin, flavonoids and antioxidative capacity were analyzed on 2-7 leaves and petiole sap of the same leaves per plant per genotype sampled at 4 months after planting (MAP)[40]. The leaves were weighed using an electronic scale and placed in a ziploc bag. Petiole sap was collected directly into 1.5ml vials according to the procedure outlined in the Hortus manual [41]. Leaf and sap samples were immediately placed in ice boxes at 4ºC and further processed according to van Bel [42] and Hortus manual [41].

2.3.1 Salicylic acid determination

Salicylic acid (SA) measurements were carried out on different clones of cassava using the standardized protocol [43] with modifications and the amount of SA in the leaf samples was determined accordingly. Leaf samples of cassava weighing 100mg were frozen in liquid nitrogen and ground to powder using a mortar. Samples were left at 24°C to thaw. Varying aliquots (10 – 1000 mg) of the sample were extracted in 1.0 mls of different solvents to assay the solubility of SA from tissues in the presence of interfering substances.

The samples were swirled well in the solvent followed by centrifugation (PrismaR, Edison, New Jersey, USA) at 1,000rpm for 10 minutes. The supernatant was stored on ice at -20°Cfor SA measurement. 100 µl of the supernatant was mixed with 0.1% freshly prepared ferric chloride. The volume of the reaction mixture was made up to 3.0 ml and the complex formed between Fe^{3+} ion and SA, which is violet in color was determined by spectrophotometry (Biowaveii+, Cambridge, England), measuring the absorbance at 540 nm.

Thereafter, the development of the standard curves was conducted with water as a solvent in aliquots of 100, 200, 300, 400 and 500 µg series. Standard curves of SA using water and the curve of best fit by regression analysis were made [43]. The standard graph had an r^2value= 0.995, closer to 1 indicating strong correlation between the x and y axis data. The curve was calculated using the formula, *y* = 0.001x, where, *y* is the absorbance at 540 nm and *x*, the amount of water equivalent (g/ml).

2.3.2 Total phenolic content determination

A 50 mg leaf sample was homogenized with distilled water to obtain aqueous extract and a standard protocol [44] was followed with minor modifications. 200 µL of extractwas collected in a test tube and made up to 3 ml with distilled water, centrifugation was done at 600 rpm for 10 minutes and the incubation procedure was followed at 24°C and 40°C consecutively at the recommended time interval.The absorbance of the sample was determined using a UV visible spectrophotometer (Biowaveii+, Cambridge, England) at 650 nm [45]. Calibration curve was constructed with different concentrations of gallic acid (0.25-0.0039 g/ml) [46] and was expressed as g of gallic acid equivalents (GAE) per ml extract.

2.3.3 Total Tannin determination

A 100 mg leaf sample was placed into a 2 ml eppendorf tube, where 0.5 mls of 5% ascorbic acetone solution was added to dissolve leaf precipitate and placed on an orbital shaker for 20 minutes. The protocol as shown by Harborne, (1998) was followed. Pigments were removed using 0.5 mls of petroleum ether and left on the bench until it all evaporated then 0.3 mls of distilled water was added and centrifuged for 10 minutes at 1,000rpm followed by adding 2.4 ml of 5% hydrochloric acid (HCL)-butanol solution. Each content in the tube was run through a 240mm filter paper and 0.5 mls of the filtrate was made up to 1ml with distilled water in a conical flask. 0.5 mls of folin ciocalteu reagent was added and mixed with 2.5ml of 20% sodium carbonate solution and mixed.0.1ml 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 550nm. A similar procedure was followed for the sap samples. A standard graph was constructed using tannic acid at concentrations ranging from 0.025 to 3.815E-07 [47]. The total tannin content was expressed as g tannic acid equivalents per ml of sample filtrate [48].

2.3.4 Total flavonoid content determination

A standard protocol [49] was followed with slight modifications [15]. A leaf sample weighing 50 mg was homogenized with 2 ml of 0.1M ice cold phosphate buffer at a pH =7.5. A 50 µLof 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 ($AlCl_3$) solution after 5 minutes of incubation at 40ºC, and then the mixture allowed to stand for 6 minutes [49]. 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. The mixture was allowed to stand for 15 minutes at 24ºC and absorbance measured at 510 nm, using UV-visible spectrophotometer. Thereafter, a calibration curve of quercetin was obtained for concentrations ranging from 0.002 to 0.0014g/ml and the total flavonoid content of each extract was expressed as g of quercetin equivalents (QE) per ml of sample mixture [50].

2.3.5 Antioxidative capacity

Antioxidative capacity was determined using Ferric reducing antioxidant potential (FRAP) [51] with modifications [40]. The sample was mixed well and absorbance read at 700 nm [24]. Since the presence of reducers (*i.e.*, antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form [51], the formation of blue-colored ferrous tripyridyltriazine complex (Fe^{2+}-TPTZ) was measured at pH 3.6 spectrophotometrically [24]. Ascorbic acid was used as a standard [50] with a stock solution of 5000 mg/L prepared in distilled water, from which dilutions were made ranging from 25 mg/L to 500 mg/L. The antioxidative capacity was recorded as g of ascorbic acid equivalents (AAE) per ml [50].

