



# OPEN Identification of stable resistant wild pigeonpea donors to multiple predominant bruchid species in India

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Identifying stable-resistant sources offers an eco-friendly and sustainable insect management strategy. Sixty-eight wild pigeonpea accessions were systematically screened against three predominant bruchid species (*Callosobruchus analis*, *C. chinensis*, *C. maculatus*) using a high-throughput screening pipeline at Kanpur, India, from 2021 to 2024. Resistance classification was based on seed damage and susceptibility index. Four *Rhyncosia bracteata* accessions consistently exhibited convergent and complete resistance across species, while few *C. scarabaeoides* accessions were resistant to *C. chinensis* and *C. maculatus* but moderately stable against *C. analis*. Multivariate analyses revealed significant species- and condition-driven variation, with seed damage, emergence and developmental period, as key discriminators. Correlation funnel confirmed antibiosis as the predominant resistance mechanism, while antixenosis was species-specific, particularly against *C. maculatus*. Machine learning model predicted seed and insect traits as major contributors. Factorial Analysis of Mixed D and hierarchical clustering validated biologically meaningful grouping of resistant versus susceptible accessions, with biochemical traits enhancing separation of intermediate classes. Collectively, *R. bracteata* emerges as a robust multi-species resistance donor, and *C. scarabaeoides* as a species-specific donor. These multi-species resistant resources act as invaluable resources for introgression breeding to develop bruchid-resistant pigeonpea cultivars and highlight opportunities to dissect biochemical pathways and QTLs underlying resistance.

**Keywords** Pigeonpea, Bruchids, *Callosobruchus*, Resistance, Stable donors

Bruchids, also known as, pulse or seed beetles, are major pests of legume crops worldwide. They attack matured or dried grains both in the field and during storage, often causing substantial economic losses, especially in stored grains<sup>1</sup>. Bruchids are cosmopolitan in distribution, with over 1700 species described worldwide across 62 genera. Among these, the predominant genera include *Callosobruchus*, *Bruchus*, *Bruchidius*, and *Acanthoscelides*<sup>2,3</sup>. In India, over 100 species under 11 genera are documented, with *Callosobruchus* being most significant<sup>4</sup>. Among them, *Callosobruchus maculatus* (F.), *C. analis* (F.), and *C. chinensis* L. are widespread causing extensive damage to stored pulses<sup>5</sup>.

Pulses are vital crops for the livelihoods of tropical and subtropical nations including India, which is both largest producer (~25 MT, 25% of global production) and the largest consumer. Despite recent progress toward self-sufficiency, imports remain substantial at 4.7 MT in 2023–24<sup>6</sup>. Government initiatives have targeted productivity gains, especially in pigeonpea, black gram, and lentils, but policy efforts alone cannot meet domestic demand; multidimensional research is also essential to reduce both field and post-harvest losses. Current post-harvest losses in pulses are estimated at 5.65–8.41% (nearly USD 1 billion), with storage losses contributing ~2%, largely due to insect damage<sup>7,8</sup>. Pigeonpea is particularly vulnerable, with storage losses of 1.54–1.9%. Bruchids alone can cause 4–60% grain damage, sometimes reaching total loss under unmanaged infestations<sup>3,9</sup>. NITI Aayog<sup>6</sup> projects pulse production to reach 30 MT by 2030 and 45.79 MT by 2050; at ~2% storage loss, this equals

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0.6–1 MT or more of annual grain loss by 2050. Thus, strengthening post-harvest protection, especially against bruchids, is crucial for safe pulse storage and for ensuring national food and nutritional security.

Traditional and modern approaches for managing bruchids remain inconsistently effective<sup>3</sup>. In India, only one contact insecticide (deltamethrin) and two fumigants (phosphine, EDCT) are available, and their overuse has led to resistance, residues, and health and environmental risks<sup>10</sup>. Reports of rising phosphine resistance<sup>11</sup> underscore the need for effective, practical, and sustainable alternative strategies.

Identification and utilization of genetic sources of resistance in grain legumes through host plant resistance (HPR) remains one of the most reliable and sustainable strategies for bruchid management. HPR relies on plant traits that adversely affect insect development, survival, and biological fitness, thereby reducing grain damage<sup>12,13</sup>. Because bruchids are oligophagous, the discovery of diverse resistance mechanisms is particularly valuable for developing durable resistant cultivars while broadening the genetic variability. Achieving this requires systematic exploration of wild relatives across multiple gene pools related to the target crop.

