Variability in morpho-biochemical traits associated with pod borer (*Helicoverpa armigera*) resistance in pigeonpea pods

ABSTRACT

Pigeonpea contributes to food and nutrition security among poor households in urban and rural areas. Globally it is characterized by stagnant and unstable yield due to its susceptibility to various stresses including the pod borer (*Helicoverpa armigera*) which causes substantial damage to the crop and may result in absolute economic yield loss. The existing cultivated pigeonpea are susceptible to pod borer with only a few genotypes reported to be tolerant based on pod and seed damage. Limited information is available on morphological and biochemical traits associated with pod borer resistance among the existing genotypes. This study was therefore carried out to identify diversified sources of resistance against pod borer damage. The study was set up to assess traits that may contribute to pod borer resistance among 12 selected elite pigeonpea genotypes in three replicates and means were separated based on LSD test using Genstat software. The field study was carried out in Kerio Valley during the long rains of April-September of 2017. The genotypes varied significantly for all the parameters measured at P≤0.05 with a mean of 608.33 g/100g (crude protein), 175.61 mg/100g (total phenol), 19.85 mg/100g (total flavonoid), 0.448 mm (trichome length), 210.6 / 4 mm^2(trichome density) and 0.353 mm(depth of locules). Significant negative correlation was also observed between total phenol, total flavonoid, depth of locules, trichome length and trichome density with pod damage. However, a positive correlation was recorded between crude proteins with pod damage. These results reveal that, host plant resistance is an association of several morphological and biochemical traits. Therefore, these genotypes with elevated levels can be selected and utilized in breeding towards improving resistance to pod borer in pigeonpea.

Keywords: biochemical traits, host plant resistance, morphological traits, pigeonpea, pod borer

1. INTRODUCTION

Food security remains a major challenge in many ASALs of sub-Sahara Africa. This is attributed to limited research efforts focused on improving locally adapted, highly nutritious and stress-tolerant crops like Pigeonpea. Pigeonpea cultivation is gaining interest in Kenya currently due to its economic importance of being highly nutritious, drought tolerant and able to give yield during dry spell when other legumes have wilted (Subbarao *et al*., 2000) . However, current statistics shows that Kenya is ranked the fourth globally by contributing 4% of the total production lead by India which contributes 67% of the production. The major growing areas in Kenya are Eastern province (Makueni, Kitui, Embu, Mbeere and Machakos counties) and coastal regions. Despite the economic importance of this crop, its potential yield has not been realized due to several stresses including pod borer that causes substantial economic loss (Cheboi *et al*., 2016).

Considerable progress has been made in increasing techniques to screen for resistance to insects in pigeonpea. Screening under natural conditions remains the long term viable options however variations on flowering period of pigeonpea genotypes and the insect populations over space and time compromises its reliability, effectiveness and stability. The use of morphological (trichomes, cell wall lignification, branching and podding habit, and pod wall hairs and trichomes) and biochemical traits (phenols, flavonoids & phytic acid) associated with insect resistance permits the rapid determination of potentially resistant plant material. These factors influence host plant selection and pest colonization. This also removes the variation associated with insect density and the effect of environmental factors on the expression of resistance to insects (Sai *et al*.,2018).

Host plant resistance is an important component for reducing losses due to insect pests. Therefore, an understanding of different morphological and biochemical components of resistance is essential in developing breeding strategies for resistance to insect pests (Sai *et al*.,2018; Sharma *et al*., 2009) to improve pigeonpea production through reduction of pod borer incidence by selecting resistant and superior genotypes for growing in semi-arid areas of Kenya.

2. MATERIAL AND METHODS

2.1 *Experimental site*

Kerio Valley is located in Elgeiyo Marakwet County. The site is a high potential area for pigeonpea and hot spot area for *Helicoverpa armigera* incidences. It is located 1°35'S, 36 °66'E at an elevation of 1890 meters A.S.L in agro-ecological zone 6 (LM 6), with low agricultural potential. Its annual rainfall ranges between 400-800mm and mean temperature ranges between 16-30°C. Soils are Vitric andosols with well drained deep to sandy loam soils. Rains are erratic and not reliable(Jaetzold and Schmidt 1983).