2.4 Data Analyses

2.4.1 Statistical analysis

Analysis of variance was conducted using the linear model in Genstat12.0. The linear equation used was: $Y_{ijk} = \mu + G_i + E_j + GE_{ij} + E_{ijk}$ [53]

Where; Y was the response in terms of whitefly count, nymph count, leaf damage, sooty mold of the j^{th} replication of genotype "i" in environment

Table 1. Genotypes used in the study

Entry	Genotype	Class	Entry	Genotype	Class	Entry	Genotype	Class
1	UG 120133	Resistant	19	UG 120133* UG 120160	Susceptible	37	NASE 13* UG 120193	Susceptible
2	UG 120191	Mresistant	20	UG 120191* UG 120293	Susceptible	38	NASE 13* UG 120257	Resistant
3	UG 120293	Mresistant	21	UG 120191* NASE 13	Mresistant	39	NASE 13* UG 120124	Mresistant
4	NASE 13	Susceptible	22	UG 120191* UG 130068	Mresistant	40	NASE 13* UG 120160	Susceptible
5	UG 130068	Susceptible	23	UG 120191* UG 120198	Mresistant	41	UG 130068* UG 120198	Susceptible
6	UG 120198	Susceptible	24	UG 120191* UG 120193	Mresistant	42	UG 130068 * UG 120193	Susceptible
7	UG 120193	Susceptible	25	UG 120191*UG 120257	Resistant	43	UG 130068 * UG 120160	Susceptible
8	UG 120257	Resistant	26	UG 120191* UG 120124	Resistant	44	UG 120198 * UG 120198	Susceptible
9	UG 120124	Resistant	27	UG 120191*UG 120160	Mresistant	45	UG 120198 * UG 120124	Resistant
10	UG 120160	Susceptible	28	UG 120293* UG 120293	Susceptible	46	UG 120198* UG120160	Mresistant
11	UG 120133* UG 120133	Susceptible	29	UG 120293*UG 130068	Susceptible	47	UG 120193* UG 120193	Susceptible
12	UG 120133* UG 120191	Mresistant	30	UG 120293* UG 120198	Susceptible	48	UG 120193* UG 120257	Resistant
13	UG 120133*NASE 13	Susceptible	31	UG 120293* UG 120193	Susceptible	49	UG 120193 * UG 120160	Susceptible
14	UG 120133* UG 130068	Susceptible	32	UG 120293* UG 120257	Mresistant	50	UG 120257 * UG 120257	Resistant
15	UG 120133* UG 120198	Mresistant	33	UG 120293* UG 120124	Mresistant	51	UG 120257 * UG120124	Resistant
16	UG 120133* UG 120193	Susceptible	34	UG 120293* UG 120160	Susceptible	52	UG 120257 * UG120160	Resistant
17	UG 120133* UG 120257	Susceptible	35	NASE 13* NASE 13	Susceptible	53	UG 120124* UG 120124	Resistant
18	UG 120133* UG 120124	Susceptible	36	NASE 13* UG 120198	Susceptible	54	UG 120124* UG 120160	Mresistant
						55	UG 120124* UG 120251	Resistant

Source: NaCRRI; Mresistant: Moderately resistant

"k"; μ = overall mean of the responses; G_i = genotype effect; E_j = environment effect; GE_{ij} = interaction effect; E_{ijk}=experimental error.

A combined analysis of variance was done on the genotypes' nymph, whitefly count, leaf damage, sooty mold, salicylic acid, tannin, flavonoid, total phenolic content and antioxidant activity across two seasons using linear mixed model (restricted maximum likelihood -REML) procedure in Genstat 12.0 software (18). A model described by Hongyu, García-Peña, Araújo, & Santos Dias, [53], was used as follows;

$$\gamma_{ijkl} = \mu + \rho_i + S_k + r_l + b_m + \rho s_{ki} + ls_{kj} + \rho ls_{ijk} + \varepsilon_{ijklm} \quad [53]$$

Nymph, whitefly count, leaf damage and sooty mold means were separated using Fisher's Least Significant Difference (LSD) test at 5% probability level [54].

The combining ability analysis was carried out following Griffing's half diallel mating method II model 2 (Griffing, 1956) which included the direct S1 crosses and parents. The estimates of GCA effect for the parents and the SCA effects for the crosses were calculated accordingly(55). The model followed was:

$$Yij = \mu + gi + gj + sij + \frac{1}{bc}\Sigma_k\Sigma_l\varepsilon ijkli, j=1,\ldots p, \quad [56]$$

Where, Y_{ij} is the observation of cross (x_{ij}), μ is the population mean, g_i and g_j are the general combining ability effect for the ith and jth parents, S_{ij} is the specific combining ability effect of the cross between the ith and jth parents such that $S_{ij} = S_{ji}$and ε_{ijkl} is the experimental error due to environmental effect associated with the i_{jklth}. The variance component was calculated using the formula:

$$\sigma^2_g=(MS_{gca}-MS_{error})/(p-2); \quad \sigma^2_s=(MS_{sca}-MS_{errror})/1 \quad [28]$$

Where, MS_{gca}= variance due to GCA; MS_{sca}= variance due to SCA;MS_{error}= error variance.