In pigeonpea, numerous wild accessions have been collected, characterized, and conserved in Indian institutes such as ICRI SAT, NBPGR, and IIPR, and these resources have been widely explored for resistance to major biotic (pod borers, wilt, SMD) and abiotic (draught, salinity) stresses<sup>14–18</sup>. Further, the utilization of wild *Cajanus* species has facilitated the introgression of productivity-enhancing and stress-resistance traits into cultivated pigeonpea. Through modern genomics and pre-breeding approaches, these wild alleles are helping broaden the crop's genetic base, thereby improving yield stability and resilience under challenging environments<sup>19–21</sup>. Although few studies<sup>22–24</sup> have identified bruchid-resistant donors in certain wild and cultivated *Cajanus* species against *C. maculatus* and/or *C. chinensis* from India, these findings were based on single-screening condition and a single resistance scoring criterion. Moreover, none of these species were evaluated against *C. analis*—a often-overlooked yet equally prevalent and more destructive species<sup>5,25,26</sup>. This gap limits the broader applicability of the previously identified resistant donors for comprehensive bruchid management.

Considering these limitations, this study aimed to identify and validate stable bruchid-resistance donors from diverse wild pigeonpea germplasms against all three major bruchid species infesting stored pulses across India<sup>5,25,26</sup>. Unlike earlier studies; a high-throughput protocol integrating multiple screening conditions and resistance scoring criteria over three consecutive years enabled systematic and comprehensive evaluation of the germplasm panel. The study also examined host-insect trait interactions to identify key predictors of resistance. Importantly, the study reports stable, multi-species, convergent resistance donors in wild pigeonpea accessions with strong potential for deployment in introgression breeding to develop bruchid-resistant cultivars.

## Material and methods

### Location of the study, plant and insect materials

The study was conducted between 2021 and 2024 at the Storage Entomology Laboratory, Division of Crop Protection, ICAR-Indian Institute of Pulses Research (IIPR), Kanpur, India. Sixty-eight diverse wild pigeonpea accessions were evaluated for bruchid resistance. These accessions, maintained in wild garden and genebank, were originally collected from different agro-ecological regions and national repositories (NBPGR & ICRI SAT) including IIPR. Seeds were screened against three major bruchid species<sup>5</sup>—*Callosobruchus chinensis*, *C. analis*, and *C. maculatus*. Species identity was confirmed using standard morphological keys and molecular characterization<sup>25</sup>. Pure cultures of the three species were maintained under controlled conditions ( $28 \pm 1^\circ\text{C}$ ,  $70 \pm 2\%$  RH, and 12 h photoperiod), with uniform-aged populations obtained through continuous subculturing on sterilized mungbean seeds.

### Screening of accessions against bruchid species

Screening was conducted following high-throughput multiple screening protocols<sup>25,27</sup>. All 68 accessions were initially evaluated under free-choice (FC) screening condition, followed by no-choice (NC) to impose maximum pressure on seeds and assess resistance under contrasting conditions. Twenty-three accessions that exhibited varied resistance reactions against the three species in these two screenings were further validated in confirmatory no-choice (CNC) screening.

The FC tests were conducted in modified screening chambers with each accession replicated three times using 30 seeds per replicate<sup>25,27</sup>. The NC and CNC tests were conducted in plastic vials ( $4 \times 5$  cm,  $W \times H$ ), enabling rigorous validation of resistance responses. Each vial contained 30 seeds per accession, with three replicates per accession.

Across all assays, freshly emerged 3–4-day-old adults were used at three pairs per replicate (one pair per ten seeds). Sexes were distinguished using antennal and pygidium morphology. Adults were removed after 72 h of exposure, initial seed infestation was recorded, and samples were incubated under standard conditions used for culturing. Post-emergence observations began once F1 adults emerged in any replicate.

### Data collection and statistical analyses

#### Seed morphological traits

Seed morphological traits were recorded for all 68 accessions prior to initiating bruchid infestation, as these traits contribute to antixenosis and influence oviposition preference. Both quantitative and qualitative traits were recorded using 10 seeds per accession with three replications. Test weight ( $x100sw$ ) was recorded by measuring the weight (g) of 100 seeds. Seed length (sl) and width (sw) were measured with a vernier caliper and expressed in millimeters. Seed surface area (ssa) was derived from length and width using the formula of McCabe et al.<sup>28</sup> and expressed in  $\text{mm}^2$ . Qualitative traits including seed texture (st), seed coat color (scc), seed coat pattern (sccp), seed shape (ss), and seed hilum (sh), were recorded using a hand lens and stereo-zoom trinocular microscope following ICAR-NBPGR descriptors<sup>29</sup>.

### Seed biochemical traits

Biochemical traits were quantified from seeds of 16 accessions that consistently exhibited resistance responses across species and screening conditions, along with a susceptible check (ICP15630, G23). This was done to understand the role of seed antibiosis, and three replications were used for each accession. The biochemical estimations were performed at ICRISAT, Hyderabad.