2.2 *Experimental material*

Twelve elite medium duration pigeonpea genotypes consisting of 3 landraces tolerant to pod borer, 3 advanced resistant genotypes, 2 moderately susceptible, 2 susceptible and 2 commercial varieties used as checks.

2.3 *Determination of biochemicals in pigeonpea pods*

The study involved evaluation of 12 medium duration pigeonpea genotypes grown in Kerio Valley (pod borer hot spot site) for one season. Planting was done at the onset of the rains during the long rains of April- September 2017 under randomized complete block design with five replications in each experimental plot measuring 5mx5m in length and width respectively, spaced 75 cm between the rows (inter-row) and 25 cm between the plants (intra-row). After podding, 50 immature pods for each genotype were harvested randomly from five tagged plants in the three middle rows of each plot and placed in ice box to maintain its viability. These samples were then transported to BeCA-ILRI Hub laboratory where they were freeze dried for two days which after were grounded using a blender© into fine homogenous samples ready for analysis (Crude protein, total phenols and total flavonoids).

2.3.1 *Determination of crude protein*

Crude protein analysis was based on Folin-Lowry method with minor modifications. Approximately 100 mg of dried seed samples was weighed in triplicate into 15 ml Falcon tubes, 5 ml of 5% Sodium Dodecyl Sulfate (SDS) was added, vortexed and incubated for 2 hours at room temperature and centrifuged at 2000 rpm for 10 min. One hundred micro-litre supernatant was aliquoted into 2ml Eppendorf tube and added with 1900 µl of distilled water to final volume of 2000 µl. Twenty micro-litre of the diluted extract and bovine serum albumin standard (20-100µg/ml) was aliquoted into respective wells in a 96 well microplate in duplicates. To each of the sample and standard, 100µl of Reagent A (Copper-tartrate-carbonate reagent, 5%SDS, 0.8M NaOH and dH_2O) and 50µl of Reagent B (0.4N Folin-Ciocalteu phenol) was added to each well after 20 seconds with gentle priming. The solution was incubated at room temperature for 30 minutes for colour

development. Absorbance/optical density (OD) readings were obtained at 630 nm using a BioTek Synergy-HT (Vermont, USA) microplate reader. The average OD for the two readings of the standards were calculated and used for linear regression analysis. The OD standards and their corresponding protein concentrations were plotted to obtain a linear calibration curve ($r^2 \geq 0.98$) and determine the protein concentration of the test samples.

For quality control purposes BCR 708, a certified reference sample from the Institute for Reference Materials and Measurement, Joint Research Center of the European Commission was included in the analysis. The test samples falling outside the expected range were retested. The relative percent difference (RPD) of each sample was calculated from the duplicate OD readings and samples with RPD values greater than 10% were retested.

2.3.2 *Determination of total phenol*

Total phenols were determined following Folin-Ciocalteu method with minor modifications (Kujala et al., 2000). A total of 0.4 g of the milled samples was weighed in a 50ml Falcon tube and added with10 ml of the 80 % methanol. The samples were incubated for 24 hours on a mechanical shaker at 25 °C. The mixture was then centrifuged at 4,000 rpm for 10 min; the supernatant was aliquoted for determination of the total phenolic contents in a 96 well microtiter plate. Upon adding 20 µl of the samples/blank/standards and 100 µl of Folin-Ciocalteu phenol reagent in duplicates at the respective wells, the solution was mixed gently by priming and after 5 minutes, 80 µl of 7 % Na_2CO_3 was added with gentle priming. The plate was covered with an aluminum foil and the reaction was incubated at room temperature for 90 min for colour development. The resulting blue colour was measured using BioTek Synergy-HT (Vermont, USA) at 725 nm. External calibration was used for quantification of total phenolics as their corresponding gallic acid equivalent.

