Journal of Agricultural Science; Vol. 15, No. 1; 2023
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Inheritance of Secondary Metabolites Associated With Cowpea Resistance to Flower Bud Thrips

Hellen Wairimu Gitonga[1,2], Samuel Kyamanywa[1] & Isaac Onziga Dramadri[1,2]

[1] Department of Agricultural Production, Makerere University, Kampala, Uganda

[2] Regional Centre of Crop Improvement, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda

Correspondence: Hellen Wairimu Gitonga, Department of Agricultural Production, Makerere University, P.O. Box 7062, Kampala, Uganda. E-mail: hellenwairimu@gmail.com

Isaac Onziga Dramadri, Department of Agricultural Production, Makerere University, P.O. Box 7062, Kampala, Uganda. E-mail: onzigaisaac@gmail.com

Received: October 3, 2022 Accepted: November 13, 2022 Online Published: December 15, 2022

doi:10.5539/jas.v15n1p1 URL: https://doi.org/10.5539/jas.v15n1p1

Abstract

Plant secondary metabolites are part of defense mechanism that form host plant resistance. Understanding the inheritance of secondary metabolites is preliquisite for conducting an effective plant breeding program. The objective of this study was to determine the mode of inheritance of secondary metabolites associated with resistance of cowpea to flower bud thrips. Five cowpea genotypes with varying level of resistance to flower thrips were crossed in full diallel method 1. The F_2 were evaluated for secondary metabolites and flower thrips damage in field and screen house. The results confirmed genotype TVU-3804 as resistant, and TVU-9820 and TVU-201 as susceptible to flower bud thrips. Flavonoids, antioxidants, proteins and reducing sugars had significant ($p < 0.001$) GCA and SCA effects indicating the importance of additive and dominance effect in controlling resistance of flower bud thrips. In addition, significant reciprocal observed for crosses such as Lori Niebe × Sanzi and Sanzi × TVU-3804 for flavonoids and antioxidants is an indication that maternal effect is key in governing resistance of cowpea to thrips. The broad sense heritability was low for secondary metabolites except flavonoid which had moderate value, an indication that delaying selection to a later generation would yield better results while breeding for resistance to flower thrips based on secondary metabolites. The results of this experiment showed that it is possible to use secondary metabolites to breed for improved cowpea resistance to flower bud thrips.

Keywords: cowpea, general combining ability, specific combining ability, inheritance, flower bud thrips, secondary metabolites

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important source of protein in developing countries where a good part of the community cannot afford animal protein. It is an important crop that could help achieve the food security goal in the developing countries especially in Uganda where the crop ranks fourth in legume, after common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L. Merr) and groundnut (*Arachis hypogaea* L.) (Fatokun et al., 2012). In Uganda, the production of Cowpea is by small holder farmers in the Northern and Eastern part of the country. Production at farmers field is low compared to research fields owing to several biotic and abiotic stresses (Oyewale & Bamaiyi, 2013). Among the major constraints to cowpea production are flower bud thrips (*Megalurothrips sjostedti* Trybom) which attack the plant at the most critical stage of flowering. The insect sucks sap of the floral structure thereby causing discoloration and abscission of the flower buds. In severe infestation, the pest can lead to 100% yield loss (Adipala et al., 2001). In order to mitigate the challenges of thrips, host plant resistance has been reported to be the long-term affordable and environment friendly option (Sobda et al., 2017).

In recent studies, plant secondary metabolites have been reported to play a significant role in host plant resistance (Singha et al., 2011). Upon attack by thrips, plants produce metabolites which deter or reduce the survival of the pest (Erb & Reymond, 2019). Consequently, metabolites such as phenolics, antioxidants, reducing sugars, carbon, flavonoids among others have been found to influence resistance of cowpea to flower bud thrips

(Agbahoungba, et al., 2018). For instance, antioxidant and phenolic compounds reduce the fecundity of flower bud thrips on cowpea hence reduction in population growth (Erb & Reymond, 2019). Secondary metabolites can therefore be targeted for improvement of cowpea resistance to flower thrips.

In order to improve resistance of cowpea to thrips, understanding how the secondary metabolites are passed from parents to the progeny is imperative. Inheritance studies for secondary metabolites were conducted in cassava under whitefly infestation where additive and dominance gene effects were reported to play major roles (Mwila et al., 2017). Elsewhere, it was reported that non-additive gene effects played a major role in inheritance of flavonoids and antioxidants in cowpea seed (Nassourou et al., 2016). Furthermore, it was found out that phenolics and the antioxidative capacity in dehulled cowpea seed was controlled by additive, dominance and non-allelic gene effects (Tchiagam et al., 2012). Previous studies on the mode of inheritance of resistance to flower bud thrips in cowpea reported additive gene effect as major contributor (Symphorien et al., 2018). However, information on the inheritance of secondary metabolites associated with resistance of cowpea to flower bud thrips is limited. Determining the mode of inheritance of secondary metabolites is important for making effective selection decisions. Besides, understanding the combining ability and gene action is essential in selecting parents for a breeding programme. The objective of this study was to determine the mode of inheritance of plant secondary metabolites associated with resistance of cowpea to flower bud thrips. The idea of combining ability analysis gives accurate estimates of the nature and magnitude of gene actions involved in the inheritance of quantitative characters, which can facilitate the selection of parents with good general combining ability effects and crosses with good specific combining ability effects.

2. Materials and Methods

2.1 Study Site and Genetic Population

The experiment was conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) in Uganda. MUARIK lie at 0°28′N, 32°37′E and 1200 m above sea level. It receives an average annual rainfall of 1150 mm, average annual temperature of 21.50 °C and the soils are sandy-clay-loam (Sserumaga et al., 2015). Five genotypes selected from previous cowpea screening nursery based on the level of resistance to flower bud thrips were used in this experiment. Genotype TVU-3804 was resistant, Sanzi and Lori Niebe were moderately resistant while TVU-201 and TVU-9820 were susceptible to flower thrips (in press). The 5 genotypes were crossed in full diallel method 1 to produce 25 crosses. Crossing was done in screen house at MUARIK. To ensure synchrony in flowering, planting of the parents was done in 3 sets, with 2 weeks interval. At flowering, emasculation was done in the evening and pollinated the following day in the morning (Tumwegamire et al., 1998). The F_1 seeds were harvested, threshed separately and advanced to F_2 population.

2.2 Evaluation of F_2 Population

The F_2 were laid down in a randomized complete block design in 3 replications in the field and in screen house. In the field, two seeds were planted per hole in plots measuring 3 × 3 m with spacing of 0.75 m and an intra-rows space of 0.25 m. To ensure sufficient population of thrips, a susceptible genotype WC36 was planted around the experiment area as well as between blocks, 2 weeks before establishment of the experiment. In the screen house, three seeds were planted in each pot with four pots representing a plot. The recommended field management practices for cowpea were applied.

