

Primary and secondary metabolite compounds in cowpea seeds resistant to the cowpea bruchid [*Callosobruchus maculatus* (F.)] in postharvest storage

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ABSTRACT

Cowpea is a one important legume crop in Sub-Saharan Africa due to its high content of protein and other nutrients. However, seeds of cowpea varieties are destroyed by *Callosobruchus maculatus* in storage. This study investigated various biochemical compounds of susceptible and resistant cowpea genotypes, to determine the biochemical compounds underlying cowpea resistance to bruchid. Six cowpea genotypes were analyzed in three replicates. One-way analysis of variance, Pearson correlation and path analysis were used to determine the influence of the biochemical compounds on the cowpea status. Amongst the cowpea genotypes assayed the biochemical compounds analyzed (Vicilin; α -amylase Inhibitor; Phenols; Condensed Tannin; Tannin; Carbohydrate; Flavonoid and Protein), were present in amounts. Resistant genotypes exhibited higher contents of Phenols (22.29 mg/g for WC66*5T and 19.74 mg/g for WC36); Tannin (2.45CEmg/g for WC36 and 2.52CEmg/g for TVU13677); and Carbohydrate (72.82% for TVU13677 and 71.09% for WC36). The most susceptible genotypes had the lowest content of Phenols (13.5 mg/g for TVU946 and 12.72 mg/g for Glessissaffodo); Tannin (0.74CEmg/g for Akounado and 0.97CEmg/g for Glessissaffodo) and Carbohydrate (60.95% for Akounado and 61.39% for TVU946), while Condensed Tannin was associated to their seed coat colour but not to their resistance status against bruchid. The resistant genotypes TVU13677; WC36 and WC66*5T were found to contain a higher amount of a subset of biochemical compounds as defensive compounds against cowpea bruchid. The susceptible cowpea genotypes (Akounado; Glessissaffodo; TVU946) screened for their biochemical content presented low amounts of these defensive compounds. The path analysis implemented highlighted the existence of causal relationships among biochemical compounds analyzed and resistance parameters, thus revealing the resistance basis of cowpea seed biochemical compounds. These latter results point out the possibilities to consider the presence of these biochemical compounds underlying the resistance of cowpea to bruchid in the breeding program toward the resistance to bruchid.

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] seeds are essential legume crops cultivated in many Sub-Saharan Africa countries, used for human food sources as well for the source of income (Phillips et al., 2003; Adam

and Baidoo, 2008), and are therefore used as a nutritional food supplement to cereals (Modu et al., 2010). Throughout the world, the cowpea crop feeds millions of people and cultivated on about 9 million ha with an annual production of 2.9×10^6 tonnes (Snapp et al., 2018; Fatkun et al., 2002). Thus, this legume is considered as a main protein

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source in rural area of West Africa where most people cannot afford protein foods such as fish and meat (Lephale et al., 2012; Dugje et al., 2009). However, in field condition, cowpea crop faces many biotic constraints like bacteria (*Xanthomonas campestris* pv *vignicola*), virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), parasitic plants (*Striga gesnenoides* and *Alectra vogeli*), insects pest (*Aphis carccivora*, *Megalurothrips sjostedti*, *Maruca vitrata*) and fungi (*Choanephora* spp) (Singh, 2005; Boukar et al., 2016). In addition, cowpea in postharvest condition faces severe attack from cowpea bruchid [*Callosobruchus maculatus* (F.)]. Indeed, bruchid is a major cosmopolitan pest in storage for all cowpea varieties (Castro et al., 2013; Badii et al., 2013). This pest induces a significant damage and causes economic losses through reducing cowpea seeds quality (Lephale et al., 2012; Jackai and Asante, 2003).

Cowpea bruchid [*Callosobruchus maculatus* (F.) (Coleoptera: Crysolmelidae)] causes approximately 60% losses or higher in storage (Ogunkanmi et al., 2018; Baoua et al., 2012). Farmers usually apply chemicals to preserve cowpea in storage from bruchid infestation. However, the use of chemical products on food grains in storage has negative effects on human and animal health, and on other organisms leading to biodiversity loss and an extensive environmental contamination (Isman, 2008). Alternatively, farmers usually use the inert products including kaolin dust and some biopesticides from local products such zest of citrus, neem extract, chili and other common botanicals (*Anacardium occidentale*, *Ocimum gratissimum* and *Artemisia annua*) (Kpoviessi et al., 2017, 2019; Ilege, 2015; Brisibe et al., 2011; Akunne et al., 2013). These local biopesticides although are proven to bear minimum health and environmental hazards, have limited efficacy. However, to effectively control the pests, huge amounts of such products are usually required, and these may not always be available. Hence, the bruchid-resistant cowpea varieties is the best alternative for efficient protection of cowpea seeds from *Callosobruchus maculatus* attack (Tripathi et al., 2015; Fawki et al., 2012).

