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Biochemical Components Conferring Resistance to Pigeonpea Genotypes against *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) under Semitropical Storage Conditions

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ABSTRACT

Background: Pigeonpea, [C*ajanus cajan* (L.) Millsp.] is one of the most common tropical and subtropical pulse crops belonging to the family Leguminosae. Bruchids, *Callosobruchus chinensis* is one of the most important storage pest of pulses in Asia and Africa. The present research focuses mainly on the possibilities of exploring biochemical sources of resistance to bruchids in cultivated and wild relatives of pigeonpea. This is an alternative method to reduce the use of insecticides and could be used in breeding programs to develop integrated pest management strategies for controlling bruchids.

Methods: Various cultivars of pigeonpea and their wild relatives were studied under laboratory conditions for biochemical resistance to *C. chinensis* at ICRISAT, Hyderabad during 2018-19 and 2019-20. The maintained culture of pulse beetle, were used for various bioassay techniques.

Result: The results revealed that seeds of wild species *C. platycarpus* ICPW 68 recorded lowest seed damage by *C. chinensis* (4.33% and 18.9%) as compared to ICPL 161 (59.78% and 74.40%) both under single and multi-choice tests, respectively. A negative correlation was observed between anti-nutritional factors and seed damage.

Key words: Biochemical compounds, Bruchids, Pigeonpea, Resistance, Weight loss.

INTRODUCTION

India is the largest producer, consumer and also the largest importer of pulses in the world. Pulses are grown in around 24-26 million hectares of area with a production of 17-19 million tonnes annually in India and accounts for over 33.33 percent of the total world area and over 20% of total world production (Upadhyay *et al*., 2017). Pigeonpea, *C. cajan* is one of the most common tropical and subtropical legume crops belonging to the family Leguminosae, mostly being cultivated for its edible seeds in Africa, Asia and Americas.

Generally, plants possess toxic secondary metabolites which act either directly on insect pests through antibiosis or develop non-preference for insects feeding on the seeds (War *et al*., 2012). The host plants contain both nutritional and anti-nutritional compounds. The nutritional compounds may enhance the growth and development and on the contrary the anti-nutritional compounds, may deter the survival and development of insects (Ding *et al*., 2000 and Cipollini *et al*., 2005).

However, the unpredictability in pigeonpea production due to poor genetic background and damage by insect pests and diseases limits the seed yield of this crop. Under storage conditions, bruchids, *C. chinensis* and *C. maculatus* crusade extensive losses to all the food legumes worldwide (Sharma *et al*., 2017) and causing 40-50% losses of pulses in storage (Gosh and Durbey, 2003). Keeping this in view, the present research focuses mainly on the possibilities of exploring biochemical sources of resistance to bruchids in cultivated as well as wild relatives of pigeonpea.

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MATERIALS AND METHODS

Test insects and maintenance

Bruchids culture was reared and maintained in Entomology unit of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. Bruchids were reared on chickpea variety (L 550) throughout the study period. Sex determination for male and female was done using key characters (Arora, 1977). The culture was maintained in the (Bio-Oxygen Demand, BOD) incubator with 28±2°C temperature and 70±5% relative humidity. Sub culturing of adult bruchids were done with one kg chickpea/pigeonpea seeds in a five-liter capacity plastic jar.

Bioassay technique

Fifteen genotypes of pigeonpea were evaluated for resistance to *C. chinensis* in multi-choice (MC) and single-choice (SC) tests under laboratory conditions. In the first test, all pigeonpea test genotypes were subjected to *C. chinensis* infestation freely (no restriction). Seeds of each genotype were kept equidistantly in circular manner in a wide mouth plastic basin measuring 9 cm in length and 2 cm in depth. Each plastic basin was considered as one replication and there were three replications for each genotype. Ten pairs of 0-24 hours old adults of *C. chinensis* were released in the center of each plastic basin using an aspirator device and covered with food wrapping film containing tiny perforations. Under single choice test (SC), *C. chinensis* was allowed incursion only to a single genotype. The seeds of each genotype were placed separately in plastic bioassay cups measuring 4 × 10 cm (L × B) and five pairs of freshly emerged 0 to 24 hours old adults of *C. chinensis* were released. Each bioassay cup was considered as one replication and thereafter the same procedure was followed for multi-choice test (MC). The whole experiment was conducted in an incubator maintained at 28±2°C and 70±5% relative humidity and 12: 12 hours (L:D) of photoperiod. Seed damage was expressed as the percentage of damaged seeds for each genotype and the percentage damage incidence was determined using the formula described by Khattak *et al*., (1995) Sathish *et al*., (2020):

$$\text{Damage incidence (\%)} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100$$