Protein content (prot) was determined by Lowry's method<sup>30,31</sup> using Folin-Ciocalteu reagent (FCR). A standard curve was prepared using bovine serum albumin (BSA), and absorbance was measured at 660 nm and expressed as mg protein per g seed sample. The tannin content (tan) using the Vanillin-Hydrochloride method, which relies on the reaction of phenolic compounds with vanillin under acidic conditions to form a red-colored complex measurable at 500 nm and expressed as mg tannin per gram of sample<sup>32</sup>. Total phenolic content (phen) in the sample was estimated using the Folin-Ciocalteu method, wherein the FCR is reduced by phenolic compounds under alkaline conditions to generate blue-colored complex measurable at 650 nm<sup>33</sup>. A catechol-based standard curve was used, and values were expressed as catechol equivalents (mg/g sample). Total soluble sugars (cho) were quantified using the Anthrone method<sup>32</sup>, in which carbohydrates are dehydrated by concentrated sulfuric acid to form furfural derivatives, which react with anthrone to produce a blue-green chromogen measurable at 630 nm. A glucose standard curve was used, and results were expressed as mg g<sup>-1</sup> seed sample. Flavonoids (flav) were estimated by the vanillin-sulfuric acid method<sup>34</sup>, where phloroglucinol-type compounds react with vanillin in the presence of concentrated sulfuric acid to form a red complex measured at 500 nm. Results were expressed as absorbance values ( $A_{500}$ ).

### Insect traits

Insect infestation traits were recorded during pre-incubation and post-emergence phases. Pre-incubation observations included oviposition (op) and egg density (ed). Post-emergence traits included seed damage (gd), adult density (ad), number of males (m), and number of females (f). Adult emergence was monitored daily for 30 days after first emergence and the mean developmental period (mdp) was calculated with this data using Howe's formula<sup>35</sup>. Oviposition and seed damage were expressed as percentages, while egg, adult, and sex counts were recorded per 30-seed sample. Adult survivability (as) percentage was estimated as per Seram et al.<sup>36</sup>.

### Categorization of resistance reactions

Resistance reactions by accessions were categorized using two established criteria: susceptibility index (SI;  $rflag\_si$ ) and seed damage (SD,  $rflag\_sd$ ). SI was calculated from adult survivability and mean developmental period<sup>35,37</sup>. Accessions were categorized as resistant (R,  $SI < 0.05$ ), moderately resistant (MR, 0.051–0.060), moderately susceptible (MS, 0.061–0.070), susceptible (S, 0.071–0.080), and highly susceptible (HS,  $> 0.081$ ). The seed damage scale directly categorized accessions as highly resistant (HR, 0% damage), resistant (R, 1–9%), moderately susceptible (MS, 10–69%), and highly susceptible (HS, 70–100%)<sup>35</sup>.

### Statistical analyses

Data from insect, seed morphological and biochemical traits were analyzed using multiple statistical approaches to understand trait interactions across accessions, screening conditions, and species. Data were first tested for normality and suitability prior to analysis wherever necessary. The detailed statistical analyses adopted for this are as follows:

- Descriptive statistics and boxplots** Descriptive statistics, including ANOVA was done ascertain the variability of different traits between accessions. These were worked out for insect, seed morphological and biochemical traits using the *TraitStats* package<sup>38</sup>. Boxplots were prepared with *ggplot2*<sup>39</sup>.
- MANOVA, Univariate analyses, Correlation funnel and Random Forest:** Multivariate analysis of variance (MANOVA) was employed to examine whether insect traits varied significantly across screening conditions (FC vs. NC) within each species and between species (when pooled across conditions). Wilks' Lambda was used as the test statistic to evaluate multivariate effects of species or condition. When MANOVA indicated significant differences, univariate ANOVA was applied on individual traits to identify the specific contributors of observed variation. Analyses were carried out in R using the base '*stats*' package for MANOVA and ANOVA and visualization through *ggplot2* and *gridExtra*<sup>40</sup>. Separate MANOVAs were run within each species (FC vs. NC) and across species (FC and NC pooled), with significance tested at  $\alpha = 0.05$ .

To investigate role of each trait on the observed bruchid resistance, the *correlationfunnel* package version 0.2.0 in R was employed<sup>41</sup>. Two resistance criterion-based flags ('*rflag\_sd*' for SD and '*rflag\_si*' for SI) were used as outcome variables representing resistant categories of accessions. The method binarizes numeric and categorical variables and quantifies their correlation with a binary target, thereby ranking the strength traits-resistance associations. Funnels were constructed separately for each of the three bruchid species, and for both SD- and SI-based resistance classifications, enabling rapid exploratory identification of key insect and seed morphological traits linked with resistance or susceptibility.