Plant defense involves both mechanical and chemical pathways (Freeman and Beattie, 2008). Understanding both pathway helps developing adequate breeding strategies. In cowpeas, reports showed that seeds color, size, hardness, texture and biochemical compounds are usually involved in the resistance pathways (Singh and Rachie, 1985). In contrast to that it has been recently reported that physical or mechanical characteristics of cowpea seeds were less significant than the biochemical components of cowpea seeds for conferring resistance to *Callosobruchus maculatus* (Tripathi et al., 2020; Kosini et al., 2019; Cruz et al., 2016). Besides, the non-protein anti-metabolites including ortho-dihydroxy phenols, tannins and total phenols have been revealed important in conferring the cowpea seed resistance to bruchid (Venugopal et al., 2000). Biochemical resistance mechanisms in cowpea seed involves the chemical characteristics in the seed coat and cotyledon exerting non-preference for oviposition and antibiosis effects on the development of larvae, thus influencing the larval hatching and prolonged the individual development (Kosini et al., 2019; Cruz et al., 2016; Arong and Usua, 2006). Biochemical compounds such as tannins, α -amylase inhibitor and phenolic acids have been identified to induce cowpea seed resistance to cowpea bruchid (Tripathi et al., 2020; Kosini et al., 2019). However, the main mechanism of cowpea seeds resistance to bruchid has been reported to be associated to the high amount of α -amylase inhibitor (Lattanzio et al., 2005). Moreover, it was also revealed that the resistance to cowpea bruchid is imparted in pulses by a high level of vicilin which is a non-nutrient molecule (Ignacimuthu et al., 2000). The presence of vicilin is furtherly shown to be refractory to digestion by *C. maculatus* in the cowpea seeds of the variety IT84S-2246-4 and other varieties bred from IT84S-2246-4 (Appleby and Credland, 2003).

There is no general consensus on the role of tannins content in the cowpea seed coat regarding oviposition and *C. maculatus* development in cowpea seed. For example, a positive correlation was found between seed coat tannin content and cowpea seed resistance to bruchid (Lale and Makoshi, 2000). In contrast, other authors reported that condensed

tannins content of seed coats were positively correlated to the seed coat color and not to the resistance status of cowpea to bruchid (Lattanzio et al., 2005). In conformity with Tripathi et al. (2020) and Cruz et al. (2016) the texture, shape, seed coat thickness or pigmentation of cowpea accessions seeds lead neither to the resistance nor to the susceptibility to cowpea bruchid. Consequently, it is clear that the cowpea resistance to bruchid might be due to the influence of the combinations of several biochemical compounds in the seeds. These findings justify the research on the various defensive roles of these chemical compounds in cowpea seeds. Further to the identification of bruchid resistant cowpea varieties in a previous study (Kpoviessi et al., 2020), the present study seeks to i) determine the chemical compounds of seed coats and cotyledons ii) assess correlation between cowpea resistance parameters and cowpea seed chemical composition parameters, and iii) implement path analysis to provide better insights on the causal relationships among resistance parameters and biochemical compounds.

2. Material and methods

The study analyzed six cowpea genotypes (Table 1) differing in their response to bruchid infestations (Kpoviessi et al., 2020). From each genotype eight biochemical compounds were extracted in three replicates according to the methodologies described below. Indeed, three replicate were used for each biochemical compound extracted from each cowpea variety. For each replicate, data were recorded on biochemical parameters. Path analysis was also implemented to determine the causal relationships among biochemical compounds and resistance parameters.

2.1. Methodologies for biochemical compounds extraction

2.1.1. Total phenolic and total flavonoids

Total phenolic content was extracted with 80% aqueous methanol containing 1% HCl (1:50, w/v) by refluxing in a boiling water bath for 30 min (Miesho et al., 2017). The refluxed material was concentrated under vacuum in a rotary flash evaporator (RU 10 C SO99, IKA, Germany) and used for determining the total phenolic content (TPC) and total flavonoid content (TFC). The TPC of each extract was determined using the method described by Chandrasekara and Shahidi (2010) and the contents expressed in milligrams of gallic acid equivalents (GAE) per gram of defatted flour. The TFC of flour was measured by the aluminum chloride colorimetric assay method described by Kim et al. (2003) by reading the absorbance at 510 nm (Biowave ii+, Cambridge, England) and the contents expressed in percent of gallic acid equivalents (GAE) per gram of defatted flour.

2.1.2. Tannins extraction and assay

Tannin analysis was carried out on 10 g of dry seeds from different cowpea genotype according to Vande Castele et al. (1981). The extraction was performed with boiling methanol—ethanol—water

Table 1

Bruchid resistance parameters for cowpea genotypes used for biochemical analyses (Kpoviessi et al., 2020).