2.3 Data Collection

Data were recorded on thrips damage from ten plants selected randomly within the plot, on a scale of 1-9, at 50, 65 and 80 days after planting (DAP). Scores were defined as: 1-3 = resistant, 4-6 = moderately resistant and 7-9 = susceptible (Jackai & Singh, 1988). Rating was based on a combination of varying intensities of thrips induced browning of the stipules and flower buds, non-elongation of peduncles, and flower bud abscission as shown in Table 1.

Table 1. Scale for rating flower bud thrips damage in Cowpea

Rating	Appearance
1	No browning/drying of stipules, leaves or flower buds; no bud abscission
3	Initiation of browning of stipules, leaves or flower buds; no bud abscission
5	Distinct browning/drying of stipules and leaves or flower buds; some bud abscission
7	Serious bud abscission accompanied by browning/drying of stipules and buds; non-elongation of peduncles
9	Very severe bud abscission, heavy browning, drying of stipules and buds; distinct non-elongation of (most or all) peduncles

Source: Jackai and Singh (1988).

Fifty days after planting, the terminal leaves, racemes, floral buds and flowers of each plot were collected for lab test. Determination of total flavonoids, total antioxidants, total phenolics, percent lignin, percent proteins, total reducing sugars and total tannins contents was done at National Crops Resources Research Institute (NaCRRI)'s biochemistry laboratory, Namulonge in Uganda.

2.3.1 Determination of Total Flavonoid Content

The total flavonoids were determined by weighing 1 g of fresh sample using a weigh balance. Using motor and pestle, the sample was crashed and 0.6 ml of 80% methanol added. The mixture was vortexed and orbital shaken for 30 min. This was followed by centrifugation at 6000 rpm at 4 ^{o}C for 10 min. The flavonoids were then estimated by Aluminium chloride ($AlCl_3$) method (Michalska et al., 2007). 1 ml of the methanolic supernatant was transferred into an empty 50 ml falcon tube. 4 ml of distilled water was added to the extract followed by 0.3 ml of 5% sodium nitrate solution. The mixture was vortexed and incubated in dark for 5 min, after which 3 ml of 10% Aluminium chloride was added. Incubation in the dark was repeated for 6 min. 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 ml using distilled water. The solution was vortexed and incubated in the dark for 10 min. The absorbance was recorded at 510 nm using UV-visible spectrophotometer. The total flavonoid content was calculated as Quercetin acid equivalent (mg/g) from the calibration curve $Y = 0.002x + 6E\text{-}05$, $R^2 = 0.9781$.

2.3.2 Determination of Total Phenolic Content

The total phenolics were determined by weighing 1 g of fresh sample using weigh balance. Using motor and pestle the sample was crashed and 0.6 ml of 80% methanol added. The mixture was vortexed and orbital shaken for 30 min. This was followed by centrifugation at 6000 rpm at 4 ^{o}C for 10 min. 1 ml of clear supernatant was transferred into an empty 50 ml falcon tube. To this, 5 ml of distilled water was added followed by 0.5 ml of Folin Ciocalten's reagent. 1.5 ml of 20% sodium carbonate was added to the sample, increased to 10 ml with distilled water and vortexed. The mixture was incubated in water bath at 40^{o}C for 30 min. Absorbance readings were done at 750 nm using spectrophotometer. The total phenolic content was calculated as gallic acid equivalent (mg/g) from the calibration curve $Y = 0.764x - 0.0152$, $R^2 = 0.9952$.

2.3.3 Determination of Protein

A 0.5 g of fresh sample was weighed, and extraction done using 5 ml ionic stress buffer (50 mM Tris. HCl and 200 mM NaCl, PH 8.5). The mixture was vortexed and orbital shaken for 30 min for maximum protein extraction. The mixture was centrifuged at 6000 rpm at 4 ^{o}C for 10 min. 1 ml of the supernatant was transferred to an empty 50 ml falcon tube. 3 ml of biuret solution was added then vortexed and absorbance readings done at 540 nm. Calibration of percentage protein was done using the standard equation $Y = 5.2858x - 0.1041$, $R^2 = 0.9942$.

2.3.4 Determination of Total Reducing Sugar

The total reducing sugars were determined by weighing 1 g of fresh sample using weigh balance. Using motor and pestle the sample was crashed and 0.6 ml of 80% methanol added. The mixture was vortexed and orbital shaken for 30 min. This was followed by centrifugation at 6000 rpm at 4 ^{o}C for 10 min. 0.5 ml of the methanolic extract was transferred to an empty 50 ml falcon tube and diluted with 1 ml distilled water. This was followed by addition of 1 ml concentrated sulphuric acid (H_2SO_4) to dehydrate the solution. 0.5 ml of 5% phenol was added for formation of the colored complex compound (mild gold color complex) of the reducing sugars. Quantification of reducing sugar was done at 490 nm using spectrophotometer. Calibration of the reducing sugar (%) was done using the standard equation $Y = 5.785x - 0.0015$, $R^2 = 0.9987$.

2.3.5 Determination of Total Tannin

The total tannin was determined by weighing 1 g of fresh sample using weigh balance. Using motor and pestle the sample was crashed and 0.6 ml of 80% methanol added. The mixture was vortexed and orbital shaken for 30 min. This was followed by centrifugation at 6000 rpm at 4 ^{o}C for 10 min. The total tannins were determined using the modification of Harbourne (1998) method. 1 ml of the extract was transferred into 50 ml empty falcon tube. 0.5 ml of 5% ascorbic acid solution was added to dissolve the precipitants. The mixture was vortexed and orbital shaken for 30 min for efficient mixing. 0.5 ml petroleum ether containing 1% acetic acid was added followed by 0.3 ml distilled water, vortexed and centrifuged at 6000 rpm at 4 ^{o}C for 10 min. The organic supernatant was transferred into 50 ml empty falcon tube. 2.4 ml of 5% Hcl-butanol was added and vortexed, and 0.5 ml of Folin Ciocaltious reagent added. 2.5 ml of 20% sodium carbonate solution was added and vortexed. The resultant mixture was incubated for 30 min at 80 ^{o}C and cooled in a cold water bath at 25 ^{o}C. Spectrophotometric absorbance reading was done at 550 nm and total tannins calibrated using the standard curve $Y = 0.0279x + 0.0001$, $R^2 = 0.9844$.