Since the parents used in the crosses were considered random, broad sense, narrow sense heritability. was determined by the formula below:

$$H^2 = \frac{\sigma^2A+\sigma^2D+\sigma^2I}{\sigma^2A+\sigma^2D+\sigma^2I+\sigma^2e+\sigma^2ge}$$

$$h^2 = \frac{\sigma^2A}{\sigma^2A+\sigma^2D+\sigma^2I+\sigma^2e+\sigma^2ge} \quad [57]$$

The baker's ratio and average degree of dominance were calculated using the formulae as shown below:

Baker's ratio/prediction ratio = $(2\ \sigma^2_{gca})/(2\sigma^2_{gca} + \sigma^2_{sca})$

Average degree of dominance= $[(\frac{\sigma 2D}{\sigma 2A})^{1/2}]$ [58]

3. RESULTS

The variance component estimates across the measured parameters are presented in Table 2. The genotypes were highly significant (P < 0.001) for all the metabolites measured while (P < 0.05) for nymph count and whitefly count and (P < 0.01) for leaf damage and sooty mold.

The general combining ability (GCA)was highly significantly (P < 0.001) for whitefly count, leaf damage and antioxidative capacity. Meanwhile significant (P < 0.05) differences for nymph count, total phenolic content (TPC), peroxidase, protein and (P < 0.01) for sooty mold, salicylic acid, tannin and flavonoid were recorded. The GCA variance was greater than SCA variance for nymph count, whitefly count, leaf damage, sooty mold, antioxidative capacity, total phenolic content, tannin, flavonoid, peroxidase and protein (Table 3). The SCA variance was greater than GCA variance for salicylic acid. The differences between GCA and SCA variance values for salicylic acid, total phenolic acid, tannin and flavonoid were low as compared to antioxidative capacity, peroxidase and protein. Additive gene effects recorded higher values in comparison to dominant gene effects for most traits. There were significant (P < 0.001) differences for nymph

Table 2. General analysis of variance for measured traits

SOV	df	NC	WC	LD	SM	SA	POD	Tan	Fl	TPC	AC
Rep	2	0.002	0.019	0.017	0.011	0.002	0.002	0.026	0.011*	0.002*	0.02*
Rep.Block	10	0.007	0.058	0.075	0.038	0.138	0.011	0.028	0.010	0.001	0.01
Genotype	54	101.00**	199.40**	0.05*	0.07*	0.12***	0.72***	0.06***	0.03***	0.01***	0.07***
Res	49	0.003	0.032	0.028	0.019	0.718	0.008	0.017	0.011	0.001	0.01
LEE	50.2	0.019	0.258	0.028	0.003	0.008	0.008	0.010	0.005	0.001	0.004
SED		35.000	24.560	17.060	21.001	6.290	4.73	12.09	7.15	8.31	3.68

**significant at P < 0.05; **significant at P < 0.01; ***significant at P < 0.001; Rep: Replication; Res: Residual; LEE: Lattice effective error; SED: standard error deviation; nc: nymph count; wc: whitefly count; ld: leaf damage; sm: sooty mold; sa: salicylic acid; pod: peroxidase; tan: tannin; fl: flavonoid; tpc: total phenolic content; ac: antioxidative capacity*

count, leaf damage, sooty mold, ($P < 0.05$) for whitefly count, flavonoid and protein, ($P < 0.01$) for antioxidative capacity, total phenolic content, tannin and peroxidase. The additive gene action for salicylic acid was higher and significant ($P < 0.001$) than dominant gene action with values of 5.1E-6 and 9.0497E-18 respectively. The average degree of dominance for traits was less than 1. Broad sense heritability was higher than narrow sense heritability for all traits measured except salicylic acid. Salicylic acid had the highest baker's ratio (0.999) while nymph count had the lowest value (0.667).