Genotypes	PWL	NEL	MNH	PSD	PBE	MDP	DSI
Akounado	48.72	80.66	53.66	66.66	61.67	24.50	5.57
Glessissaffodo	38.51	68.33	40	66.66	57.57	25.00	5.11
TVU13677	0	1.66	0	0	0	0	0
TVU946	67.61	46.66	31	100	80.46	27.67	5.68
WC36	11.74	26.33	3.33	10	8.47	35.00	1.15
WC66*5T	2.77	0	0	0	0	0	0
LSD	38.63	58.08	41.33	60.41	53.25	16.03	4.67
CV%	49.24	57.00	60.49	49.95	49.56	48.09	51.18

PBE = Percentage of Bruchid Emergence; PWL = Percentage of Weight Loss; MDP = Median Development Period; DSI = Dobie Susceptibility Index; NEL = Number of Eggs Laid; MNH = Mean Number of Hole; PSD = Percentage of Seed Damage.

(4:4:2, v/v/v) under nitrogen; the solvent was evaporated under the vacuum and the residue dissolved in water (crude extract). Tannins were separated from non-tannins with 1% gelatin in 10% NaCl solution and the pellet, containing tannins, was solubilized in 50% pH 3 acetone at 37 °C. Gelatin was then removed, increasing the acetone concentration up to 90%. The acetone was evaporated under vacuum, total tannins was dissolved in water and the hydrolysable fraction separated from the condensed one using formaldehyde at 1.6 mg ml⁻¹ final concentration. Tannins were assayed by Folin—Ciocalteu spectrophotometric method and results were expressed as catechin equivalents. The presence of condensed tannins was also proved by the red colour formation after heating the crude extracts with n-butanol—HCl—Fe (III).

2.1.3. Content of condensed tannins

The content of condensed tannins in such obtained materials was determined. Tannin fractions were dissolved in methanol (0.5 mg/mL). To 1 mL of prepared solution, 5 mL of vanillin/HCl reagent (0.5 g vanillin in 4% hydrochloric acid in methanol [v/v]) was added. Samples and controls (without vanillin) was allowed to stand for 20 min in darkness and then absorbance at 500 nm was read. Results were expressed as absorbance units per 1 mg of tannin fractions.

2.1.4. Flavonoids

About 10 g of seeds of cowpea genotypes powder (or 0.1 g of shoot) was weighed and mixed with 50 mL of acidified methanol which was prepared from a ratio of 79:20:1 MeOH:H₂O:HCl. The mixture was incubated for 72 h in darkness for auto-extraction, and was filtered through Whatman paper Number 2 and absorbance of the clear supernatant measuring spectrometrically at 300, 530, and 657 nm using acidified methanol as standard. Concentrations of flavonoids were measured at 300 nm and expressed as Abs g DM⁻¹ (Flint et al., 1985) while anthocyanin concentration in seed extracts was measured as Abs₅₃₀ - 1/3Abs₆₅₇ (Lindoo and Caldwell, 1978) and was expressed as Abs g DM⁻¹.

2.1.5. Total protein

Total protein was quantified using the Bradford method (Bradford, 1976). A sample of 0.1 g of cotyledon flour was added into a falcon tube containing 5 ml of distilled water. The solution was agitated for 30 min at 50 °C on a thermomixer (Eppendorf AG, Hamburg, Germany). From this protein solution, 0.1 ml was pipetted off and stained with 3 ml of Bradford reagent (Commassie brilliant blue + 95% Ethanol + 85% Phosphoric acid + Deionized water). The stained proteins were quantified by measuring absorbance in a spectrophotometer (Biowave ii+, Cambridge, England) at a wave length of 595 nm against standard albumin.

2.1.6. Total carbohydrate

Total carbohydrate was determined as total starch and sugar content by hydrolysis of 0.1 g sample with 5 ml of 10% (v/v) Sulfuric acid at 80 °C in a water bath (Grant TXF 200, England) for 30 min. The sample was left to cool at room temperature and the resultant sugar as well as the original sugar was quantified using the method of Dubois et al. (1956). 0.5 ml of solution was diluted with 1 ml of deionized water and dehydrated using 1 ml of concentrated sulfuric acid. The resulting furfural compound was estimated by adding 0.5 ml of 5% phenol and the resulting-colored compounds quantified by measuring its absorbance at a wavelength of 490 nm in a spectrophotometer (Biowave ii+, Cambridge-England) against a starch soluble standard.

2.1.7. α -Amylase inhibitor assay

The inhibitory activity assay was performed by adding different volumes of inhibitors extract to 50 μ l of insect α -amylase preparation in a total volume of 1.2 ml of barbital buffer solution, pH 5.4. The mixture was incubated at 20 °C for 10 min before the addition of 0.2 ml of substrate solution (0.1% potato starch solution in water). After

incubation at 20 °C for 10 min, the reaction was stopped with 0.2 ml 3 M HCl. The undigested starch was determined by adding 0.4 ml of an I₂—KI solution (1.2 and 1.8 mM, respectively) and by measuring the change in absorbance at 620 nm. Controls without inhibitors were included to determine amylase activity of each preparation (expressed as amylase units, i.e. the amount of enzyme that gave 50% hydrolysis of the added starch) according to Silano et al. (1975). The α -amylase inhibitory activity (percentage of control) was expressed as a percentage of α -amylase activity values in the absence of pre-incubation with the seed extract. The Bradford method was used for protein determination of α -amylase inhibitor extracts using bovine serum albumin as a standard (Bradford, 1976).