The identification of key traits strongly associated with resistance was further validated using Random Forest (RF). RF was selected because it is particularly effective for high-dimensional biological datasets, offering the ability to model complex nonlinear interactions while minimizing risks of overfitting. In this study, RF classification was applied to predict bruchid-resistance traits in wild pigeonpea using two independent indicators: resistance categories based on seed damage (*rflag\_sd*) and susceptibility index (*rflag\_si*). Two datasets were analyzed: (i) insect and seed morphological traits across 68 germplasms, and (ii) insect, seed morphological and biochemical traits across 17 germplasms. Resistance categories were binarized by grouping "HR" and "R" as Resistant and "MS", "S", and "HS" as Susceptible, while "MR" accessions excluded to avoid ambiguity. Models were implemented

in R using the *caret* package<sup>42</sup> for training and resampling, and *randomForest* for classification<sup>43</sup>. Repeated five-fold cross-validation was used for the 68-accession dataset, while leave-one-out cross-validation was employed for the smaller 17-accession dataset. Data preprocessing, reshaping, and visualization were performed using the *tidyverse* suite<sup>44</sup>. For each classification model, the area under the ROC curve (AUC) and performance metrics (accuracy, sensitivity, specificity) and compiled into a single summary table (Table 6). All intermediate outputs (confusion matrices, ROC plots, partial dependence plots, and variable importance rankings) are provided in the supplementary files (Supplementary files 3–5) for reproducibility.

c. *Grouping of accessions through FAMD and Clustering* Since the study recorded both qualitative and quantitative data, the Factorial Analysis of Mixed Data (FAMD) was used to jointly analyse quantitative insect and seed traits through PCA and qualitative resistance categories through MCA, thereby providing a unified ordination framework. Analyses were conducted in R using *FactoMineR*<sup>45</sup> and *factoextra*<sup>46</sup>, with plots refined using *ggplot2*<sup>39</sup>. Two levels of analysis were performed: (i) all 68 germplasms per species, using insect traits data from FC and NC conditions, and (ii) a subset of 17 germplasms, incorporating insect traits from FC, NC, and CNC conditions along with biochemical traits. In both analyses, accessions were grouped by resistance categories (HR, R, MR, MS, S, HS), and 95% confidence ellipses were plotted to visualize within-group variance.

To further explore variability and grouping pattern among pigeonpea germplasms, hierarchical clustering was performed using both insect and seed morphological traits, and subsequently incorporating biochemical traits. For each bruchid species, datasets from FC and NC tests were combined by averaging trait values per germplasm. Morphological traits were merged by germplasm identity, and in a second analysis, biochemical traits were integrated for the 17 germplasms. All quantitative traits were standardized (Z-score) to eliminate scale effects. Hierarchical clustering was done based on Euclidean distances and Ward's minimum variance in R with *phatmap* 1.0.13 package<sup>47</sup>. For each species, two heatmaps were generated: (i) insects + morphological data of 68 germplasms, and (ii) insects + morphological + biochemical data of 17 germplasms. Dendrograms were cut into four clusters, enabling identification of germplasm groups with similar insect response patterns and evaluation of whether morphological or biochemical traits improved resistance discrimination.

d. *Role of antibiosis in accessions' resistance against bruchid species* Correlation analyses were performed between insects and seed biochemical traits recorded for 17 germplasms under FC, NC, & CNC conditions to ascertain the influence of seed antibiosis on insect development. Pearson correlation coefficients ( $r$ ) were computed using *Hmisc* package in R<sup>48</sup> with adjusted  $p$ -values, and heatmaps (*ggplot2*<sup>39</sup>) were generated for each bruchid species, combining all three screening conditions into a single correlation matrix, with significance levels indicated by stars (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

e. *Influence of accessions' resistance levels on sex ratio of bruchid species* This analysis tested whether sex ratio (male/(male + female)) varied across germplasm resistance classifications (SD- and SI- based) using male and female data from two FC and NC conditions for each species. Sex ratio values  $< 0.5$  indicated female-biased emergence, while  $> 0.5$  indicated male-biased emergence.

Analyses followed a three-tier approach. First, exploratory visualization was performed using *ggplot2*<sup>39</sup>, generating boxplots of sex ratio distributions per resistance class, faceted by species (CA, CC, CM) and screening condition (FC, NC). Second, linear mixed-effects models (LMMs) were fitted using *lme4*<sup>49</sup> and *lmerTest*<sup>50</sup>, with sex ratio as the response, resistance flags  $\times$  condition  $\times$  species as fixed effects, and germplasm as random intercept. Since sex ratio is a proportion, LMMs were used rather than arcsine transformation, which is unsuitable for ecological proportion data<sup>51</sup>. Post-hoc Tukey comparisons were obtained using *emmeans*<sup>52</sup>, and model fit was evaluated using DHARMA<sup>53</sup>. Third, validation analyses were conducted with classical ANOVA with Tukey HSD. Given that sex ratio is a proportion, beta-regression models (*betareg*<sup>54</sup>) and beta-family GLMMs (*glmmTMB*<sup>55</sup>) were fitted as alternatives, particularly for data with many zero/one values.