Oviposition preference

In multi choice test, the ovipositional preference of the female *C. chinensis* to pigeonpea grains of different genotypes was studied. The seeds of each genotype were randomly placed equidistantly in a plastic cup; ten pairs (male, female) of *C. chinensis* were introduced into the center of the plastic basin and covered with a perforated food wrapping plastic film. The total number of eggs laid on each genotype were counted after 7 days for the assessment of ovipositional preference. In single choice test, to study the ovipositional preference of the female *C. chinensis,* pigeonpea grains of each genotype were placed in a bioassay cup separately and five pairs of *C. chinensis* were released. Thereafter, the same procedure was followed as in the multi-choice test.

Adult emergence (%)

The freshly emerged beetles were counted at 30 days after release and removed daily to avoid the chances of their recounting and the data was pooled to get the total number of adults emerged. The number of eggs laid on each genotype were counted after 7 days of bruchids release. The per cent adult emergence was recorded using the formula (Howe, 1971):

$$\text{Adult emergence (\%)} = \frac{\text{Total number of adults emerged}}{\text{Total number of eggs laid}} \times 100$$

Seed weight loss (%)

Seed weight loss was measured with a digital balance (Sartorius, CP124S, Sartorius AG Gottingen, Germany) before and after 45 days of bruchid infestation. The weight loss % was calculated using the formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of grains-Final weight of grains}}{\text{Initial weight of grains}} \times 100$$

Number of exit holes

The genotypes were examined on a bi-weekly basis after F_1 progeny emergence to record the number of exit holes per genotype by visual observation.

Biochemical basis of resistance to bruchids

Biochemical compounds were estimated using standard protocols. The total phenols were estimated using the method described by Bray and Thorpe (1954); tannins by Vanillin-Hydrochloric acid method (Burns, 1971); total of flavonoids by Vanillin reagent method (Swain *et al*., 1959) and the total soluble sugars by Phenol-Sulphuric acid method (Dubois *et al*., 1956).

Statistical analysis

The experiments were designed statistically by completely randomized design (CRD) with three replications. Results from the data were subjected to analysis of variance (ANOVA) and the 'F' tests were carried at 5% level of significance using SPSS 16^{th} version. The Pearson's correlation coefficient (r) analysis was performed using GenStat 14^{th} edition. The diversity among the genotypes was assessed based on seed damage percentage under single choice and multi choice tests using principal coordinate analysis (Genstat, 2014).

RESULTS AND DISCUSSION

Seed damage by the bruchids on different pigeonpea genotypes

The seed damage percent was significantly different among pigeonpea genotypes both under multi-choice and single choice conditions (Fig 1). The results revealed that seeds of wild species *C. platycarpus* ICPW 68 were highly resistant to damage by *C. chinensis*. It kept the *C. chinensis* population under check under storage conditions. The lowest percentage of seed damage was recorded on ICPW 68 (4.33% and 18.9%) while, ICPL 161 exhibited highest seed damage percent (59.78% and 74.40%) under multi-choice and no choice conditions respectively (Fig 1). In the present study, none of the 15 pigeonpea genotypes tested were found to be free from the damage by pulse beetle, but there were appreciable differences in their susceptibility to *C. chinensis.* The results of the present studies are in corroboration with the findings of Jadhav *et al*., (2012) who reported that bruchid damage ranged between 14 and 30% in wild relatives of pigeonpea, compared to 78% in the commercially cultivated