2.1.8. Vicilin determination in cowpea seed

Cowpea seedlings were dried in an air forced oven up to a moisture content lower than 14%. The dried seedlings were ground in a hammer mill fitted with a 1 mm mesh screen. The resultant was treated meals twice with chloroform/methanol (2:1 v/v) for lipid extraction and dried at air. For the protein extraction, the defatted meal (1:10 w/v) was extracted with 0.2 mol. L⁻¹ borate buffer (pH 8) containing 0.5 mol. L⁻¹ NaCl and centrifuge (30 074×g, 30 min, 4 °C). The supernatant (supernatant A) was retained and there after the borate insoluble sediment was re-extracted followed by centrifugation. The new sediment was recovered by freeze-drying. The new extract (supernatant B) was combined with the supernatant A, and adjusted to pH 4.5 with glacial acetic acid in the cold, Stir the resultant solution for 30 min and follow with centrifugation at (30 074×g, 30 min, 4 °C). The supernatant was dialyzed extensively against distilled water and centrifuged (30 074×g, 30 min, 4 °C). The new sediment that contains vicilins was frozen freeze-dried (vicilins 7S) for recovery.

2.2. Data collection and data analysis

Data on biochemical parameters were collected for each replicate. The differences for cowpea accession in biochemical characteristics were tested with one-way ANOVA. Fisher protected Least Significant Differences (LSD) test were used at 5% significance level. Pearson correlation analysis was implemented using R with package PerformanceAnalytics (<https://cran.r-project.org/web/packages/PerformanceAnalytics/index.html>), to indicate the correlation measure and the power of the relationship amongst cowpea seed biochemical parameters and specific life parameters of *Callosobruchus maculatus*. Path analysis was also performed with R software using a subset of packages namely, lavaan (<https://CRAN.R-project.org/package=lavaan>), OpenMx (<https://CRAN.R-project.org/package=OpenMx>), semPlot (<https://CRAN.R-project.org/package=semPlot>), GGally (<https://CRAN.R-project.org/package=GGally>) and corplot (<https://CRAN.R-project.org/package=corplot>) to determine the causal relationship among both biochemical parameters and resistance parameters.

3. Results

3.1. Variation of cowpea genotypes biochemical compounds

Amongst the cowpea genotypes analyzed, significant difference ($P < 0.001$) were found in the seeds biochemical contents for vicilin, α -amylase inhibitor, condensed tannin, carbohydrate, flavonoid and protein. Significant difference ($P < 0.01$) were also observed among the studied genotypes for phenols and tannin (Table 2). These types of variations were also observed with the phenotypic parameters recorded (Table 1).

3.2. Influence of *C. maculatus* activity on cowpea seeds

Variability of bruchid activity on cowpea genotypes used is highlighted by the phenotypic parameters recorded (Table 1). Therefore, the

Table 2
Analysis of variance of biochemical parameters for the cowpea genotypes studied.

Source of Variation	df	Biochemical Parameters							
		Vic	α -Aln	Phe	C-Tan	Tan	Carb	Flav	Prot
Genotypes	5	0.00124***	2230.9***	48.54**	0.013***	1.679**	84.15***	205.07***	10.65***
Residuals	12	0.00003	12.2	5.86	0.001	0.21	7.81	0.09	0.55
Range		0.3–0.36	28.35–89.8	11.35 - 24.61	0.02 - 0.27	0.11 - 2.95	55.95 - 77.00	18.46 - 37.86	17.58 - 22.50
CV(%)		5.97	43.87	25.32	41.16	47.38	8.21	28.12	9.46

Vic: Vicilin; α -Aln: α -Amylase Inhibitor; Phe: Phenols; C-Tan: Condensed Tannin; Tan: Tannin; Carb: Carbohydrate; Flav: Flavonoid; Prot: Protein. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '.' 1.

percentage of seed damage (PSD) was upper than 50% for the susceptible genotypes with a percentage of seed damage of 100% for TVU946 a susceptible genotype. Meanwhile, the resistant genotype presented a lower percentage of weight loss, with a PSD of 0% for WC66*5T and TVU13677 and a PSD of 10% for WC36. The number of egg laid (NEL) recorded on the susceptible genotypes was greater, with a NEL of 46.66, 68.33 and 80.66 for TVU946, Glessissaffodo and Akounado respectively. The lowest NEL was recorded for the resistant genotypes with a NEL of 0, 1.66 and 26.33 for WC66*5T, TVU13677 and WC36 respectively. Consequently, the mean number of hole (MNH) recorded was high on the susceptible genotypes when compared with the resistant genotypes. Thus, the high MNH was recorded on a susceptible genotype, Akounado with a MNH of 53.66 and the lowest MNH was recorded on TVU13677 and WC66*5T the resistant genotypes with a MNH of 0.