2.3.6 Determination of Total Antioxidant

The total antioxidants were determined by weighing 1 g of fresh sample using weigh balance. Using motor and pestle, the sample was crashed and 0.6 ml of 80% methanol added. The mixture was vortexed and orbital shaken for 30 min. This was followed by centrifugation at 6000 rpm at 4^{o}C for 10 min. 0.5 ml of the supernatant was picked and transferred into an empty 50 ml falcon tube. 2.5 ml of 0.2 M sodium sulphate buffer was added, followed by additional 2.5 ml potassium phosphate fericyanide. The mixture was vortexed and incubated in water bath at 50^{o}C for 20 min. After cooling, 2.5 ml tricholoroacetic acid was added and mixed well by shaking. The mixture was centrifuged at 6000 rpm at 4^{o}C for 10 min. From the clear supernatant, 5 ml was transferred into clean empty 50 ml falcon tube and 5 ml of distilled water added. 1 ml ferric chloride was added and the solution mixed well by shaking. The absorbance readings were done at 700 nm using spectrophotometer and calibration done using the standard equation $Y = 0.0022x + 3E\text{-}05$, $R^2 = 0.9642$.

2.3.7 Determination of Total Lignin Content

Acid insoluble lignin also known as Klason lignin was determined by Klason method (Moreira-Vilar et al., 2014) through subjecting lignin to an acid hydrolysis process. The acidic hydrolysis was carried out by adding 3.75 ml of sulphuric acid (72%) to 0.375 g powder of lignin in digestion tubes (50 ml falcon tubes) and the uniform mixture generated by stirring. The mixture was left for 1 hour at 30°C in the water bath and the resultant mixture diluted with 36.25 ml of distilled water and incubated at 100°C for 3 hours in a water bath. The mixture was cooled in cold water bath for 15 min and then filtered under vacuum. The resulting solid, which was the insoluble lignin was calculated as follows:

$$\text{Acid Insoluble Lignin} = \left[\frac{B - A}{C}\right] \times 100 \quad (1)$$

Where, A is the weight of empty 50 ml falcon tube (g), B is the weight of the 50 ml falcon tube plus dried lignin residue (g) and C initial weight of the lignin sample (g). Acid soluble lignin (ASL) was determined spectrophotometrically (UV absorption at 280 nm). The filtrate was diluted with 1M H_2SO_4 until the absorbance reached between 0.1 to 0.8 cm^{-1}. The acid-soluble lignin was calculated as follows:

$$\text{Acid Soluble Lignin(ASL)} = \left[\frac{A \times B \times C}{D \times E}\right] \times 100 \quad (2)$$

Where, A is the absorbance at 280 nm, B is dilution factor, C is filtrate volume (L), D is extinction coefficient of lignin (110 g L^{-1} cm^{-1}) and E is the initial lignin weight (g). Percentage total lignin content was thereafter determined by the sum of the insoluble and soluble lignin and expressed as percentage of total weight of powder analysed.

2.4 Data Analysis

Secondary metabolites and flower bud thrips severity data were subjected to analysis of variance using the linear mixed model using R version 4.2.1 computer software. Before analyzing, the assumptions of analysis of variance were tested. The linear model for RCBD design was as follows

$$y_{ijk} = \mu + g_i + e_j + b_k + ge_{ij} + \varepsilon_{ijk} \quad (3)$$

Where, y_{ijk} is the observed trait value for the i^{th} genotype from j^{th} environment in the k^{th} block; μ is the overall mean effect, g_i is the i^{th} genotype effect, e_j is the j^{th} environment effect, b_k is the effect of k^{th} block, ge_{ij} is the interaction effect of i^{th} genotype and j^{th} environment, ε_{ijk} is the experimental error.

Mean separation for the traits were done following Fisher protected least significant difference test at $\alpha = 0.5$.

To estimate inheritance, the coefficient of genetic determination (CGD) for secondary metabolites was calculated. CGD is a fixed parent equivalent of heritability, because heritability is strictly applied to a random population resulting from random parents. The data was analyzed in Analysis of genetic designs with R for Windows (AGD-R) version 5.0 (Rodríguez et al., 2015), using model 1 method 1 of Griffing to determine the general and specific combining ability (GCA and SCA) (Griffing, 1956) which provide an unbiased approximations of population parameters. The statistical model for the analysis was as follows,

$$y_{ijkl} = \mu + g_i + g_j + s_{ij} + r_{ij} + l_k + b_l + lg_m + ls_{ijk} + lr_{ijk} + e_{ijkl} \quad (4)$$

Where, μ is the overall mean, g_i is the GCA effect of the i^{th} parent, g_j is the GCA effect of the j^{th} parent, s_{ij} is the SCA effect of the ij^{th} genotype, r_{ij} is the reciprocal effect of the ij^{th} genotype, l_k is the effect of k^{th} environment, b_l is the effect of l^{th} block, lg_m is the effect of m^{th} interaction between environment and genotype, ls_{ijk} is the effect of the interaction between k^{th} environment and SCA of the ij^{th} genotype, lr_{ijk} is the effect of the interaction between k^{th} environment and reciprocal of the ij^{th} genotype, and e_{ijkl} is the environmental effect of the $ijkl^{th}$ observation.

Baker's ration was estimated as a ratio of GCA and SCA (Baker, 1978) as follows,

$$y = \frac{2\sigma_g^2}{2\sigma_g^2 + \sigma_s^2} \quad (5)$$

Where, $2\sigma_g^2$ is the GCA variance and σ_s^2 is SCA variance

The variance ratios were then used to calculate the narrow-sense coefficient of genetic determination (NSCGD) and the broad-sense coefficient of genetic determination (BSCGD) as follows,

$$NSCGD = \frac{2\sigma_g^2}{2\sigma_g^2 + \sigma_s^2 + \sigma_e^2} \quad (6)$$

$$BSCGD = \frac{2\sigma_g^2 + \sigma_s^2}{2\sigma_g^2 + \sigma_s^2 + \sigma_e^2} \quad (7)$$

3. Results

3.1 Performance of Cowpea F_2 Genotypes for Flower Thrips Damage and Secondary Metabolites

There was a significant ($p < 0.001$) variation among the genotypes for flower thrips damage at 50, 65, and 80 DAP. On the other hand, significant ($p < 0.001$) variation was observed for flavonoids, proteins, ($p < 0.01$) tannins and ($p < 0.05$) reducing sugars contents (Table 2). Conversely, genotypes were non-significant for antioxidants, phenolics and lignin contents. Environments significantly ($p < 0.001$) influenced thrips damage at 80 DAP, ($p < 0.01$) 50 DAP, but non-significant for damage at 65 DAP. Equally, environments significantly ($p < 0.001$) influenced flavonoids, ($p < 0.01$) phenolics but non-significant for antioxidants, proteins, lignin, tannins and reducing sugars contents. The interactions between genotypes and environments significantly ($p < 0.001$) influenced flower bud thrips damage at 80 DAP and ($p < 0.05$) 50 DAP, but non-significant for all the metabolites.