## Results and discussion

### Germplasm details

The study utilized 68 wild pigeonpea accessions belonging to two genera: *Cajanus* (49 accessions) and *Rhynchosia* (19 accessions). The genera *Cajanus* represented by five species, including *C. scarabaeoides* (30 accessions), *C. platycarpus* (9), *C. crassus* (6), *C. cajanifolius* (3), and *C. mollis* (1). The genus *Rhynchosia* was represented by three species: *R. minima* (9 accessions), *R. rothii* (6), and *R. bracteata* (4). Together, the panel encompassed accessions from the primary, secondary, tertiary, and quaternary gene pools of pigeonpea, reflecting broad genetic diversity with considerable variability for resistance reported to diverse biotic and abiotic stresses (Table 1, Fig. 1).

### Variability of seed morphological and biochemical traits

The variation was highly significant among accessions for all the quantitative seed morphological traits ( $p < 0.001$ ). Seed length varied  $\sim 2$ – $6$  mm while averaged at 4 mm, width ranged  $\sim 1.4$ – $4.4$  mm ( $\sim 2.4$ ), surface area ranged  $\sim 2.8$ – $19$  mm<sup>2</sup> ( $\sim 7.9$ ), and 100-seed weight 3.45 g. The highest variability was observed for 100-seed weight (ANOVA = 440.2, CV = 53.2%) and surface area (314.7, 40.9%) followed by other two traits (Table 2, Fig. 2, Supplementary file 1).

Significant genotypic differences were also observed in biochemical composition ( $p < 0.001$ ). Proteins ranged between 139–223 mg/g while averaged at  $\sim 191$ , tannins ranged  $\sim 18$ – $47$  mg/g (28), phenols 0.93–1.96 mg/g (1.23), total sugars 25–59 mg/g ( $\sim 45$ ), and flavonoids 0.1–0.39 mg/g (0.27). Variability was greatest for sugars,

| Sl. No    | Species                                             | Collection/ accession no | Germplasm Code | Collection source                                                       | Gene pool  | Useful traits reported                                                                                                                                                                                                                                             | Source                                                                                                                                                                                                                                                                             |
|-----------|-----------------------------------------------------|--------------------------|----------------|-------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.        | <i>Cajanus scarabaeoides</i> (L.) Thouars           | ICP15799                 | G1             | NBPGR, ICRISAT, Chattisgarh, West Bengal, Madhya Pradesh                | Secondary  | Tolerance to <i>C. maculatus</i> , pod borer, pod wasp and pod fly, Fusarium resistance; SMD, cyst nematode; dwarfism, high protein content, draught, salinity and aluminium toxicity tolerance; CMS (A2 cytoplasm)                                                | Sharma et al. <sup>56</sup> ; Saxena et al. <sup>57</sup> ; Mallikarjuna <sup>58</sup> , 2011b <sup>22</sup> ; Srivastava et al., 2006b <sup>59</sup> ; Nagaraja et al. <sup>15</sup> ; Lamichaney et al. <sup>60</sup> ; Bohra et al. <sup>14</sup> ; Khoury et al. <sup>61</sup> |
|           |                                                     | ICP15886                 | G2             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15688                 | G3             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15713                 | G4             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15699                 | G5             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15724                 | G6             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15687                 | G7             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15710                 | G8             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | DD/SPD-3                 | G9             |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15731                 | G10            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15695                 | G11            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15690                 | G12            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | SG/DD-13                 | G13            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | IC15698                  | G31            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15700                 | G32            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15725                 | G33            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15882                 | G34            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15883                 | G35            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15685                 | G36            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15689                 | G37            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15711                 | G38            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | SG/DD-701                | G39            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15922                 | G40            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15712                 | G41            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15727                 | G42            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | SG/DD-71                 | G48            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | DD/SPD-2                 | G49            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| ICP15633  | G65                                                 |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| SG/DD-15  | G66                                                 |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| SG/DD-64  | G67                                                 |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| 2.        | <i>Cajanus cajanifolius</i> (Haines) van der Maesen | ICP15630                 | G23            | NBPGR, ICSRIST                                                          | Secondary  | Salinity and pod borer tolerance; source of CMS (A5 cytoplasm)                                                                                                                                                                                                     | Lamichaney et al. <sup>60</sup>                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15631                 | G24            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15632                 | G25            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| 3.        | <i>Cajanus crassus</i> (King) Maesen)               | ICP15767                 | G14            | NBPGR, ICRISAT                                                          | Tertiary   | SMD resistance; high protein content; source of A3 cytoplasm                                                                                                                                                                                                       | Remanandan <sup>62</sup> ; Khoury et al. <sup>61</sup> ; Lamichaney et al. <sup>60</sup>                                                                                                                                                                                           |
|           |                                                     | ICP15774                 | G15            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15773                 | G16            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15771                 | G17            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15616                 | G18            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| ICP15776  | G19                                                 |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| 4.        | <i>Cajanus platycarpus</i> (Benth.) Maesen          | ICP15663                 | G20            | NBPGR, ICRISAT, Chhattisgarh, West Bengal, Jharkhand and Madhya Pradesh | Tertiary   | Tolerance to bruchid, pod borer and salinity, Cyst nematode resistance, resistance to <i>Phytophthora</i> and <i>Alternaria</i> blight, SMD, extra early flowering, vigour and maturity, photoperiod insensitivity; Higher pod set and seed protein; source of CMS | Mallikarjuna et al. <sup>63</sup> , 2011a <sup>64</sup> , 2011b <sup>22</sup> ; Srivastava et al., 2006a <sup>65</sup> ; Lamichaney et al. <sup>60</sup> ; Jadhav et al. <sup>23</sup> ; Mishra et al. <sup>24</sup>                                                               |
|           |                                                     | ICP15703                 | G21            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15661                 | G22            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | DD-SPD-1                 | G50            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15662                 | G44            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15664                 | G45            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15665                 | G46            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15666                 | G47            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| ICP15921  | G43                                                 |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| 5.        | <i>C. mollis</i> (Benth.) Maesen                    | ICP15653                 | G63            | NBPGR, ICRISAT                                                          | Tertiary   | High protein content, more seeds per pod                                                                                                                                                                                                                           | Remanandan <sup>62</sup> ; Upadhyaya set al. <sup>66</sup> ; Lamichaney et al. <sup>60</sup>                                                                                                                                                                                       |
| 6.        | <i>Rhynchosia bracteata</i> Baker                   | ICP15816                 | G60            | NBPGR, ICRISAT                                                          | Quaternary | Pod borer and pod fly tolerance                                                                                                                                                                                                                                    | Mallikarjuna et al., <sup>67</sup> ; Sharma et al. <sup>56</sup> ; Lamichaney et al. <sup>60</sup>                                                                                                                                                                                 |
|           |                                                     | IC618509                 | G61            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15817                 | G26            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
|           |                                                     | ICP15815                 | G27            |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |
| Continued |                                                     |                          |                |                                                                         |            |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                    |