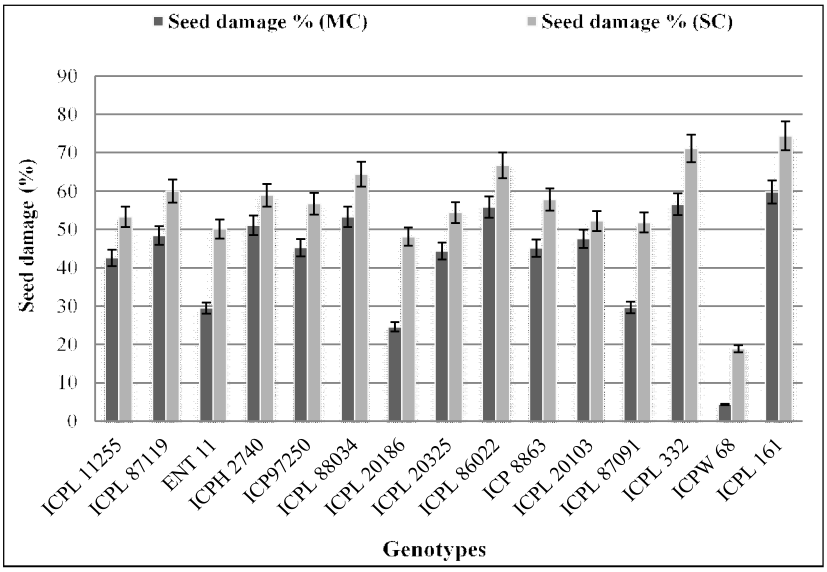

Fig 1: Evaluation of host resistance reaction of pigeonpea genotypes to *C. chinensis*.

pigeonpea variety ICPL 85010. The present findings are also in confirmation with the reports of Eker *et al*., (2018) who evaluated the kabuli accessions of chickpea YAR and ILC 8617 and reported 100% seed damage in multi-choice test, but in no-choice test, the accession CA 2969 exhibited 93% seed damage followed by the accession YAR (87.0%) and in desi chickpea, the accession ICC 4957 and *C. reticulatum* accession AWC 612, the seed damage was 83.0 per cent.

Oviposition preference of the bruchids on grains of Pigeonpea genotypes

The oviposition response of *C. chinensis* both under multi-choice and single choice conditions varied significantly (p=<0.005) across different pigeonpea genotypes (Fig 2). The results revealed that minimum number of eggs were laid on ICPW 68 both under single choice (49.37) and multi–choice (21.33) conditions, followed by ICPL 20186 (28.40) and ENT 11 (33.67). The highest number of eggs were recorded on ICPL 161 (171.38 and 68.00) under single and multi-choice tests, respectively. In other words, none of the test genotypes were found resistant to *C. chinensis* in terms of oviposition preference due to their physical and morphological traits. However, ICPW 68 was found to be statistically superior genotype among all the genotypes tested. The number of eggs deposited by *Callosobruchus* spp. was affected by seed size, curvature, colour, thickness of the seed coat and smoothness of the seeds (Mphuru, 1981). Oviposition is a paramount behavior exhibited by an insect for continuation of its race and establishment of their population (Sehgal and Sachdeva, 1985). The ovipositional responses of *C. chinensis* seem to be governed by several biotic and ecological factors. The differential preference for oviposition of *C. chinensis* on different accessions might be due to odour of the seed which could emanate from its chemical constituents, may provide the stimulus for oviposition (Howe and Curie, 1964).

Number of exit holes on seeds

There were significant differences in mean number of holes per ten grains among the genotypes. The lowest mean number of holes were observed in ICPW 68 (3.00), followed by ICPL 20186 (5.67) and ENT 11 (6.00) while highest mean number of holes were observed in ICPL 161 (12.33) (Fig 3). The results of the present studies are in agreement with the reports of Kuldeep *et al*., (2015) who recorded minimum number of emergence holes of *C. chinensis* in Pusa Komal (18.33) and maximum in IC313300 (84.67) followed by IC326996 (78.67).

Adult emergence %

The per cent adult emergence was found to be lowest on ICPW 68 (6.25), while the highest adult emergence percent was observed on ICPL 161 (31.44) (Fig 3). These findings are similar to Rosemond and Khan (2013) who reported that the adult emergence per cent ranged from 90.20 in B26 to 23.88 in B59. They screened eighteen cultivars for resistance to *C. chinensis* and reported that B26PL2, B59, B6PL2 and B194 exhibited less than 50% adult emergence. B26PL2, B59, B6PL2 and B194 did not differ significantly from each other (z-test, P>0.05). Low adult emergence coincided with low egg to adult survival on these varieties.