The average OD for the two readings of the gallic acid standards (10-100 µg/ml) were calculated and used for linear regression analysis. The obtained OD standards versus their corresponding gallic acid concentrations were plotted to prepare a linear calibration curve ($r^2 \geq 0.98$). The RPD between two readings was calculated as described for total phenolics.

The total phenolic content was determined after dilution factor correction and expressed as mg gallic acid equivalent per 100 grams of dry sample.

2.3.3 *Determination of total flavonoids*

The total flavonoid content was determined using Aluminum chloride colorimetric procedure (Kujala et al., 2000). A total of 0.4 g of the milled samples was weighed into clean 50ml Falcon tubes. 10 ml of the 80 % methanol was added to each sample. The samples were shaken on a mechanical shaker at 25 °C for 24 hours. The mixture was then centrifuged at 4,000 rpm for 10 min then the supernatant was aliquoted for determination of the total flavonoid contents. 20 µl of sample extracts or standard solution of catechin (10-100µg/ml) was aliquoted in duplicate into respective wells of the microplate. 80 µl of ddH_2O was added followed by 10 µl of 5% $NaNO_2$ with gentle priming. After 5 minutes, 10 µl of 10 % $AlCl_3$ was added and gently mixed by priming. After another 5 minutes, 80 µl of 2 M NaOH was added and gently mixed by priming. The reaction was incubated at room temperature for 30 min and the absorbance of the samples and standards was measured using a BioTek Synergy-HT (Vermont, USA) microplate reader at a wavelength of 510 nm.

The average OD for the two readings of the catechin standards (10-100 µg/ml) were calculated and used for linear regression analysis. The obtained standards OD versus their corresponding catechin acid concentrations were plotted to prepare a linear calibration curve ($r^2 \geq 0.98$). The relative percent difference (RPD) for each sample was calculated from two OD readings. Sample with RPD value greater than 10 % were retested.

The total flavonoid content was determined after dilution factor correction and the results expressed as mg of catechin equivalent per 100 g of dry sample.

2.4 *Data analysis*

Morphological and biochemical composition data were analyzed using SAS version 9.2 (SAS Institute Inc., second edition, 2013). Three replicates of each sample were used for statistical analysis and resulting values are expressed as mean± S.D. One way analysis of variance (ANOVA) and F-test was carried out to assess any significant differences between the

means ($p \leq 0.05$). Correlation analyses of biochemical and morphological data with pod damage were carried out using Pearson correlation programme in SAS.

3. RESULTS AND DISCUSSION

1. Results

3.1 *Variation in biochemical and morphological traits associated with pod borer resistance*

The genotypes varied significantly (P≤0.001) in total phenols, total flavonoids and crude proteins. ICEAP 01154/2 (tolerant) recorded the highest amount of total phenols (773.9) while KAT 60/8 (susceptible check) recorded the lowest mean (238.8). High amount of total flavonoids was reported in ICEAP 00902 (231.6a) which is one of the tolerant genotype and KAT 60/8 reported the lowest value (85). However, KAT 60/8 recorded highest crude protein compared to Mthawajuni (landrace) which recorded the lowest value (13.78). Significant variation (P≤0.001) was also observed in trichome length, density and depth of locules in pods among the pigeonpea genotypes. Trichome length recorded a range of 0.49-0.821mm with a mean of 0.448mm. ICEAP 01150 (moderately susceptible) recorded the highest length (0.821) and ICEAP 00850 (resistant check) recorded the lowest (0.181mm). Trichome density exhibited a diverse range of 24-347 and a mean of 210.6. ICEAP 01154/2 (tolerant) reported the highest number (347) and ICEAP 00554 the lowest number (24). However, a range of 0.101- 0.622 mm with a mean of 0.353mm was observed in depth of locules. MZ 2/9 recorded high depth (0.622) and KAT 60/8 recorded low depth (0.101) mm (**Table 1**)