| Sl. No | Species                          | Collection/ accession no | Germplasm Code | Collection source                                              | Gene pool  | Useful traits reported                                                                          | Source                               |
|--------|----------------------------------|--------------------------|----------------|----------------------------------------------------------------|------------|-------------------------------------------------------------------------------------------------|--------------------------------------|
| 7.     | <i>Rhynchosia minima</i> (L.) DC | ICP15830                 | G28            | NBPGR, Chhattisgarh, West Bengal, Jharkhand and Madhya Pradesh | Quaternary | Medicinal value                                                                                 | Lamichaney et al. <sup>60</sup>      |
|        |                                  | SG/DD/4-1                | G29            |                                                                |            |                                                                                                 |                                      |
|        |                                  | SG/DD/07                 | G30            |                                                                |            |                                                                                                 |                                      |
|        |                                  | SG/DD-02                 | G54            |                                                                |            |                                                                                                 |                                      |
|        |                                  | SG/DD-03                 | G55            |                                                                |            |                                                                                                 |                                      |
|        |                                  | ICP15838                 | G56            |                                                                |            |                                                                                                 |                                      |
|        |                                  | SG/DD-76                 | G57            |                                                                |            |                                                                                                 |                                      |
|        |                                  | SG/DD-4                  | G58            |                                                                |            |                                                                                                 |                                      |
|        |                                  | DD/SPD-12                | G62            |                                                                |            |                                                                                                 |                                      |
| 8.     | <i>Rhynchosia rothii</i> Aitch   | ICP15851                 | G51            | NBPGR, ICRISAT                                                 |            | Early and high flowering, high protein content, drought tolerance and photoperiod insensitivity | Lamichaney et al. 2024 <sup>60</sup> |
|        |                                  | SG/DD-05                 | G52            |                                                                |            |                                                                                                 |                                      |
|        |                                  | ICP15853                 | G53            |                                                                |            |                                                                                                 |                                      |
|        |                                  | ICP15857                 | G59            |                                                                |            |                                                                                                 |                                      |
|        |                                  | ICP15658                 | G64            |                                                                |            |                                                                                                 |                                      |
|        |                                  | ICP15858                 | G68            |                                                                |            |                                                                                                 |                                      |

**Table 1.** Details of wild pigeonpea accessions screened for their resistance against three bruchids species.

flavonoids and tannins (CV > 25%), while lower for phenols. Among accessions, protein content was highest in G33 and lowest in G34. Tannin content was highest in G34 and lowest in G40. Phenol content was maximum in G26 and minimum in G23, with G26, G60, G27, and G61 remaining on the higher side. Soluble sugars varied widely, with the maximum in G36 and the minimum in G37. Flavonoids peaked in G38, followed by G36, G33, and G34, whereas the lowest value was in G67. Similarly, tannins were abundant in G34, G36, G33, and G32, while G40 exhibited the least (Table 2, Fig. 2, Supplementary file 1.xlsx).