The performance of crosses compared to best parent revealed variations where some progenies performed better than the best parent while others had intermediate performance (Table 3). Crosses involving resistant genotypes generally had lower scores while those involving susceptible parents had higher thrips damage scores. However, for secondary metabolites, majority of the crosses had intermediate levels between the two parents. Genotype TVU-3804 had the lowest scores for thrips damage of 3 while TVU-9820 had the highest scores of 9. Genotype Sanzi recorded highest flavonoids and reducing sugars, TVU-201 had the highest content of antioxidants and proteins, TVU-3804 had highest content of lignin and tannins while Lori Niebe recorded the highest phenolics content.

3.2 Combining Ability and Inheritance of Traits

The results of Griffings diallel indicated a significant ($p < 0.001$) environment effect on thrips damage at 80 DAP and ($p < 0.01$) 50 DAP, and non-significant at 65 DAP (Table 4). In the same way, there was a significant ($p < 0.001$) environment effect on flavonoids and ($p < 0.01$) phenolics content, while non-significant for antioxidants,

lignin, proteins, sugars and tannins. GCA mean squares were significant ($p < 0.001$) in flower bud thrips damage at 50, 65 and 80 DAP. For secondary metabolites, GCA mean squares were significant ($p < 0.001$) in flavonoids, antioxidants, proteins, ($p < 0.01$) tannins and ($p < 0.05$) reducing sugars, and non-significant for lignin and phenolics. The SCA mean square was significant ($p < 0.001$) in thrips damage at 50, 65 and 80 DAP. Then again, for secondary metabolites, SCA mean square was significant ($p < 0.001$) in flavonoids, antioxidants, proteins, ($p < 0.01$) reducing sugars and ($p < 0.05$) lignin, and non-significant in phenolics and tannins. The reciprocal mean squares were significant ($p < 0.001$) in thrips damage at 50, 80 DAP and ($p < 0.05$) 65 DAP. For metabolites, the reciprocal mean squares were significant ($p < 0.001$) in flavonoids, antioxidants, ($p < 0.05$) proteins and tannins, and non-significant for lignin phenolics and reducing sugars. GCA by environment interaction means square was significant ($p < 0.01$) for thrips damage at 50 DAP and non-significant for damage at 65, 80 DAP. On the other hand, the GCA by environment interaction mean square was significant ($p < 0.001$) for antioxidants, ($p < 0.05$) flavonoids, reducing sugars, and non-significant for lignin, phenolics, proteins and tannins. SCA and environment interaction significantly ($p < 0.05$) influenced thrips damage at 50, 80 DAP and was non-significant for thrips damage at 65 DAP. In addition, the interaction significantly ($p < 0.001$) influenced antioxidants but was non-significant for flavonoids, lignin, phenolics, proteins, reducing sugars and tannins content. The reciprocal and environment interacted significantly ($p < 0.001$) in thrips damage at 80 DAP and ($p < 0.05$) at 50 and 65 DAP. For secondary metabolites, the interaction was significant ($p < 0.001$) in antioxidants, and non-significant in flavonoids, lignin, phenolics, proteins, reducing sugars and tannins. The GCA/SCA ration varied from 0.95 to 1.98 for flower thrips damage, and 0.03 to 1.98 for secondary metabolites (Table 4). The baker's ratio ranged between 0.65 and 0.8 for flower thrips damage and 0.05 and 0.8 for secondary metabolites. The NSCGD ranged from 0.31 to 0.56 for thrips damage and 0.01 to 0.42 for secondary metabolites. The BSCGD varied from 0.48 to 0.79 for thrips damage and 0.14 to 0.53 for secondary metabolites contents.

Table 5 represents the GCA, SCA and reciprocals for the flower bud thrips damage and secondary metabolites contents. Genotypes Lori Niebe, Sanzi and TVU-3804 had significant ($p < 0.05$) negative GCA effect while TVU-201 and TVU-9820 had significant ($p < 0.01$) positive GCA effect for flower bud thrips damage. In all the genotypes, lignin, phenolics and reducing sugars had non-significant GCA effect. Flavonoids were significantly ($p < 0.01$) different in all parents except TVU-9820. Analysis of combining ability showed that all the crosses had significant ($p < 0.05$) SCA effect in thrips damage at 80 DAP except Sanzi × TVU-9820 and TVU-201 × TVU-9820. There was non-significant SCA effect on secondary metabolites in most of the crosses except phenolics which was significant ($p < 0.05$) in Lori Niebe × Sanzi, Sanzi × TVU-201, Lori Niebe × TVU-3804, Sanzi × TVU-3804, Lori Niebe × TVU-9820, Sanzi × TVU-9820 and TVU-201 × TVU-9820. At 80 DAP, thrips damage was significant ($p > 0.05$) for reciprocal cross between Lori Niebe × TVU-201, Sanzi × TVU-201, Sanzi × TVU-3804, Sanzi × TVU-9820 and non-significant in Lori Niebe × Sanzi, Lori Niebe × TVU-9820 and TVU-3804 × TVU-9820. Secondary metabolites had non-significant SCA effect in most of the reciprocal crosses except some like antioxidants which was significant in Lori Niebe × Sanzi, Lori Niebe × TVU-201, Sanzi × TVU-201, Lori Niebe × TVU-3804, Sanzi × TVU-3804, flavonoids which was significant in Lori Niebe × Sanzi, Lori Niebe × TVU-201, Sanzi × TVU-201 and Sanzi × TVU-3804.

Table 2. Analysis of variance for flower thrips damage and secondary metabolites in F_2 generation across environments

SoV	df	50DAP	65DAP	80DAP	Flavonoid (mg/g)	Antioxidant (%)	Phenolic (mg/g)	Protein (%)	Lignin (%)	Tanin (mg/g)	Sugar (%)
Rep	2	2.26	0.40	0.10	8124.00	98.95	0.26	23.24	19.90	7.61	18.05
Rep:Block	12	0.35	0.50	0.49	738.25	91.45	0.57	1.74	42.01	3.07	16.76
Geno	24	5.54***	4.41***	10.71***	12498.54***	400.42^{ns}	1.19^{ns}	27.09***	185.10^{ns}	8.95**	48.95*
Env	1	4.65**	1.91^{ns}	10.76***	99588.00***	130.80^{ns}	9.43**	0.04^{ns}	8.40^{ns}	10.60^{ns}	22.73^{ns}
Geno × Env	24	1.14*	1.09^{ns}	1.56***	2136.79^{ns}	423.59^{ns}	1.05^{ns}	2.50^{ns}	111.89^{ns}	3.57^{ns}	22.45^{ns}
Residuals	86	0.64	0.72	0.44	1493.58	69.72	0.91	5.25	138.70	3.63	25.53
CV (%)		25.52	17.84	9.91	16.14	10.87	29.50	33.51	22.31	22.47	31.17

Note. *, **, *** = significant at 0.05, 0.01, 0.001 probability levels respectively, ns = not significant, SoV = Sources of variation, Rep = replication, Env = environments, Geno = Genotypes, CV = Coefficient of variation, df = degrees of freedom, DAP = Days after planting.