Table 1. ***Biochemical and morphological traits associated with pod borer resistance in varied pigeonpea genotypes with their status of resistance.***

		Biochemical factors			Morphological traits		
Genotypes	**Status of resistance**	**Total Phenols**	**Total flavonoids**	**Crude proteins**	**Trichome length(mm)**	**Trichome density (no/4mm^2)**	**Depth of locules (mm)**
ICEAP 00068	MS	596.9^{f}	174.9^{f}	22.6^{ab}	0.752^{b}	213^{g}	0.103^{i}
ICEAP 00554	S	497.7^{g}	155.9^{h}	20.63^{cd}	0.36h^{l}	24^{j}	0.118^{g}
ICEAP 00557	S	408.2^{h}	147^{i}	22.51^{a-c}	0.539^{d}	42.3^{i}	0.105^{hi}
ICEAP 00850	RC	685.5^{c}	196.7^{d}	19.51^{d}	0.181^{k}	328^{b}	0.426^{d}
ICEAP 00902	T	747.5^{b}	231.6^{a}	20.01^{d}	0.421^{f}	312^{c}	0.408^{f}
ICEAP 01150	MS	661.4^{d}	180.7^{e}	19.82^{d}	0.821^{a}	129.3^{h}	0.106^{h}
ICEAP 01154/2	T	773.9^{a}	220.9^{b}	16.21^{e}	0.689^{c}	347^{a}	0.604^{c}
ICEAP 01541	T	744.4^{b}	203.8^{c}	19.04^{d}	0.378^{g}	291.3^{d}	0.421^{e}
KAT 60/8	SC	238.8^{i}	85^{j}	23.22^{a}	0.49^{e}	46.3^{i}	0.101^{i}
Mthawajuni	T	619.9^{e}	164.1^{g}	13.78^{f}	0.222^{i}	275^{e}	0.614^{b}

MZ 2/9	MS	665.8[d]	178.5[ef]	20.09[d]	0.195[j]	233.3[f]	0.622[a]
UGACC 22	MS	660[d]	168.2[g]	20.76[b-d]	0.331[d]	285.3[de]	0.603[c]
Mean		**608.33**	**175.61**	**19.85**	**0.448**	**210.6**	**0.353**
Lsd		**6.045**	**4.185**	**1.825**	**0.001**	**10.81**	**0.004**
CV%		**0.1**	**0.6**	**2.6**	**0.1**	**3**	**0.7**
Genotype		***	***	***	***	***	***

*** significance at (P≤0.001), MS-moderately susceptible, S-susceptible, RC-resistant check, T-tolerant, SC-susceptible check

3.2 *Correlation analysis*

Correlation analysis was undertaken fitting pod damage with morpho-biochemical traits associated with pod borer resistance to study their relationship with resistance/susceptibility to pod borer. Significant correlation was observed in all parameters analyzed with some traits correlating negatively and others positively. Positive significant correlation was found in crude protein (r=0.896**) with pod damage. Moreover, negative significant correlation was recorded in total phenols (r = -0.923***), total flavonoids (r = -0.918***), trichome density (r= -0.936***), trichome length (-0.628**) and length of locules (-0.872***) with pod damage (Table 2).

Table 2. Simple correlation coefficient of morpho-biochemical traits with pod damage of pigeonpea genotypes

Morpho-biochemical traits	Total % pod damage
Total Phenols	-0.923***
Total flavonoids	-0.918**
Crude protein	0.896**
Trichome density	-0.936***
Trichome length	-0.628**
Length of locules	-0.872***