Percent weight loss

All the pigeonpea genotypes differed significantly in terms of percent weight loss due to *C. chinensis*. The data revealed that genotype ICPW 68 recorded significantly lowest average percent grain weight loss (2.01). ICPL 161 recorded highest average percent weight loss (24.39) (Fig 3). The findings of the present study are in corroboration with the findings of Alok *et al*., (2005) who studied the weight loss due to *C. chinensis* on green gram which incurred highest weight loss (22.88) followed by arhar (20.79), peas (15.19), Vijay (13.14), Vishal (13.03) and Kabuli (11.71). While in

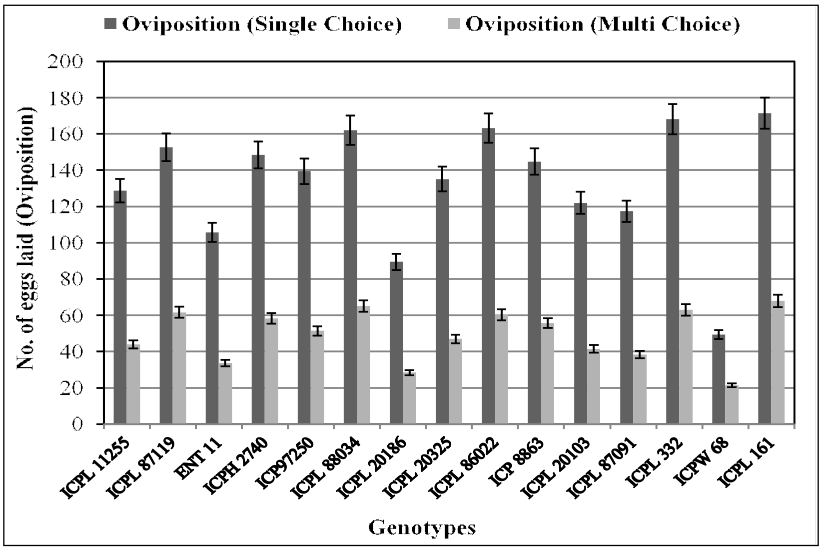

Fig 2: Ovipositional preference of *C. chinensis* towards pigeonpea genotypes.

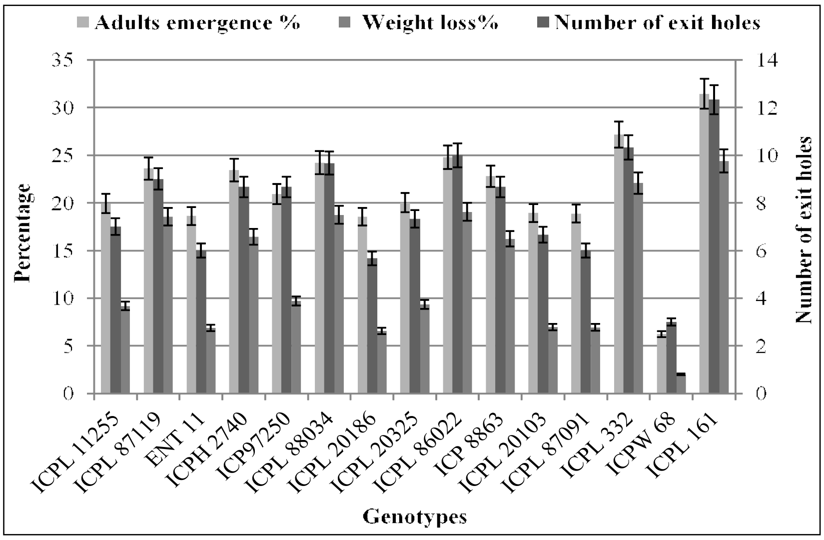

Fig 3: Evaluation of different pigeonpea genotypes against *C. chinensis*.

kidney bean no weight loss was observed as there was no infestation.

Total flavonoids content

ICPW 68 recorded high flavonoid content (1.28 mg/g) followed by ICPL 87091 (1.21 mg/g), ENT 11 (1.19 mg/g) and ICPL 20103 (1.18 mg/g). The lowest amounts of total flavonoids were recorded in ICPL 161 and ICPL 332 (0.40 and 0.36 mg/g respectively) (Table 1). Similarly, Ahmad *et al*., (2019) reported that flavonoids content of different chickpea varieties ranged from 0.283 to 0.466 mg/g. Rani *et al*., (2014) observed that flavonoids content in whole seed extract was (8.65±0.6 mg/g) in pigeonpea.

Total tannin content

The total tannin content in pigeonpea genotypes ranged from 9.69 mg/g to 16.75 mg/g with a mean value of 12.57 mg/g. The highest tannin content (mg/g) was found in ICPW 68 (16.75) followed by ICPL 20186 (15.00), ICPL 20103 (14.38) and ENT 11 (14.06). ICPL 161 recorded lowest tannin content (9.69) (Table 1). Singh and Jambunathan (1981) reported that the tannin content in pigeonpea genotypes ranged from 4.3-11.4 mg/g. Tannin content in chickpea has been reported to range from 1.9 mg/g to 6.1 mg/g (Singh *et al*., 1982) and in lentils from 0.80 mg/g to 6.48 mg/g (Ahuja *et al*., 2015).