**Variability in insect infestation traits**

Significant variance in insect traits was recorded between accessions across screening conditions and species (Table 3, Figs. 3, 4, Supplementary file 1). Egg density (ed) varied significantly higher ( $p < 0.001$ ) among accessions within each species and screening condition. However, the average egg density per seed by three species across accessions was > 1 (1.15–2.5) indicating good egg laying under all screening conditions. Between species, *C. chinensis* consistently recorded higher average density (~ 2.3–2.5 per seed) under three screening conditions, followed by *C. analis* (~ 1.5–2.0). While *C. maculatus* (CM) had lower (1.1–1.5).

Oviposition (op) differed significantly ( $p < 0.001$ ) across accessions. For *C. analis* and *C. chinensis*, the mean oviposition exceeded 90% under three screening conditions, while *C. maculatus* displayed more variable oviposition with < 83% in FC and NC (3.33–100%), and higher in CNC (98%, range 90–100%). Seed damage (gd) exhibited the strongest differentiation between accessions ( $p < 0.001$ ). Overall, the average seed damage (%) did not cross 60, 50 and 26 for *C. analis*, *C. chinensis*, and *C. maculatus*, respectively. Few of the prominent accessions suffered nil damage against three species in three conditions and while susceptible ones suffered higher up to 100%. Between screening conditions, the mean seed damage was higher in FC and NC, while it was lower in CNC. *C. analis* inflicted consistently higher mean seed damage across accessions and screening conditions followed by *C. chinensis* and *C. maculatus*. *C. maculatus* recorded overall lowest damage, with many accessions sustaining < 10% under CNC.

Total adults (ad), male (m) and female (f) densities also differed significantly between accessions. *C. analis* and *C. chinensis* produced > 15 adults per replication ( $n = 30$  seeds) in FC and NC, but numbers were slightly reduced under CNC. *C. maculatus* recorded far fewer adults overall (mean < 8), with several resistant accessions none. Between species, *C. analis* produced the highest adult densities, *C. chinensis* intermediate, and *C. maculatus* the lowest. However, based on the range values, *C. chinensis* record up to 55 adults per 30 seeds while against 30 for remaining two species. Adult survivability (as) varied significantly among accessions within each species. In *C. analis*, survivability averaged ~ 31% in FC, rose to ~ 42% in NC, but dropped to 26% under CNC. *C. chinensis* recorded > 50% survivability in NC, while *C. maculatus* was often below 20%. Between conditions, CNC consistently recorded reduced survivability as it contained majority of resistant accessions with one check. Overall, across species, *C. analis* exhibited the highest survivability, *C. chinensis* moderate, and *C. maculatus* the lowest.

Mean developmental period (mdp) also differed significantly. *C. analis* completed development in ~ 28 days, *C. chinensis* in ~ 29 days, and *C. maculatus* required longer (~ 34 days). CNC assays tended to prolong development compared to FC and NC. Susceptibility index (si) index values differed markedly among accessions within species. In *C. analis* and *C. chinensis*, the mean SI clustered around 0.04–0.05 under FC and NC but declined in CNC, whereas CM accessions often recorded < 0.04. Overall, the mean SI values were not more than 0.5 across screening conditions and species.

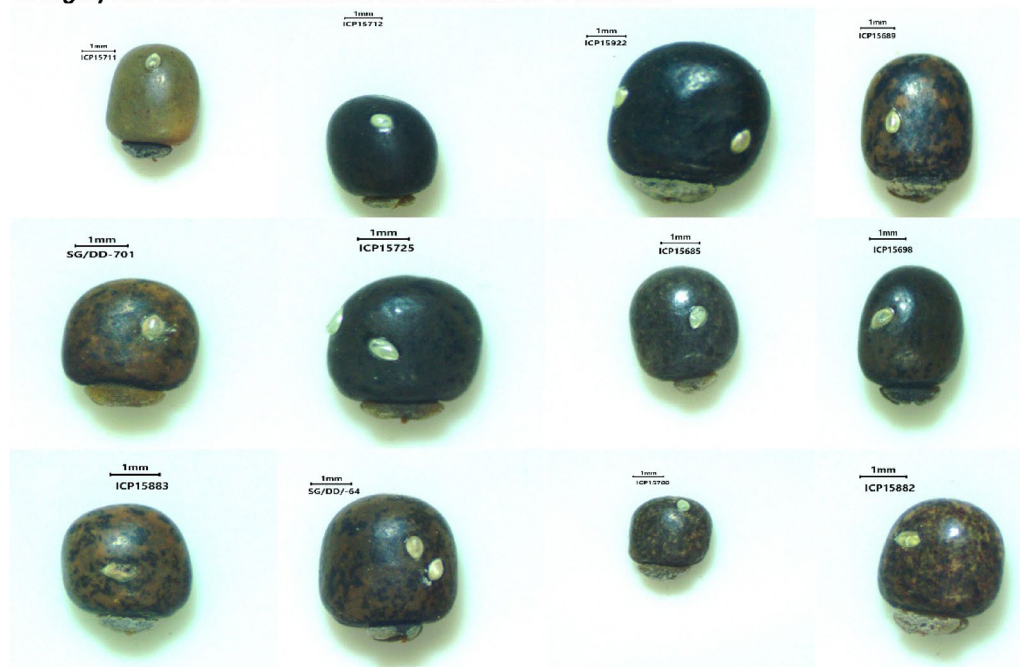
**Categorization of resistance reactions**

Based on susceptibility index (SI) and seed damage (SD), wild pigeonpea accessions showed wide variability in their resistance to three bruchid species under three screening conditions (Tables 4 and 5).