Table 3. Mean performance of the parents and F_2 population to flower bud thrips and secondary metabolites

	50DAP	65DAP	80DAP	Flavonoid (mg/g)	Antioxidant (%)	Phenolic (mg/g)	Protein (%)	Lignin (%)	Tanin (mg/g)	Sugar (%)
Parents										
Lori Niebe	3	5	6	176.47	82.62	1.37	5.43	58.36	1.70	15.48
Sanzi	3	5	6	303.22	79.99	1.03	6.27	48.52	3.35	22.66
TVU-201	5	6	8	279.47	86.48	0.92	9.76	44.43	3.41	16.19
TVU-3804	1	3	3	193.30	80.06	1.11	6.83	59.19	3.69	16.15
TVU-9820	5	6	9	208.72	79.02	0.57	5.91	51.82	2.65	10.29
Crosses										
Lori Niebe × Sanzi	3	5	6	174.64	80.79	1.20	4.93	55.03	1.63	14.31
Lori Niebe × TVU-201	4	5	8	289.72	66.46	2.15	8.89	56.25	5.93	18.43
Lori Niebe × TVU-3804	3	5	6	192.89	65.59	1.72	4.90	46.79	1.87	12.90
Lori Niebe × TVU-9820	3	4	6	187.00	84.58	1.46	5.97	49.74	2.32	13.86
Sanzi × Lori Niebe	3	5	6	284.30	67.72	0.98	4.52	50.92	2.85	17.69
Sanzi × TVU-201	4	5	7	299.47	79.48	1.11	6.00	58.07	4.37	18.91
Sanzi × TVU-3804	2	4	5	239.22	65.64	0.88	3.35	38.58	2.55	13.44
Sanzi × TVU-9820	4	5	8	280.89	77.82	1.61	7.98	58.13	2.54	17.72
TVU-201 × Lori Niebe	3	4	6	182.42	86.02	0.94	6.49	47.71	2.25	14.91
TVU-201 × Sanzi	4	6	9	264.72	64.66	1.07	6.79	55.61	1.72	13.58
TVU-201 × TVU-3804	3	5	8	251.97	83.04	1.00	6.91	57.64	3.09	15.45
TVU-201 × TVU-9820	4	6	8	289.47	74.12	1.68	11.26	50.93	4.91	21.19
TVU-3804 × Lori Niebe	3	4	6	189.39	69.61	1.59	5.19	43.79	1.84	11.56
TVU-3804 × Sanzi	1	3	3	185.30	74.32	1.76	5.49	54.03	2.69	13.99
TVU-3804 × TVU-201	4	5	7	265.97	54.40	1.99	7.92	55.48	2.14	18.87
TVU-3804 × TVU-9820	3	6	7	222.22	80.13	1.19	6.85	52.85	2.71	15.31
TVU-9820 × Lori Niebe	3	5	6	188.67	82.84	2.13	6.37	51.40	3.65	19.65
TVU-9820 × Sanzi	3	5	6	281.47	83.21	1.32	6.90	55.17	3.74	16.02
TVU-9820 × TVU-201	4	6	8	302.47	91.81	2.45	13.76	60.43	5.41	19.19
TVU-9820 × TVU-3804	3	5	7	253.55	79.92	1.11	6.30	58.93	3.23	17.52
LSD	0.26	0.28	0.22	12.55	2.71	0.31	0.74	3.82	0.62	1.64

Table 4. Mean squares for flower thrips damage and metabolites in F_2 population across environments

SoV	df	50DAP	65DAP	80DAP	Flavonoid (mg/g)	Antioxidant (%)	Lignin (%)	Phenolic (mg/g)	Protein (%)	Sugar (%)	Tanin (mg/g)
Env	1	4.64**	1.91^{ns}	10.76***	99587.65***	130.76^{ns}	8.43^{ns}	9.43**	0.04^{ns}	22.73^{ns}	10.59^{ns}
Rep(Env)	4	3.33***	0.82^{ns}	0.28^{ns}	4256.17***	79.61***	41.43^{ns}	0.15^{ns}	12.74*	21.73^{ns}	3.96^{ns}
Cross	24	5.80***	4.35***	11.34***	13297.72***	459.81^{ns}	184.93^{ns}	1.31^{ns}	29.34***	53.15*	7.99*
GCA	4	24.67***	15.03***	43.12***	47082.31***	517.78***	46.19^{ns}	0.73^{ns}	106.27***	56.12*	12.49**
SCA	10	2.71***	3.25***	7.28***	4376.24**	287.56***	279.22*	1.53^{ns}	21.85***	71.73**	6.19^{ns}
Recip	10	1.35***	1.17*	2.69***	8705.36***	608.87***	146.12^{ns}	1.32^{ns}	6.04*	33.38^{ns}	7.99*
Env × Cross	24	1.12**	1.12^{ns}	1.73***	2413.79^{ns}	426.99***	111.00^{ns}	0.97^{ns}	3.19^{ns}	25.83^{ns}	3.57^{ns}
Env × GCA	4	1.87**	0.46^{ns}	0.17^{ns}	4367.49*	549.16***	125.23^{ns}	1.19^{ns}	2.77^{ns}	64.04*	3.65^{ns}
Env × SCA	10	0.82*	1.02^{ns}	0.86*	2042.69^{ns}	384.99***	141.77^{ns}	1.09^{ns}	3.59^{ns}	18.93^{ns}	3.26^{ns}
Env × Recip	10	1.11*	1.48*	3.22***	2003.39^{ns}	420.12***	74.53^{ns}	0.76^{ns}	2.97^{ns}	17.45^{ns}	3.85^{ns}
Residual	72	0.52	0.67	0.44	1422.19	64.15	128.19	0.89	4.88	24.44	3.63
δ2g (GCA)		0.33	0.20	0.57	627.76	6.90	0.62	0.01	1.42	0.75	0.17
δ2s (SCA)		0.17	0.21	0.47	317.36	25.18	22.34	0.12	1.64	6.13	0.48
δ2g (GCA)/δ2s(SCA)		1.88	0.95	1.22	1.98	0.27	0.03	0.08	0.86	0.12	0.35
δ2r(Recip)		0.11	0.08	0.19	723.31	50.71	11.95	0.11	0.49	2.78	0.65
Baker ratio		0.79	0.65	0.71	0.80	0.35	0.05	0.14	0.63	0.20	0.41
NSCGD = h^2		0.49	0.31	0.56	0.42	0.13	0.01	0.02	0.30	0.05	0.08
BSCGD = H		0.61	0.48	0.79	0.53	0.38	0.16	0.14	0.48	0.24	0.18

Note. *, **, *** = significant at 0.05, 0.01, 0.001 probability levels respectively, ns = not significant, SoV = Sources of variation, DAP = Days after panting, Env = Environment, GCA = general combining ability, SCA = specific combining ability, Recip = reciprocal, NSCGD and BSCGD = Narrow and Broad sense coefficient of genetic determination.