Phenol content

The average content of total phenols was found to be higher in ICPW 68 (1.38 mg/g), followed by ICPL 87091 (1.05). ICPL 161 recorded lowest phenol content (0.44), followed by ICPL 332 (0.46), ICPL 86022 (0.48) and ICPL 88034 (0.48) (Table 1). Phenolic compounds have been reported to lower the activity of digestive enzymes such as amylase, trypsin and chymotrypsin and could also damage the mucosa of the digestive tract. The present results are in corroboration with earlier (Japjot *et al*., 2017) studies where in the wild type species *(C. scarabaeoides,* ICP15683/W15) with smallest seed size exhibited highest phenol content. Nwosu *et al.,* (2013), reported the total phenol content value of 1.6 mg/g in pigeonpea.

Total soluble sugars

ICPL 161 (47.25 mg/g) had maximum amount of sugars (mg/g) followed by ICPL 332 (45.22) and ICPL 86022 (43.09). The lowest sugar content was recorded in ICPL 20186 (27.00) followed by ENT 11 (32.14) and ICPW 68 (35.41) and these genotypes were statistically superior to the rest of the genotypes. Similarly, Japjot (2017) categorized the sugar content higher than 52.23 mg/g under higher total soluble sugars group, values between 38.44-52.23 mg/g under medium total soluble sugars group and lower than 38.84 mg/g under low total soluble sugars group (H $\geq$ 52.23; L $\leq$ 38.44). High combined larval and pupal mortality reduced adult eclosion in some cultivars and this might be due to

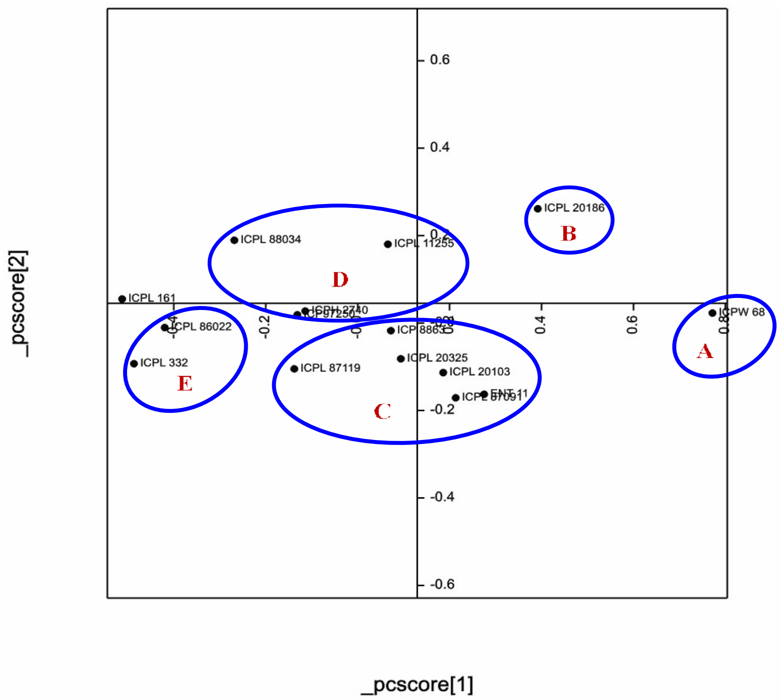

Fig 4: Principal Coordinate Analysis (PCA) based on biochemical resistance of pigeonpea in terms of per cent seed damage to *C. chinensis.*