The GCA effects estimates of all parameters measured are shown in Table 4. Several genotypes showed a combination of negative and positive GCA estimates for various traits. Genotype UG 120124 recorded significant ($P<0.001$) negative GCA effects for whitefly count ($P< 0.05$) for nymph count, leaf damage with values of -27.93, 9.62, -0. 627 respectively. Meanwhile the effects were significant ($P<0.001$) and positive for salicylic acid, antioxidative capacity and peroxidase ($P< 0.05$) for total phenolic content and flavonoid; ($P<0.01$) for tannin. The values were; salicylic acid (0.025), antioxidative capacity (0.043), peroxidase (0.119), total phenolic content (0.009), flavonoid (0.07) and tannin (0.0003) respectively. UG 120257 genotype had negative significant ($P<0.001$) effects for leaf damage, ($P<0.05$) for nymph count, and ($P < 0.01$) for sooty mold while significant ($P < 0.001$) for tannin, ($P < 0.05$) for antioxidative capacity, peroxidase and protein ($P < 0.01$) for salicylic acid. UG 120133 had significant ($P < 0.05$) negative effects for nymph count, leaf damage, sooty mold, and peroxidase, ($P < 0.01$) for whitefly count and salicylic acid, ($P < 0.01$) positive effects for antioxidative capacity and total phenolic content. UG 120191 had significant ($P < 0.001$) positive effects for antioxidative capacity and flavonoid, ($P < 0.05$) for peroxidase and protein and ($P < 0.01$) effects for salicylic acid, total phenolic content and tannin meanwhile had significant ($P < 0.05$) negative effects for leaf damage and ($P < 0.01$) for sooty mold. UG 120160 had significant ($P < 0.001$) positive effects for salicylic acid, ($P < 0.05$) for sooty mold but negative effects for nymph and whitefly count at significance of $P < 0.05$ and $P < 0.01$ for antioxidative capacity, total phenolic content and tannin.

However, UG 130068 had positive significant ($P < 0.01$) effects for whitefly count, leaf damage, sooty mold; ($P < 0.01$) for nymph count and negative significant ($P < 0.05$) effects for salicylic acid, tannin and ($P < 0.01$) for antioxidative capacity, total phenolic content and peroxidase. The values of the traits were 20.17 (whitefly count), 0.499 (leaf damage), 0.0024 (sooty mold), 10.862 (nymph count), -0.015 (salicylic acid), - 0.0005 (tannin), -0.015 (antioxidative capacity) -0.001 (total phenolic content) and -0.025 (peroxidase) respectively. Similarly, UG 120198 had significant ($P < 0.05$) positive effects for nymph count and sooty mold and negative effects for salicylic acid, total phenolic content, ($P < 0.01$) for antioxidative capacity, tannin and flavonoid.

The estimates of SCA effects of biochemical parameters measured were determined and presented in Table 5. Significant positive SCA effects were evident on several crosses for salicylic acid, antioxidative capacity, total phenolic content, tannin, flavonoid, peroxidase and protein.

UG 120191* UG 120257 showed positive significant ($P < 0.05$) SCA effects for salicylic acid, ($P < 0.01$) for antioxidative capacity, total phenolic content and peroxidase. UG 120191* NASE 13 had positive significant ($P < 0.001$) SCA effects for salicylic acid and tannin; ($P < 0.05$) for total phenolic content and peroxidase and ($P < 0.01$) for antioxidative capacity and protein. UG 120133*UG 120124 had positive significant ($P < 0.001$) for antioxidative capacity, total phenolic content and protein, ($P < 0.01$) for salicylic acid, flavonoid and peroxidase.

Meanwhile UG 120193* UG 120160 had negative significant ($P < 0.05$) for total phenolic content, ($P < 0.01$) for antioxidative capacity, tannin, peroxidase and protein. UG 130068* UG 120198 showed negative significant ($P < 0.05$) estimates for salicylic acid and peroxidase ($P < 0.01$) for antioxidative capacity, total phenolic content and protein.

The highest SCA negative value (-0.098) was shown in a cross between UG 120198 * UG 120198 for salicylic acid and the lowest value (0.001) was recorded in UG 130068 * UG 120160 and UG NASE 13 * UG 120198 for protein; NASE 13 * UG 120124 for total phenolic content and peroxidase. The highest positive SCA value (0.178) was recorded by UG 120191* NASE 13 for TPC while the lowest (0.001) for crosses UG 120133* UG 120198 and UG 120133 * NASE 13.

Table 3. Variance component estimates on parameters measured

Variance components	Nymph count	Whitefly count	Leaf Damage	Sooty Mold	Salicylic acid(g/ml)	Antioxidative capacity (g/ml)	Total phenolic content(g/ml)	Tannin (g/ml)	Flavonoid (g/ml)	Peroxidase (g/ml)	Protein (g/ml)
σ^2GCA	2719.56**	1177.41***	0.6497***	0.5314*	1.50E-06*	5.80E-03***	0.003577**	0.0014*	0.0019*	0.00774**	0.022952**
σ^2SCA	1576.9918***	389.004	0.0078	0.1336	2.2684E-6***	0.0044*	0.0035*	0.0013	0.0016*	0.0031*	0.0029*
σ^2A	8103.5406***	3323.711**	2.3401***	0.9793***	5.01E-6***	0.0230*	0.0143*	0.0053*	0.0072**	0.0297**	0.0918**
σ^2D	6307.9674*	1556.016*	0.0312	0.5344**	9.0497E-18	0.0176*	0.0138*	0.0052	0.0062*	0.0125*	0.0118*
σ^2_E	5832.0574	323.0103	0.0349	0.1422	0.0220***	0.0266**	0.0105	0.0044	0.0079*	0.0370*	0.0199*
$[(\frac{\sigma 2D}{g 2A})^{1/2}]$	0.3892	0.2340	0.0066	0.2728	2.82E+11	0.3826	0.4825	0.4905	0.4305	0.2104	0.0644
H^2	0.7119	0.9379	0.9854	0.9141	0.67447	0.7905	0.8138	0.6791	0.8096	0.6882	0.8389
h^2	0.4003	0.6388	0.9724	0.59139	0.67447	0.4471	0.4136	0.3424	0.4352	0.4840	0.7431
Bakers ratio	0.6666	0.8188	0.9944	0.8883	0.9994	0.725	0.6734	0.6725	0.7045	0.8327	0.9395