Table 5. GCA, SCA and reciprocal effects for flower thrips damage and metabolites in parents and F_2 generations across environments

	50DAP	65DAP	80DAP	Flavonoid (mg/g)	Antioxidant (%)	Lignin (%)	Phenolic (mg/g)	Protein (%)	Sugar (%)	Tanin (mg/g)
Parents					**GCA effect**					
Lori Niebe	-0.27**	-0.13^{ns}	-0.29**	-35.28***	0.07^{ns}	-0.96^{ns}	0.12^{ns}	-1.03**	-0.78^{ns}	-0.47*
Sanzi	-0.23*	-0.24*	-0.45**	22.17**	-1.45^{ns}	-0.53^{ns}	-0.17^{ns}	-0.99**	0.89^{ns}	-0.17^{ns}
TVU-201	0.78***	0.59**	1.19***	31.04**	0.48^{ns}	0.31^{ns}	0.02^{ns}	1.91***	1.08^{ns}	0.61*
TVU-3804	-0.80***	-0.64**	-0.96***	-20.77**	-3.54*	-0.15^{ns}	-0.03^{ns}	-0.78*	-1.08^{ns}	-0.30^{ns}
TVU-9820	0.52***	0.41**	0.50**	2.84^{ns}	4.43**	1.33^{ns}	0.04^{ns}	0.88*	-0.1 ^{ns}	0.33^{ns}
Crosses					**SCA effect**					
Lori Niebe × Sanzi	0.32^{ns}	0.31^{ns}	0.49**	3.11^{ns}	-1.18^{ns}	1.67^{ns}	-0.23*	-0.09^{ns}	-0.31^{ns}	-0.16^{ns}
Lori Niebe × TVU-201	-0.38*	-0.39*	-0.73***	0.83^{ns}	-1.12^{ns}	-0.16^{ns}	0.008^{ns}	-0.04^{ns}	0.16^{ns}	0.90*
Sanzi × TVU-201	0.43*	0.12^{ns}	0.44*	-10.59^{ns}	-3.78*	4.28*	-0.16*	-1.37*	-1.93^{ns}	-0.45^{ns}
Lori Niebe × TVU-3804	0.61**	0.56**	0.91***	7.71^{ns}	-5.75**	-6.40*	0.19*	0.02^{ns}	-2.12^{ns}	-0.42^{ns}
Sanzi × TVU-3804	-0.68**	-0.69**	-1.09***	-28.62**	-1.85^{ns}	-5.81*	0.15*	-0.65^{ns}	-2.31*	0.04^{ns}
TVU-201 × TVU-3804	0.38*	0.16^{ns}	0.80***	9.22^{ns}	-5.04*	3.61^{ns}	0.09^{ns}	-0.56^{ns}	0.95^{ns}	-0.75^{ns}
Lori Niebe × TVU-9820	-0.63**	-0.65**	-0.88***	-19.20*	2.39^{ns}	-2.59^{ns}	0.27*	-0.52^{ns}	1.44^{ns}	0.08^{ns}
Sanzi × TVU-9820	-0.001^{ns}	-0.06^{ns}	0.23^{ns}	16.69*	0.7^{ns}	3.06^{ns}	0.23*	0.71^{ns}	-0.12^{ns}	-0.07^{ns}
TVU-201 × TVU-9820	-0.21^{ns}	-0.08^{ns}	0.09^{ns}	22.61*	1.24^{ns}	1.25^{ns}	0.61*	2.87***	3.01*	1.17*
TVU-3804 × TVU-9820	0.21^{ns}	0.85***	0.70***	16.33*	2.32^{ns}	1.91^{ns}	-0.23^{ns}	-0.37^{ns}	1.39^{ns}	-0.11
					Reciprocal effect					
Lori Niebe × Sanzi	0.28^{ns}	0.02^{ns}	0.14^{ns}	54.83***	-6.53**	-2.05^{ns}	-0.11^{ns}	-0.21^{ns}	1.69^{ns}	0.61^{ns}
Lori Niebe × TVU-201	-0.68**	-0.66**	-0.69**	-53.65***	9.78***	-4.27^{ns}	-0.61**	-1.20*	-1.76^{ns}	-1.84**
Sanzi × TVU-201	0.37*	0.27^{ns}	0.64**	-17.38*	-7.41**	-1.23^{ns}	-0.02^{ns}	0.39^{ns}	-2.66*	-1.32**
Lori Niebe × TVU-3804	0	0	0	-1.75^{ns}	2.01ns	-1.50ns	-0.06ns	0.15ns	-0.67ns	-0.01^{ns}
Sanzi × TVU-3804	-0.42*	-0.57**	-0.74**	-26.96**	4.34*	7.73**	0.43*	1.07*	0.28^{ns}	0.07^{ns}
TVU-201 × TVU-3804	0.35*	-0.04^{ns}	-0.48**	7.00^{ns}	-14.32***	-1.08^{ns}	0.49*	0.50^{ns}	1.71^{ns}	-0.48^{ns}
Lori Niebe × TVU-9820	0.08^{ns}	0.23^{ns}	-0.05^{ns}	0.83^{ns}	-0.87^{ns}	0.83^{ns}	0.33^{ns}	0.19^{ns}	2.896*	0.66^{ns}
Sanzi × TVU-9820	-0.05^{ns}	-0.14^{ns}	-0.75**	0.29^{ns}	2.69^{ns}	-1.48^{ns}	-0.15^{ns}	-0.54^{ns}	-0.85^{ns}	0.60^{ns}
TVU-3804 × TVU-9820	0.25^{ns}	0	0	6.50^{ns}	8.85**	4.75^{ns}	0.38^{ns}	1.25*	-1.00^{ns}	0.25^{ns}
TVU-3804 × TVU-9820	0.28^{ns}	-0.26^{ns}	0.01^{ns}	15.67^{ns}	-0.10^{ns}	3.04^{ns}	-0.04^{ns}	-0.28^{ns}	1.10^{ns}	0.26^{ns}

Note. *, **, *** = significant at 0.05, 0.01, 0.001 probability levels respectively, ns = not significant, DAP = Days after planting, GCA = General combining ability, SCA = Specific combining ability.