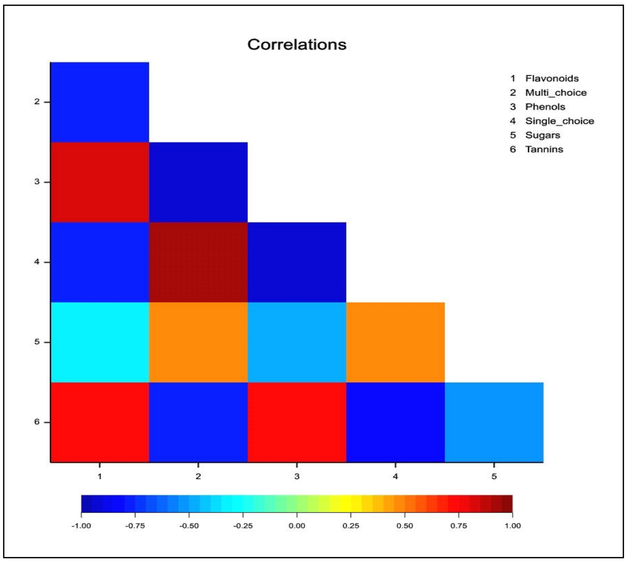

Fig 5: Correlation studies between qualitative parameters and biochemical constituents in pigeonpea against *C. chinensis.*

Table 1: Various biochemical traits of pigeonpea genotypes for resistance to *C. chinensis.*

Genotypes	Total tannins (mg/g)	Total phenols (mg/g)	Total soluble sugars (mg/g)	Flavonoids (mg/g)
ICPL 11255	13.13 (21.24)	0.88 (5.38)	32.64 (34.84)	0.77 (5.03)
ICPL 87119	12.13 (20.370	0.52 (4.13)	42.41 (40.63)	0.90 (5.44)
ENT 11	14.06 (22.01)	0.99 (5.71)	42.18 (40.50)	1.19 (6.26)
ICPH 2740	13.07 (21.19)	0.53 (4.17)	38.64 (38.43)	0.82 (5.19)
ICP97250	10.00 (18.43)	0.75 (4.97)	37.18 (37.57)	0.76 (5.00)
ICPL 88034	12.63 (20.81)	0.48 (3.97)	36.05 (36.90)	0.48 (3.97)
ICPL 20186	15.00 (22.78)	1.01 (5.77)	27.00 (31.31)	1.06 (5.91)
ICPL 20325	13.00 (21.13)	0.74 (4.93)	38.73 (38.49)	1.14 (6.13)
ICPL 86022	10.38 (18.79)	0.48 (3.97)	43.09 (41.03)	0.44 (3.80)
ICP 8863	12.75 (20.91)	0.73 (4.90)	36.23 (37.01)	1.11 (6.05)
ICPL 20103	14.38 (22.28)	0.91 (5.47)	40.32 (39.42)	1.18 (6.23)
ICPL 87091	11.31 (19.64)	1.05 (5.88)	40.00 (39.23)	1.21 (6.31)
ICPL 332	10.25 (18.66)	0.46 (3.89)	46.95 (43.25)	0.40 (3.62)
ICPW 68	16.75 (24.15)	1.38 (6.74)	35.41 (36.52)	1.28 (6.49)
ICPL 161	9.69 (18.13)	0.44 (3.80)	40.82 (39.71)	0.36 (3.44)
SE d±	0.21	0.17	0.92	0.58
C.D at 0.05%	0.53	0.42	2.31	1.46

Figures in the parentheses are angular transformed values.

biochemical composition of the seeds which also appear to play a role in reducing populations of bruchids on *C. cajan*.

Association of biochemical components with expression of resistance to *C. chinensis* using principal coordinate analysis

Principal coordinate analysis placed the test genotypes into different clusters. The resistant genotype ICPW 68 was placed distinctly in cluster A. The moderately resistant genotype ICPL 20186 was placed in cluster B, while the susceptible genotypes were placed in clusters C, D and E. (Fig 4) suggesting the possibilities for developing lines resistant to *C. chinensis.*

Correlation studies of pigeonpea genotypes resistant to bruchids

The correlation studies between per cent seed damage and biochemical parameters of pigeonpea genotypes revealed that percent seed damage in both multi-choice and single choice conditions exhibited significant and negative correlation with flavonoids (r = -0.752 and r = -0.776), phenol (r = -0.942 and r = -0.921) and tannins (r = -0.751 and r = -0.832). A significant positive correlation was observed with total soluble sugars (r = 0.479 and r = 0.461) (Fig 5). Similarly, Ambidi *et al*., (2022) reported that phenolic content and pod damage by pod borer complex showed highly significant negative association with r-value (-0.729**). This clearly indicates that high phenol content played a critical role in resisting pod borers under field conditions.

CONCLUSION

The results revealed that pigeonpea genotypes and their wild relatives varied significantly in terms of high anti nutritional contents, which exhibited greater resistance to the *C. chinensis*. Hence, promising genotypes could be used in breeding programs to develop hybrids/ cultivars resistant to *C. chinensis.*

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