3.3. Cowpea genotypes biochemical compounds analysis

The cowpea genotypes studied showed differences in the various biochemical compounds determined. Therefore, the results of biochemical parameters estimate for the cowpea seeds revealed that vicilin content ranged from 0.31 ppm for Akounado and TVU13677 to 0.36 ppm for Glessissaffodo. High amount of α -amylase was recorded in the susceptible cowpea genotype, Glessissaffodo 81.07% compared to the resistant one, WC66*5T 31.78% (Table 3). The highest per cent of protein content was recorded in the susceptible cowpea genotype, Akounado 21.3% and the lowest value was recorded in the resistant genotype, WC36 17.66%. The condensed tannin content varied from 0.10 CE mg/g in WC36 a resistant cowpea genotype to 0.22 CE mg/g in Glessissaffodo a susceptible one. The highest flavonoid content 37.27 mgQUE/g was recorded in the susceptible cowpea genotype (TVU946) (Table 3). The resistant cowpea showing a high amount of tannin content was TVU13677 with 2.52 CE mg/g. The percentage of carbohydrates recorded with the cowpea genotypes studied ranged from 55.95% to 77% (Table 2) with WC36 showing the high percentage (Table 3). The highest amount of phenolic compound was also recorded among the resistant cowpea genotypes with WC66*5T presenting 22.29 mg/g.

3.4. Correlation studies between resistance parameters and cowpea biochemical compounds

Relationship between cowpea biochemical compounds and phenotypic parameters (Fig. 1) indicated that percentage of weight loss had a

Table 3
Estimates of cowpea genotypes biochemical contents.

Genotypes	Vic (ppm)	α -Aln (%Inhibition)	Phe (mg/g)	C-Tan (mgCE/100g)	Tan (CEmg/g)	Carb (%)	Flav (mgQUE/g)	Prot (%)
Akounado	0.31	80	14	0.09	0.74	60.95	20.79	21.3
Glessissaffodo	0.36	81.07	12.72	0.22	0.97	64.77	36.08	21.28
TVU13677	0.31	35.98	19.43	0.16	2.52	72.82	18.75	19.22
TVU946	0.32	89.3	13.5	0.20	1.52	61.39	37.27	21.82
WC36	0.34	34.47	19.74	0.10	2.45	71.09	21.49	17.66
WC66*5T	0.34	31.78	22.29	0.26	1.96	71.09	31.37	17.72
LSD	0.009	6.21	4.3	0.068	0.82	4.97	0.54	1.32

Vic: Vicilin; α -Aln: α -Amylase Inhibitor; Phe: Phenols; C-Tan: Condensed Tannin; Tan: Tannin; Carb: Carbohydrate; Flav: Flavonoid; Prot: Protein.

significant positive correlation with the mean number of holes ($r = 0.83$; $p < 0.05$). Dobie susceptibility index was significant ($p < 0.01$) and positively correlated with the percentage of weight loss ($r = 0.96$), number of eggs laid ($r = 0.93$), percentage of seed damage ($r = 0.97$), mean number of holes ($r = 0.94$), and percentage of bruchid emergence ($r = 0.98$). Dobie susceptibility index had a significant positive correlation with α -Amylase Inhibitor content ($r = 0.98$; $p < 0.001$) and protein content ($r = 0.92$; $p < 0.01$). Additionally, Dobie susceptibility index had a significant negative correlation with phenols content ($r = -0.96$; $p < 0.01$), tannins content ($r = -0.87$; $p < 0.05$) and protein content ($r = -0.97$; $p < 0.01$). Meanwhile, condensed tannin was non-significant and weakly correlated to resistance parameters.

3.5. Path analysis among resistance parameters and cowpea biochemical compounds extracted

The path analysis was used in this study to examine and test the causal relationship among biochemical parameters and resistance parameters. Parameters DSI (Dobie Susceptibility Index) and AAI (α -amylase inhibitor) were set as endogenous parameters while all other parameters were set as exogenous parameters (Fig. 2). The path coefficients of 0.46 recorded between PWL and AAI indicated a positive causal relationship. Meanwhile, -81.95 and -101.57 were recorded between PWL and Phe relationship and NEL and Phe relationship respectively, showing that the increase in Phe (phenol) content causes a decrease in resistance parameters (PWL and NEL) in cowpea seeds. There is a positive causal relationship between endogenous parameters (DSI and AAI) and exogenous parameters (PBE and MNH). Path coefficients of 31.82 and 52.53 were recorded for Prot and MNH relationship and Prot and PBE relationship respectively. Additionally, parameters Tan (tannin) and Carb (carbohydrate) had strong negative causal relationship with parameter PSD with path coefficient values of -20.26 and -179.35 respectively. Thus, exhibiting that the increase of Tan (tannin) and Carb (carbohydrate) reduces the percentage of seed damage (PSD) and conversely. Path coefficients of 16.02 and 0.92 were recorded between MDP and Flav relationship and MDP and AAI relationship respectively showed the implication of biochemical compounds in the developmental period of bruchid in cowpeas.