4. Discussion

The significant difference observed for flower bud thrips damage among genotypes is an indication that the genotypes have different genetic background which could be utilized for improving cowpea for flower bud thrips resistance. Similarly, significant difference was observed among genotypes for flower thrips in previous studies in different cowpea populations (Toyinbo et al., 2021; Agbahoungba et al., 2017; Oladejo et al., 2017; Abudulai et al., 2006). The low flower thrips damage scores observed in genotype TVU-3804, Lori Niebe and Sanzi confirms that these genotypes have genes conferring resistance to thrips. Genotype Sanzi has consistently showed low thrips damage scores in several studies in different countries (Symphorien et al., 2018; Dormatey et al., 2015; Omo-ikerodah et al., 2009), an indication that the genes responsible for thrips resistance is stable across environments. The observed difference in flavonoids, proteins, tannins and reducing sugars contents among the genotypes indicate that the genetic makeup of these genotypes is diverse and could be utilized in breeding for resistance of cowpea to flower bud thrips. Genetic diversity is prerequisite for improving crops for specific traits (Shewry & Lucas, 1997). The genotypes were however not different in the production of antioxidants, phenolics and lignin. This indicates that even though the genotypes are diverse, and apart from flower bud thrips, other factors such as temperature, rainfall or soil could be contributing to the production of these metabolites. The significant variation in flavonoids and phenolics across environments shows that these metabolites are highly influenced by rainfall, temperature and humidity. This variation is also seen in flower thrips damage at 50 and 80 DAP.

Across environments, there was significant GCA and SCA for flower bud thrips damage, an indication that in the crosses, additive and non-additive gene effects were present in control of resistance of thrips. Correspondingly, there was significant GCA and SCA for some of the secondary metabolites an indication that, additive and non-additive gene effects were important in production of secondary metabolites. These observations were confirmed by the GCA/SCA and bakers ration (Table 3) which clearly indicated the importance of both additive and non-additive effects in inheritance of these traits. Large GCA/SCA ratios is an indication of importance of additive gene effect while small ratio indicates that the trait is under dominance or epistatic gene effect (Bi et al., 2015). Consequently, the inheritance of thrips damage, flavonoids and proteins are controlled mainly by additive effect, while antioxidants, lignin, phenolics, reducing sugars and tannins are controlled mainly by dominance gene effect. This is an indication that the metabolites could be used as selection criteria in breeding for resistance of cowpea to flower bud thrips. Similar findings were reported on resistance of flower thrips in cowpea (Symphorien et al., 2018). Also, additive gene effect was observed to play a major role in inheritance of secondary metabolites in cassava under whitefly infestation (Mwila et al., 2017). In addition, the interaction between GCA and environment were non-significant for thrips damage at 65 and 80 DAP, proteins and tannins, an indication that the additive gene effect was not influenced by environment hence stable. Non-additivity in secondary metabolites has been reported in past studies and it is associated with plant stress, to help the plant adapt to environmental fluctuations (Cubillos et al., 2018). The significant reciprocal variation observed in thrips damage, flavonoids, antioxidants, proteins and tannins contents suggested that cytoplasmic genes had major role in altering resistance across environments and minor role for lignin, phenolics and reducing sugars. The former was observed in an experiment on inheritance of flower thrips resistance in cowpea where reciprocal differences were significant in controlling of resistance cowpea to flower thrips (Omo-ikerodah et al., 2009). The later was also observed by Symphorien et al. (2018) where reciprocal was not significant in controlling resistance of cowpea to flower thrips. Nevertheless, the significant interaction between reciprocal and environment for thrips damage and antioxidants indicate that the cytoplasmic activities on these traits are influenced by environment, while those of flavonoids, lignin, phenolics, proteins, reducing sugars and tannins are not affected by the environment as suggested by Oladejo et al. (2017). As a result, resistant genotypes could be used as female parents when environment favours the expression of maternal factors (Symphorien et al., 2018).

Flower thrips damage scores at 50 and 80 DAP and flavonoids showed high values of broad sense coefficient of genetic determination. This implies that more than 61% of the phenotypic variance was genetic hence it can be passed to the progeny and early selection would be effective. This finding was contrary to that of Symphorien et al. (2018) who reported low genetic contribution to the phenotypic variance. They were however similar to findings of Dormatey et al. (2015) who reported high broad sense coefficient of genetic determination for resistance of cowpea to flower thrips. The difference in the findings could be tied to the fact that the studies used different genotypes, different population and environments (Singh & Miklas, 2015). It is therefore necessary to determine the inheritance of traits for each set of parents used in breeding program. On the other hand, except flavonoids, the secondary metabolites had low broad sense coefficient of genetic determination, an indication that their production is highly influenced by environment rather than genetic makeup of the genotype. Consequently, selection of such should be delayed to a later generation to reduce the error due to environmental effects. The narrow sense coefficient of genetic determination was low for secondary metabolites confirming that early selection would not be effective when breeding for these traits. Generally, Sanzi and TVU-3804 were the best transmitters of genes and could be used as parents in improvement of cowpea for resistance to flower bud thrips.

5. Conclusion

The results of this experiment suggested that both additive and non-additive gene actions play significant role in inheritance of secondary metabolites. Flavonoids and antioxidants which were significant in resistant genotypes are less influenced by environment hence, could yield positive results in selection. Genotypes Sanzi and TVU-3804 were the best transmitters of flower thrips resistance genes hence could be used as parents to introgress the resistant genes into susceptible well adapted genotypes.

References

Abudulai, M., Salifu, A. B., & Haruna, M. (2006). Screening of Cowpea for Resistance to the Flower Bud Thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera'Thripidae). *Journal of Applied Science, 6*(7), 1621-1624. https://doi.org/10.3923/jas.2006.1621.1624

Adipala, E., Nampala, P., Karungi, J., & Isubikalu, P. (2001). A review on options for management of cowpea pests: Experiences from Uganda. *Isubikalu, 1998*, 185-186. https://doi.org/10.1023/A:1011334312233

Agbahoungba, S., Karungi, J., Badji, A., Sadik, K., & Gibson, P. (2018). Inheritance of cowpea resistance to flower thrips in Uganda germplasm. *Journal of Plant Breeding and Crop Science, January*. https://doi.org/10.5897/JPBCS2017.0698

Agbahoungba, S., Karungi, J., Odong, T. L., Badji, A., Kumi, F., Mwila, N., & Rubaihayo, P. R. (2018). Biochemical constituents influencing the resistance to flower bud thrips in cowpea [*vigna unguiculata* (L.) walp] germplasm. *Journal of Animal and Plant Sciences, 28*(1), 128-137.

Agbahoungba, S., Karungi, J., Odong, T. L., Badji, A., Sadik, K., Rubaihayo, P. R., & Zonal, A. (2017). Stability and Extent of resistance of Cowpea lines to flower thrips. *African Crop Science Journal, 25*(1), 1-24. https://doi.org/10.4314/acsj.v25i1.1

Baker, R. J. (1978). Issues in diallel analysis. *Crop Sci., 18*, 533-536. https://doi.org/10.2135/cropsci1978.0011183X001800040001x

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist, 127*(4), 617-633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x

Bi, Y., Li, W., Xiao, J., Lin, H., Liu, M., Liu, M., ... Lai, Y. (2015). Heterosis and combining ability estimates in isoflavone content using different parental soybean accessions: Wild soybean, a valuable germplasm for soybean breeding. *PLoS ONE, 10*(1), 1-13. https://doi.org/10.1371/journal.pone.0114827

Cubillos, A. E. R., Tong, H., Alseekh, S., de Abreu E Lima, F., Yu, J., Fernie, A. R., ... Laitinen, R. A. E. (2018). Inheritance patterns in metabolism and growth in diallel crosses of Arabidopsis thaliana from a single growth habitat. *Heredity, 120*(5), 463-473. https://doi.org/10.1038/s41437-017-0030-5

Dormatey, R., Atokple, I. D. K., & Ishiyaku, M. F. (2015). Genetics of thrips resistance in cowpea. *Int. J. Agric. Sci. Res, 2*, 2348-3997.