**significant at P<0.05; **significant at P<0.01; ***significant at P<0.001; σ^2A: additive genetic action variance; σ^2D: dominance genetic action variance; σ^2_e: error variance; H^2: broad sense heritability; h^2: narrow sense heritability.*

Table 4. Estimates of GCA effects of the parameters measured

Genotypes	Nymph Count	Whitefly Count	Leaf Damage	Sooty Mold	Salicylic acid (g/ml)	Antioxidative capacity(g/ml)	Total phenolic content(g/ml)	Tannin (g/ml)	Flavonoid (g/ml)	Peroxidase (g/ml)	Protein (g/ml)
UG 120133	-8.2044**	-11.461*	-0.314**	-1.3566**	-0.0112*	0.024*	0.0359*	0.007	0.0043	-0.0211**	0.0083
UG 120257	-4.7629**	8.626	-1.549***	-0.2031*	0.0112*	0.065**	-0.0007	0.7211***	0.053	0.0414**	0.2326**
UG 120293	-8.9155**	-5.229**	1.444***	-0.1743*	0.0186**	0.0319***	-0.0040*	0.0922*	0.085**	-0.0515**	0.0024
UG 120191	2.4832	5.187	-0.456**	-0.55013*	0.0100*	0.0366***	0.0045*	0.0002*	0.098***	0.0398**	0.2404**
UG 120124	-9.6204**	-27.933***	-0.627**	-0.00463	0.0246***	0.0427***	0.0086**	0.0003*	0.070**	0.1139***	0.0090
UG 120160	-13.427**	-22.914**	0.057*	0.5606**	0.009***	-0.0184*	-0.0012*	-0.0001*	-0.0010	0.0002	-0.0478
UG 120193	2.3804*	7.497	-0.003*	2.2118***	-0.0026*	-6.69E-17*	-0.0052	-3.275E05	-0.0005	-0.0016	0.0148
UG 120198	0.28609**	5.510	0.016	0.8471**	-0.0229**	-2.298E-16*	-0.0634**	-0.0009*	-0.0038*	-0.0017	-0.0663
UG 130068	10.8628*	20.769**	0.499**	0.02421**	-0.0150**	-0.0326*	-0.0010*	-5.855E04**	-0.0021	-0.0247*	-0.0948
NASE 13	13.0757**	10.354	0.0208	0.2106	0.0053	0.0145	-0.0007	0.0002	0.0062	-0.0159	-0.0085
SE	16.5304	7.542	0.040	0.1450	0.0213	0.0266	0.0105	0.0021	0.0118	0.0370	0.04497

**significant at P < 0.05; **significant at P < 0.01; ***significant at P < 0.001; g/ml: gram per mililitre; SE: standard error*

Table 5. Estimates of SCA effects of the parameters measured

Genotypes	Salicylic Acid	Antioxidative capacity	Total phenolic content	Tannin	Flavonoid	Peroxidase	Protein	Genotypes	Salicylic acid	Antioxidative capacity	Total phenolic content	Tannin	Flavonoid	Peroxidase	Protein
g/ml								g/ml							
UG 120133* UG 120133	0.04*	0.23*	0.012*	0.045*	0.059*	0.08*	0.009	UG 120293* UG 120124	-0.049*	-0.03	-0.009	-0.006	-0.004	-0.02	0.015*
UG 120133* UG 120191	0.090***	0.017	0.09	0.086***	0.051*	0.074**	0.004	UG 120293* UG 120160	0.02	0.112	0.074**	0.02	0.051*	0.046	0.037*
UG 120133 *NASE 13	-0.006	-0.004	0.001	0.027	-0.004	-0.011	0.018	NASE 13* NASE 13	0.018	0.049	-0.084**	-0.038*	-0.029	-0.024**	-0.005
UG 120133* UG 130068	0.046	-0.015	-0.004	-0.001	0.003	0.031	-0.020	NASE 13* UG 120198	0.019	-0.034	-0.016	-0.006	-0.012	-0.032*	-0.001