4. Discussion

The lack of adequate knowledge on crop characterization limit the

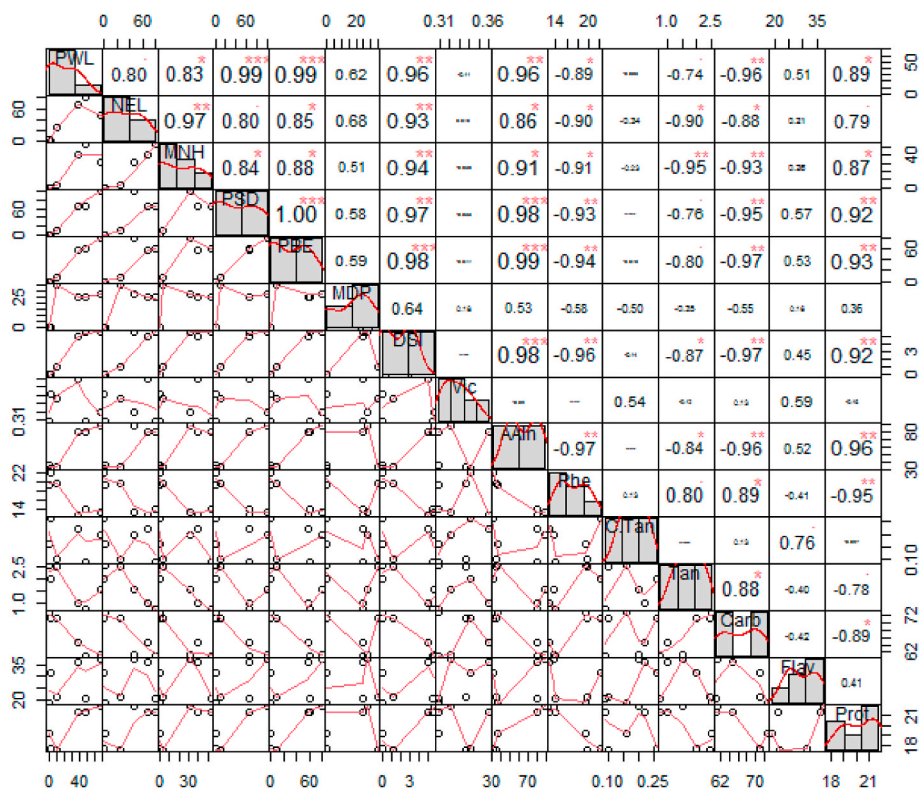


Fig. 1. Matrix of correlation between cowpea seed biochemical parameters with bruchid resistance phenotypic parameters. PBE = Percentage of Bruchid Emergence; PWB = Percentage of Weight Loss; MDP = Median Development Period; DSI = Dobie Susceptibility Index; NEL = Number of Eggs Laid; MNH = Mean Number of Hole; PSD = Percentage of Seed Damage. Vic: Vicilin; AAI: α -Amylase Inhibitor; Phe: Phenols; C-Tan: Condensed Tannin; Tan: Tannin; Carb: Carbohydrate; Flav: Flavonoid; Prdt: Protein. Signif. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '*' 0.1 '.' 1.