Erb, M., & Reymond, P. (2019). Molecular Interactions between Plants and Insect Herbivores. *Annual Review of Plant Biology, 70*, 527-557. https://doi.org/10.1146/annurev-arplant-050718-095910

Fatokun, C. A., Boukar, O., Kamara, A., Coulibaly, O., Alene A., & Boahen, S. (2012). *Enhancing cowpea productivity and production in drought-prone areas of Sub-Saharan Africa, in Four Seasons of Learning and Engaging Smallholder Farmers*.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci., 9*, 463-493. https://doi.org/10.1071/BI9560463

Harbourne, J. B. (1998). A Guide to Modern techniques of Plant Analysis. Kluwer Academic Publishers, USA.

Jackai, L. E. N., & Singh, S. R. (1988). Screening techniques for host plant resistance to insect pests of cowpea. *Trop. Grain Leg. Bull., 35*, 1-18.

Michalska, A., Ceglinska, A., Amarowicz, R., Piskula, M. K., Szawara-Nowak, D., & Zielinski, H. (2007). Antioxidant contents and antioxidative properties of traditional rye breads. *Journal of Agricultural and Food Chemistry, 55*(3), 734-740. https://doi.org/10.1021/jf062425w

Moreira-Vilar, F. C., Siqueira-Soares, R. D. C., Finger-Teixeira, A., de Oliveira, D. M., Ferro, A. P., da Rocha, G. J., ... Ferrarese-Filho, O. (2014). The acetyl bromide method is faster, simpler and presents best recovery of lignin in different herbaceous tissues than klason and thioglycolic acid methods. *PLoS ONE, 9*(10). https://doi.org/10.1371/journal.pone.0110000

Mwila, N., Rubaihayo, S., Kyamanywa, S., Odong, T. L., Nuwamanya, E., Mwala, M., … Badji, A. (2017). Biochemical factors associated with cassava resistance to whitefly infestation. *African Crop Science Journal, 25*(3), 365. https://doi.org/10.4314/acsj.v25i3.9

Nassourou, M. A., Njintang, Y. N., Noubissié, T. J. B., Nguimbou, R. M., & Bell, J. M. (2016). Genetics of seed flavonoid content and antioxidant activity in cowpea (*Vigna unguiculata* L. Walp.). *The Crop Journal, 4*(5), 391-397. https://doi.org/10.1016/j.cj.2016.05.011

Oladejo, A. S., Boukar, O., Fatokun, C. A., & Obisesan, I. O. (2017). Genetic analysis of thrips resistance in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica, 213*(9). https://doi.org/10.1007/s10681-017-2001-6

Omo-ikerodah, E., Fatokun, C., & Fawole, I. (2009). Genetic analysis of resistance to flower bud thrips (*Megalurothrips sjostedti*) in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica, 165*, 145-154. https://doi.org/10.1007/s10681-008-9776-4

Oyewale, R. O., & Bamaiyi, L. J. (2013). Management of Cowpea Insect Pests. *Sch. Acad. J. Biosci., 1*(5), 217-226.

Rodríguez, F., Alvarado, G., Pacheco, A., Crossa, J., & Burgueño, J. (2015). *AGD-R (Analysis of Genetic Designs with R for Windows)* (Version2.0).

Salifu, A. B., Singh, S. R., & Hodgson, C. J. (1988). Mechanism of resistance in cowpea (*Vigna unguiculata* (L.) Waip.) genotype, TVx 3236, to the bean flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae). 2. Non-preference and antibiosis. *Tropical Pest Management, 34*(2), 185-188. https://doi.org/10.1080/09670878809371239

Shewry, P. R., & Lucas, J. A. (1997). Plant Protein that Confer Resistance to Pests and Pathogens. *Advances in Botanical Research, 26*(C). https://doi.org/10.1016/S0065-2296(08)60120-2

Singh, S. P., & Miklas, P. N. (2015). Breeding common bean for resistance to common blight: A review. *Crop Science, 55*(3), 971-984. https://doi.org/10.2135/cropsci2014.07.0502

Singha, I. M., Kakoty, Y., Unni, B. G., Kalita, M. C., Das, J., Naglot, A., … Singh, L. (2011). Secondary metabolites in plant defence mechanisms. *World Journal of Microbiology & Biotechnology, 27*(11), 617-633. https://doi.org/10.1071/AP99008

Sobda, G., Boukar, O., Tongoona, P. B., Ayertey, J., & Offei, K. S. (2017). Quantitative trait loci (QTL) for cowpea resistance to flower bud thrips (*Megalurothrips sjostedti* Trybom). *International Scholars Journal, 4*(6), 292-299.

Sserumaga, J. P., Oikeh, S. O., Mugo, S., Otim, G. A. M., Beyene, Y., Abalo, G., & Kikafunda, J. (2015). Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (*Zea mays* L.) hybrids. *Euphytica, 205*, 1-15. https://doi.org/10.1007/s10681-015-1549-2

Symphorien, A., Jeninah, K., Arfang, B., Kassim, S., Paul, G., Richard, E., … Patrick, R. R. (2018). Inheritance of cowpea resistance to flower thrips in Uganda germplasm. *Journal of Plant Breeding and Crop Science, 10*(1), 21-32. https://doi.org/10.5897/jpbcs2017.0698

Tchiagam Noubissié, J. B., Youmbi, E., Njintang, N. Y., Abatchoua, M. A., Nguimbou, R. M., & Bell, J. M. (2012). Inheritance of phenolic contents and antioxidant capacity of dehulled seeds in cowpea (*Vigna unguiculata* L. Walp.). *International Journal of Agronomy and Agricultural Research,* 2(3).

Toyinbo, J. O., Fatokun, C., Boukar, O., & Fakorede, M. A. B. (2021). Genetic variability and trait association under thrips (*Megalurothrips sjostedti* Trybom) infestation in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica, 217*(6), 1-9. https://doi.org/10.1007/s10681-021-02849-1

Tumwegamire, S., Rubaihayo, P. R., & Adipala, E. (1998). Genetics of resistance to Sphaceloma scab of cowpea. *African Crop Science Journal, 6*(3). https://doi.org/10.4314/acsj.v6i3.27796