Genotypes	Salicylic Acid	Antioxidative capacity	Total phenolic content	Tannin	Flavonoid	Peroxidase	Protein	Genotypes	Salicylic acid	Antioxidative capacity	Total phenolic content	Tannin	Flavonoid	Peroxidase	Protein
UG 120133* UG 120198	0.54*	0.08**	0.06*	0.001	-0.005	0.04*	-0.016*	NASE 13* UG 120193	0.038	0.098	-0.018	-0.006	-0.013	-0.043**	-0.010
UG 120133* UG 120193	0.023	-0.01	0.008	0.003	0.003	0.005	0.015	NASE 13* UG 120257	-0.051*	-0.04	-0.022	-0.009	-0.018	-0.023*	-0.018*
UG 120133* UG 120257	0.04*	0.19*	0.041*	0.012	0.038*	0.012	0.007	NASE 13* UG 120124	0.001	0.003	-0.001	0.000	-0.003	-0.001	0.030
UG 120133* UG 120124	0.05*	0.124***	0.131***	0.028	0.067*	0.057*	0.058***	NASE 13* UG 120160	0.023	-0.034	-0.032*	-0.014	-0.023	0.011	-0.012*
UG 120133* UG 120160	0.022	0.005	-0.008	-0.001	-0.009	0.043*	0.010*	UG 130068* UG 120198	-0.077**	-0.044*	-0.061*	-0.051	-0.019	-0.072**	-0.04*
UG 120191* UG 120293	0.091***	0.14*	0.174**	0.045*	0.112*	0.052	-0.008	UG 130068 * UG 120193	0.027	0.019	0.021	-0.004	-0.007	-0.002	-0.013
UG 120191* NASE 13	0.084***	0.138*	0.178**	0.076***	0.110	0.062**	0.016*	UG 130068 * UG 120160	0.008	-0.063	0.0071	0.028	-0.056*	-0.077	-0.001
UG 120191* UG 130068	-0.037	-0.04*	-0.025	-0.013	-0.017	-0.03*	-0.028	UG 120198 * UG 120198	-0.095**	-0.005	-0.011	-0.004	-0.010	-0.003	-0.006
UG 120191* UG 120198	0.065**	0.074**	0.064	0.015	0.032*	0.033*	-0.008	UG 120198 * UG 120124	0.053	0.047	0.061**	0.01	0.044	0.012	0.019*
UG 120191* UG 120193	0.042	0.007	0.022	0.009	0.022*	0.003	-0.017*	UG 120198* UG120160	-0.009	-0.006	-0.006	-0.051*	-0.07	-0.055	-0.037*
UG 120191*UG 120257	0.075**	0.058*	0.036*	0.002	0.019	0.042*	0.002	UG 120193 * UG 120193	0.003	-0.004	0.000	-0.001	-0.001	0.039	0.016*
UG 120191* UG 120124	0.083**	0.069**	0.028*	0.018*	0.013	0.07*	0.008	UG 120193* UG 120257	0.019	0,0366*	-0.009	-0.014	-0.002	-0.002	-0.029*
UG 120191*UG 120160	-0.08***	-0.019	-0.031*	-0.014	-0.02	-0.012	-0.017*	UG 120193 * UG 120160	-0.063	-0.069	-0.08**	-0.03*	-0.049	-0.039*	-0.018*
UG 120293* UG 120293	0.061**	0.046*	-0.017	-0.008	-0.013	0.021	-0.008	UG 120257 * UG 120257	0.062**	0.27*	0.29*	0.013	-0.017	-0.056*	0.024*
UG 120293*UG 130068	-0.023	-0.027	-0.021	-0.01	-0.01*	-0.014	0.018*	UG 120257 * UG120124	0.073*	0.28*	0.03	0.013	0.017	0.016	0.004
UG 120293* UG 120198	0.071**	0.046*	0.065*	0.037*	0.037*	0.02	0.012*	UG 120257 * UG120160	0.024	-0.028	-0.03*	-0.014	-0.016	-0.014	-0.002
UG 120293* UG 120193	0.088***	0.079**	0.024	0.001	0.015	0.042*	0.012*	UG 120124* UG 120124	0.064**	0.22*	0.105*	0.03*	-0.004*	0.047	0.003
UG 120293* UG 120257	0.058*	0.27*	0.039*	0.015	0.026*	0.003	0.028*	UG 120124* UG 120160	0.023	0.032	0.038*	0.018	-0.013	0.017	0.014*
								UG 120124 * UG 120251	0.073**	0.007	0.23*	0.014	0.021*	0.012	0.015*
SE SCA	0.0128	0.0565	0.0476	0.0324	0.0118	0.0625	0.0512	SE SCA	0.0128	0.0565	0.0476	0.0324	0.0118	0.0625	0.0512