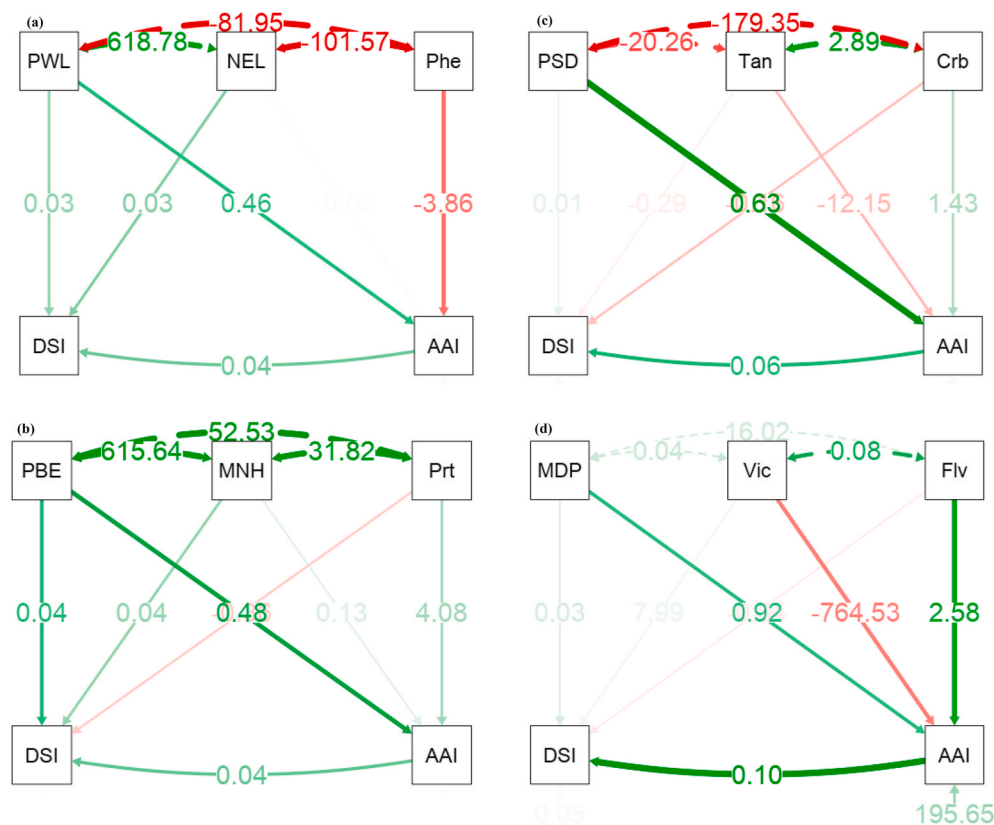


Fig. 2. Path diagram between cowpea seed biochemical parameters with bruchid resistance phenotypic parameters. (a): path analysis between DSI, AAI, Phe, NEL, PWB; (b): path analysis between DSI, AAI, PBE, MNH, Prt; (c): path analysis between DSI, AAI, PSD, Tan, Carb (d): path analysis between DSI, AAI, Vic, MDP, Flav. PBE = Percentage of Bruchid Emergence; PWB = Percentage of Weight Loss; MDP = Median Development Period; DSI = Dobie Susceptibility Index; NEL = Number of Eggs Laid; MNH = Mean Number of Hole; PSD = Percentage of Seed Damage. Vic = Vicilin; AAI = α -Amylase Inhibitor; Phe: Phenols; C-Tan = Condensed Tannin; Tan = Tannin; Carb = Carbohydrate; Flav = Flavonoid; Prt: Protein.

breeding possibility of the available germplasm (Kell et al., 2017; Kehel et al., 2020). Consequently, the evaluation of genotypes for the potential traits is very important (de Carvalho et al., 2013; Byrne et al., 2018;

Anglin et al., 2018). Screening of diverse cowpea germplasm is a relevant step to determine sources of bruchid resistance, this kind of information is essential for germplasm choices focusing the crop

improvement (Kpoviessi et al., 2020; Carrillo-Perdomo et al., 2019). The biochemical attributes revealed their importance in cowpea resistance to *C. maculatus* through this study. In addition, variation recorded in phenotypic parameters depicted on the different contents of biochemical compounds determined. Variability observed is due to the incidence of bruchid on cowpea genotypes used. This bruchid-cowpea interference exhibited the negative effects of resistant cowpea genotypes on bruchid activity in cowpea seeds. The biochemical compounds, vicilin content, condensed tannins and flavonoid content were not associated to the cowpea resistance to the bruchid. Meanwhile, biochemical compounds like α -Amylase Inhibitor content, carbohydrate content, tannin content, phenols and protein contents were associated to the bruchid resistance in cowpea. The amount of vicilin recorded within the cowpea genotypes studied revealed that this compound was not associated to the resistant status of these cowpea genotypes. This finding based on the cowpea genotypes used in this experiment, was not in accordance with the previous studies where vicilin compound, at a high concentration in the seed has been suggested to induce smaller adult size, slower development and lower survival specifically in cultivar IT84S-2246 (Messina et al., 2019; Adesina and Idoko, 2014).

For the condensed tannins, high content was recorded for susceptible (Glessissafodo) as well for resistant cowpea (WC66*5T). It was also revealed that condensed tannin content was not correlated to the resistance parameters, thus showing that condensed tannin didn't impart the bruchid-resistance in cowpea seed, but might be implicated in seed coat colour. These results are in agreement with earlier study where it has been reported that cowpea seed condensed tannin was not correlated to the insect-resistance but significantly correlated to the cowpea seed coat colour (Lattanzio et al., 2005). Additionally, the greatest amount of flavonoid was recorded for two susceptible cowpeas (Glessissafodo and TVU946). These findings corroborate those of Miesho et al. (2017) where the condensed flavonoid was not associated to the resistant status of cowpea genotypes used.

The highest amount of protein was obtained on susceptible cowpea meaning that these types of protein were not toxic and could not adversely affect the bruchid larvae development in cowpea seeds. These findings are not in accordance with those of Souza et al. (2011), where they found the toxic protein for *C. maculatus* larval development. The highest phenol content was recorded on resistant cowpea (WC66*5T) and the lowest were recorded on susceptible one (Glessissafodo). Phenol content was significant and negatively correlated with Dobie susceptibility index, suggesting that higher phenol content is essential to protect cowpea seeds. This result is also in accordance with other studies where it has been reported that phenol content was significant and negatively correlated with resistance parameters (Tripathi et al., 2020). The total carbohydrate content was higher for resistant cowpea genotypes, these indicated that the higher concentrations of total carbohydrates in cowpea seed resulted in a lower incidence of *C. maculatus*. Total carbohydrate content had significant and negative relationship with Dobie susceptibility index, indicating the crucial role of carbohydrates in imparting cowpeas seeds resistance to bruchid (Miesho et al., 2017). Cowpea seed carbohydrate content imparted resistance to cowpea bruchid by amplifying seed hardness thus making difficult seed penetration by bruchid (Ajeigbe et al., 2008).