** Significance (P≤ 0.01), *** (P≤ 0.001)

1. Discussions

4.1 *Variation in biochemical factors contributing to pod borer resistance*

Secondary metabolites (phenols, flavonoids) have been reported to influence host finding, oviposition, feeding, survival and development of insects. Significant variation in total phenols and flavonoids among the genotypes was observed in this study. Total phenols ranged from 408.2-773.9 and average of 608.33 mg/100g .Genotype ICEAP 01154/2 (tolerant) recorded the highest mean (773.9) while ICEAP 00557 one of the susceptible genotypes recorded the lowest value (408.2). Total flavonoids exhibited a similar trend as phenols with a range of 85 in KAT 60/8 and 231 in ICEAP 00902 with an average of 175.61. Similarly, KAT 60/8 (susceptible check) recorded the highest mean crude protein (23.22) while Mthawajuni (landrace) recorded the lowest mean (13.78). These results are similar to results by (Singh *et al*., 2018) who reported significant genotypic variation for total phenolic content. However, variation in biochemical compounds on pigeonpea pod surface have been reported to affect larval feeding behaviour, both electrophysiological responses of chemosensory neurons on the ovipositor and sites of oviposition selected by the pod borer (Green *et al*.,2006). This is seen in this study that the tolerant genotypes (ICEAPs 01541, 01154/2, 00902 and Mthawajuni) recorded high levels of

polyphenols (phenols, flavonoids) with low pod damages compared to the susceptible genotypes (ICEAPs 00554, 00557 &KAT 60/8).

4.2 *Variation in morphological traits associated with pod borer resistance in pigeonpea*

Morphological traits like trichome length, trichome density and biochemicals like presence of phenols, sugars and proteins are reported to influence resistance/susceptibility of pigeonpea crop to pod borer (Sai *et al*., 2018).

From this study, significant variation was observed in trichome length, density and depth of locules in pods among the pigeonpea genotypes. Trichome length recorded a range of 0.181-0.821mm with a mean of 0.448 mm. ICEAP 01150 (moderately susceptible) recorded the highest length (0.821) and ICEAP 00850 (tolerant) recorded the lowest (0.181mm). Trichome density exhibited a diverse range of 24-347/4mm and a mean of 210.6/4mm. ICEAP 01154/2 (tolerant) reported the highest number (347) and ICEAP 00554 the lowest number (24). However, a range of 0.101- 0.622 mm with a mean of 0.353mm was observed in depth of locules. MZ 2/9 recorded high depth (0.622) and KAT 60/8 recorded low depth (0.101) mm with a mean of 0.353mm. Sai *et al*., 2017 reported similar results in trichome length (0.4mm - 0.59mm) but slightly higher results in trichome density where he reported a range of 416 to 816 with a mean of 585 in a study carried out in India. The consistency of these results may be explained by the fact that trichomes are potential factors in providing potential resistance mechanism to insect pests.

4.3 *Correlation analysis for biochemical and morphological components associated with pod borer resistance with pod damage*

Significant correlation was observed in all parameters analyzed (Total phenols, total flavonoids and crude protein) with pod damage by pod borer. Some of the components correlated negatively and others positively .Significant negative correlation explains that genotypes with high phenolic and flavonoid contents in the pods offered resistance against pod borer. However, positive correlation explains genotypes with high protein content were more susceptible to pod borer. This is explained by (Sai *et al*., 2018) who reported significantly high crude protein (25.5%) in susceptible genotype when compared with resistant genotype (16.5%).These results are in accordance with Jadhav *et al.* (2012)who reported less damage in ICPL 85010 genotype which had high levels of flavonoids (chlorogenic acid). Similarly, Sharma *et al*.(2009) reported high resistance of H. armigera in wild relative with high polyphenols. Positive correlation of trichome density and trichome length with resistant genotype (ICPL 98003) was also reported by (Sai *et al*., 2018; Sharma *et al*., 2009).

4. CONCLUSION

This study shows variations among test genotypes for total phenolic, total flavonoid contents, crude protein, trichome density, trichome length and depth of locules exhibiting utility of these genetic resources for improving host plant resistance which is an association of several morphological and biochemical traits.

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COMPETING INTERESTS

The authors declare that they have no competing interests

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