SE: Standard error; SCA: specific combining ability

4. DISCUSSION

4.1 Gene Action of Cassava Metabolite Traits in Response to *Bemisia tabaci*

GCA effects were found to be highly significant ($P < 0.001$) for whitefly count, leaf damage and antioxidative capacity, ($P < 0.05$) for total phenolic content, peroxidase and protein, ($P < 0.01$) for sooty mold, salicylic acid, tannin and flavonoid (Table 3) indicating a preponderance of additive than non-additive gene action among the genotypes. The results corresponded with the significant additive variance which was significant ($P < 0.001$) for leaf damage and sooty mold, ($P < 0.05$) for whitefly count, flavonoid and protein, ($P < 0.01$) for antioxidative capacity, total phenolic content and peroxidase. The significance of additive gene action for the metabolite traits implied that clonal metabolite variance may be utilized early in the selection process as part of a breeding strategy to achieve greater gain, particularly for the improvement of cassava against *Bemisia tabaci*. Since traits controlled by additive gene action are fixable, the pedigree method would be useful in selecting for these metabolites as desirable traits that are controlled by additive gene action since they are fixable [59]. Results of GCA and additive variance being highly significant for traits such as dry matter content [60], dry matter and reaction to thrips [61] mealybug [58] and cassava green mite (CGM) resistance have been reported in cassava [62] as well as various mineral components which are vital for secondary metabolism in cacao [63].

In addition, the high differences observed between additive and non-additive genetic variance for antioxidative capacity, peroxidase and protein implied that clonal testing in estimation of genetic parameters and increasing genetic gain would be efficient [58]. The results obtained were parallel with Isik et al. [64] who reported an increase in additive genetic variance of metabolites in loblolly pine. However, different studies have obtained varying results in *Arabidopsis thaliana* [65]. The discrepancy between studies could originate from scale effect as well as sampling effect. Also, different genes involved at different ages in metabolite expression may cause different responses between studies [66]. The varying stresses and plant tissues could affect the gene action and expression of the metabolites triggered as reported by Kliebenstein [67].

4.2 Heritability of Metabolite Traits Associated to *B. tabaci* in Cassava

All heritability variance estimates of metabolites were high but broad sense heritability was higher than narrow sense heritability for all traits except for salicylic acid (Table 3). Narrow and broad sense heritability value for salicylic acid was the same and indicated that additive gene action was greatly influencing SA.

High variance estimates for both broad and narrow heritability were an indication of complexity in the traits and that both additive and dominance genetic effects affected the traits. The results were in agreement with Abney et al. [68] who documented that both broad and narrow sense heritability are vital in secondary metabolite evaluation because the additive variance does not always give an adequate assessment of the influence of genetics on metabolite, therefore it is important to consider the dominance variance and the broad heritability (H^2). High heritability variance estimates indicated that the parameters had high genetic variance, a higher frequency of genes controlling metabolite traits and the potential to improve these traits with traditional breeding strategies [62]. The variations in estimate response could also be attributed to varying environmental effects. According to Courtney et al. [69], heritability differs from one population to another, and with the test environment. Heritability for nutrient composition would vary due to soil nutrients such as the macro- and micro-elements [11].

High broad sense heritability for metabolites indicated the presence of large components of heritable portion of variation, which is the portion, exploited by plant breeders while narrow sense implied the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation of the offspring. Chipeta & Bokosi [70] and Ntawuruhungu et al. [52] documented similar findings where lower narrow sense heritability were caused by lower additive genetic effects and high dominant effect in cassava for dry matter content (DMC). Heritability in narrow sense was high for most traits except salicylic acid, antioxidative capacity, total phenolic content, tannin, flavonoid, peroxidase, implying that the traits had high heritability and the parents contributed highly to those traits. Where narrow sense heritability was low, indicated non-additive (dominance or epistasis) behavior

playing an important role suggesting that the progeny performance could not be predicted only on the GCA values of the parents [59]. The results further implied that there would be of high benefit to the farmer as the traits of interest in clonally propagated cassava could easily be transferred and fixed. Farmers have the ability to select successfully visually both in the early and later breeding stages of a breeding programme, although more efficient in earlier stages than in later stages due to the larger genetic variation in early selection [71].

As regards the *Bemisia tabaci* associated traits on the plants, high broad sense heritability was shown for leaf damage (0.9854) as compared to nymph count (0.7119) (Table 3). High broad sense heritability has been reported in cassava for CGM which has a similar feeding behavior as *B. tabaci* [62].

The baker ratio's for traits associated to *B. tabaci* was highest for leaf damage (0.9984) while nymph count (0.6667). The results implied the involvement of both additive and non-additive component of heritable variance in inheritance. Since the BR/PR was less than 1, the non-additive genetic effects were predominant for these traits and are mostly referred to in cassava it does not preclude predominance of additive effects that the best progeny might be derived from crosses with genotypes having greatest GCA effects as documented by Arunga et al. [72]. In addition, Chipeta [70] reported similar results to determine the role of additive and non-additive variance in genetic control of traits related to CGM.