The tannin content recorded in resistant cowpea genotypes was greater than tannin content recorded in susceptible cowpeas. Tannin content was significant and negatively correlated with Dobie susceptibility index, indicating that greater amount of tannin content imparted resistance to the bruchid in cowpea seed. These results were also in agreement with other studies where tannin content of cowpea seed was involved in biochemical defense mechanisms by deterring and poisoning *C. maculatus* (Lattanzio et al., 2005). The highest inhibition of α -Amylase was recorded on susceptible cowpea genotypes with around 50% inhibition than the cases of resistant cowpea genotypes. α -amylase inhibitor content had significant and negative relationship with Dobie susceptibility index, thus exhibiting the role of α -amylase inhibitor in resistance

spreading in cowpea seed. α -amylase inhibitor from cowpea as well from kidney bean (*Phaseolus vulgaris*) have been found to inhibit bruchid beetle amylases, interfering with insect developmental period by reducing the carbohydrate assimilation, thereby conducting to the reduction of the levels of adult emergence (Lattanzio et al., 2005; Ishimoto and Kitamura, 1989). The inhibition mode of α -amylase has been reported as one of the principal factors of seed defense against bruchid infestation on cowpea, as well on mungbeans (Wisessing et al., 2010; Lattanzio et al., 2005).

Amongst the cowpea genotypes studied, α -amylase inhibitor content had significant and negative relationship with carbohydrate content indicating that a low percent amylase inhibition and high carbohydrate content is required for the resistance to the bruchid in cowpea seeds. Meanwhile, within the cowpea genotypes, α -amylase inhibitor content was significantly and negatively correlated with tannin content and phenols content respectively. Knowing that tannin is a type of plant polyphenols playing a role of insect antifeeding actions (Quideau et al., 2011; Lattanzio et al., 2006), it has been showed that in cowpea seeds, the tannin content (tannic acid) significantly reduce the larval growth and development of *C. maculatus* and consequently influencing the percentage of bruchid adult emergence (Oigiangbe and Onigbinde, 1996; Lattanzio et al., 2005, 2006). Moreover, these phenolics compounds play a vital role in cowpea seeds protecting from *C. maculatus* attack (Tripathi et al., 2020; Oigiangbe and Onigbinde, 1996). Additionally, the estimates provided by the path analysis in this study, exhibited the relative strengths of the causal effects and other associations between biochemical and resistance parameters studied. This revealed the existence of the causal relationships between biochemical and resistance parameters, thus strengthening the fact that studied biochemical compounds induced the cowpea resistance to the cowpea bruchid *C. maculatus*. Accordingly, the development of cowpea resistant varieties to *C. maculatus* must focus on a high amount of phenols and tannin content respectively, thus resulting a cowpea genotype expressing a α -amylase inhibitor inhibited in cowpea seeds the development of *C. maculatus*. Lattanzio et al. (2005) stated that the resistance management strategy could take into account the cowpea seeds biochemical defenses to achieve an appropriate bruchid resistance.

5. Conclusion

This study highlighted the knowledge on the chemical characteristics of defensive systems of cowpeas against *C. maculatus*, thus providing cowpea breeders possibilities to raise the bruchid resistance of cultivated cowpea varieties. The resistance mechanism of cowpeas seeds against bruchid strongly suggests that bruchid-cowpea relationship is a complex one. At the sight of the results, cowpea genotypes TVU13677, WC36 and WC66*5T previously known as resistant genotypes were found to be good for bruchid resistance imparting in cowpea seeds. However, the biochemical compounds including α -amylase inhibitor; phenols; tannin; and carbohydrate are the key primary and secondary metabolites that are associated with the resistance of cowpea to the bruchid, based on the causal relationships occurred when cowpea expressed resistance to bruchid. Thus, the feeding retardation and developmental delay would be due to the combined effect of the biochemical compounds. These characteristics could be used as biochemical indicators for quick and accurate cowpeas genotype selection for their resistance to *C. maculatus*.

CRedit authorship contribution statement

Akovognon Dieudonné Kpoviessi: Methodology, Software, Data curation, Formal analysis, Writing – original draft, preparation, Writing – review & editing, Conceptualization. **Symphorien Agbahoungba:** Methodology, Writing – review & editing, preparation Reviewing. **Eric E. Agoyi:** Methodology, Writing – review & editing, preparation Reviewing. **Ephraim Nuwamanya:** Methodology, Data curation,

Formal analysis, Biochemical, Formal analysis, Writing – original draft, preparation, Writing – review & editing. **Achille E. Assogbadjo**: Methodology, Supervision, Project administration. **Daniel C. Chougourou**: Methodology, Supervision. **Hubert Adoukonou-Sagbadja**: Methodology, Supervision.

Declaration of competing interest

None.

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