The average degree of dominance for all traits measured was less than 1 an indication of partial dominance. The results suggested that other factors affect the expression of the traits such as additivity and environmental factors. According to Nzuki et al. [73] value of between 0 to 1 indicated partial dominance for pests (CGM, CMB) and diseases (CBSD, CMD) in cassava. For selected traits controlled by non-additive gene action, it would, therefore, be desirable to maintain a certain degree of heterozygosity to exploit the additive gene effects and recurrent selection involving crossing desirable segregants alternated with selection in order to increase the magnitude of additive genetic variance and at the same time to maintain heterozygosity [34]. The selection of these traits are normally delayed to later stages of segregation in order to reduce the number of heterozygous genes that would then be fixed to homozygosity [59].

4.3 Genotype General Combining Ability Effects

The results showed that the genotypes varied in direction and magnitude for general combining ability (Table 4). UG 120124 had significant ($P < 0.001$) negative effects for whitefly count ($P < 0.05$) for nymph count and leaf damage. UG 120293 had negative ($P < 0.001$) for leaf damage ($P < 0.05$) for nymph and whitefly count and ($P < 0.01$) for sooty mold. The result indicated that the genotype contributed to the progenies by reducing the whitefly and leaf damage [55]. Parent UG 120160 had significant negative ($P < 0.05$) for nymph and whitefly count, but positive ($P < 0.05$) for sooty mold and ($P < 0.01$) for leaf damage. The results implied that although the genotype reduced the number of nymph and whitefly counts in the offspring the leaf damage was still significant and thus would not be considered a desirable parent for resistance to *B. tabaci*. The results agree with Chipeta et al. [58] where other genotypes in cassava were found to have significant GCA effects for green leaf mite, a pest which has a similar mode of feeding with *B. tabaci* Jeppson et al. [74] and high heritability estimates in cabbage against *B. tabaci* [75].

The genotype UG 120124 had positive significant ($P < 0.001$) for salicylic acid, antioxidative capacity and peroxidase; ($P < 0.05$) for total phenolic content and flavonoid, ($P < 0.01$) for tannin which resulted in increased metabolite content and reduced *B. tabaci* effects in the progeny and would be considered as a good combiner when breeding for cassava resistance to *B. tabaci*. Meanwhile, UG 130068 showed a negative significant ($P < 0.05$) for salicylic acid, tannin and ($P < 0.01$) antioxidative capacity, total phenolic content and peroxidase. The results showed that metabolites in certain genotypes as UG 120124 were being controlled by additive gene action and were highly heritable. The findings were in tandem with those documented by Soltis & Kliebenstein [76] where secondary metabolites such as flavonoids had high additive gene effect and high heritability in rice and Bi et al. [26] in soybean an indication of ease of transferability in the particular metabolites. The results showed that the genotype (UG 120124) concomitantly had negative *B. tabaci* effects on the plant, indicating that it would be a desirable parent in a crossing program to improve *B. tabaci* resistance. Fürstenberg-hägg et al. [13] reported that phenolics and peroxidase reduced *B. tabaci* effect in maize [20] and salicylic acid was increased to reduce *B. tabaci* effects [77].

4.4 Specific Combining Ability Effects

Magnitude and direction for SCA effects of crosses for the metabolite traits were variable as such none of the hybrids exhibited superior SCA effects for all traits measured (Table 5). UG 120191 * UG 120257 recorded the highest positive significant ($P < 0.001$) for salicylic acid. UG 120191 * UG 120124 had high positive values for salicylic acid and antioxidative capacity significant ($P < 0.05$), and ($P < 0.01$) for total phenolic content, tannin and peroxidase. As regards tannin content, UG 120133 * UG120191 were significant ($P < 0.001$) and UG 120133* UG 120133 was significant ($P < 0.01$) for peroxidase. UG 120191* UG 120124 would be considered as desirable as most metabolite traits showed significance except for tannin and protein but were still in a positive direction and of high magnitude indicating it would be considered as a good genotype in a breeding program.

One of the parents (UG 120124) in the suggested desirable cross (UG 120191 * UG 120124) was among the best general combiners (Table 4). The results indicated that the performance of the progeny was dependent on the performance of the at least one of the parents. Results obtained were consistent with Ceballos et al. [78] and Chipeta et al. [58] where it was documented that not only additive effects were important in determining of derived progenies but a large component of dominance effects could translate into significant heterosis for various traits.

5. CONCLUSIONS

There were high levels of broad sense heritability of traits showing significant GCA (>50.0%) than SCA effects for salicylic acid, antioxidative capacity, total phenolic content, tannin, flavonoid, peroxidase and protein indicated that there was high genetic variance among the traits. Although both additive and non-additive genetic effects were high for the metabolites, additive gene action played a more important role in controlling the expression of most of the traits. The traits can be improved through conventional breeding by recurrent selection. The best general combiner was UG 120124 and the best cross of UG 120191*UG 120124.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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