# a. Highly resistant to all the three bruchid species



# B. Highly resistant to *C. maculatus* and resistant to *C. chinensis*



# C. Resistant to *C. maculatus* and *C. chinensis*



# E. Susceptible check



# D. Resistant to *C. maculatus* and moderately susceptible to *C. chinensis*



**Fig. 1.** Contrasting accessions of wild pigeonpea screened against three bruchid species.

For *C. analis* (as per SI), 30 accessions were resistant under FC, 33 under NC, and 20 under CNC. Notable donors included *R. bracteata* (G26, G27, G60, G61) and several *C. scarabaeoides* entries (G31–G42, G66, G67, G13, G12, G8, G9). However, SD revealed that *R. bracteata* (G26, G27, G60, G61) were the only highly resistant (HR) accessions across all test conditions, while *C. scarabaeoides* accessions were resistant only in FC and shifted to MS or HS in NC and CNC. For *C. chinensis* (SI), 36 accessions were resistant under FC, 26 under NC, and 22

| Descriptor \ Trait | Seed length (sl) (mm) | Seed surface area (ssa) (mm <sup>2</sup> ) | Seed width (sw) (mm) | 100 seed weight (x100sw) (g) | Total soluble sugars (cho) (mg/g) | Total flavonoids (flav) (A <sub>500</sub> ) | Total phenols (phen) (mg/g) | Total proteins (prot) (mg/g) | Total tannins (tan) (mg/g) |
|--------------------|-----------------------|--------------------------------------------|----------------------|------------------------------|-----------------------------------|---------------------------------------------|-----------------------------|------------------------------|----------------------------|
| Mean               | 4.05                  | 7.95                                       | 2.41                 | 3.45                         | 44.79                             | 0.27                                        | 1.23                        | 191.32                       | 28.01                      |
| Min                | 2.21                  | 2.82                                       | 1.39                 | 0.90                         | 25.66                             | 0.10                                        | 0.93                        | 139.16                       | 18.20                      |
| Max                | 6.08                  | 18.90                                      | 4.37                 | 8.00                         | 69.59                             | 0.39                                        | 1.63                        | 223.15                       | 47.51                      |
| SE(m)              | 0.09                  | 0.18                                       | 0.06                 | 0.09                         | 3.30                              | 0.02                                        | 0.09                        | 11.21                        | 2.03                       |
| SE(d)              | 0.13                  | 0.26                                       | 0.08                 | 0.12                         | 4.67                              | 0.03                                        | 0.12                        | 15.86                        | 2.87                       |
| CV                 | 23.94                 | 40.94                                      | 22.23                | 53.25                        | 32.57                             | 36.49                                       | 16.41                       | 12.60                        | 28.88                      |
| CD                 | 0.26                  | 0.51                                       | 0.17                 | 0.24                         | 9.52                              | 0.06                                        | 0.25                        | 32.30                        | 5.85                       |
| Rep_DF             | 2.00                  | 2.00                                       | 2.00                 | 2.00                         | 2.00                              | 2.00                                        | 2.00                        | 2.00                         | 2.00                       |
| Trt_DF             | 67.00                 | 67.00                                      | 67.00                | 67.00                        | 16.00                             | 16.00                                       | 16.00                       | 16.00                        | 16.00                      |
| MSrep              | 4.35                  | 14.83                                      | 0.37                 | 0.04                         | 72.65                             | 0.00                                        | 0.02                        | 1533.25                      | 3.05                       |
| MStrt              | 2.82                  | 31.77                                      | 0.86                 | 10.12                        | 638.44                            | 0.03                                        | 0.12                        | 1743.02                      | 196.40                     |
| MSerror            | 0.03                  | 0.10                                       | 0.01                 | 0.02                         | 32.73                             | 0.00                                        | 0.02                        | 377.09                       | 12.37                      |
| F-value            | 108.75                | 314.75                                     | 80.60                | 440.23                       | 19.50                             | 19.89                                       | 5.27                        | 4.62                         | 15.88                      |
| P-value            | 1.49e-94              | 5.79e-125                                  | 4.18e-86             | 1.19e-134                    | 4.04e-12                          | 3.08e-15                                    | 3.27e-08                    | 0.0001                       | 6.76e-14                   |

**Table 2.** Statistical summary of insect parameters recorded on seeds (n = 30 seeds) of 68 wild pigeonpea accessions under three different screening conditions.

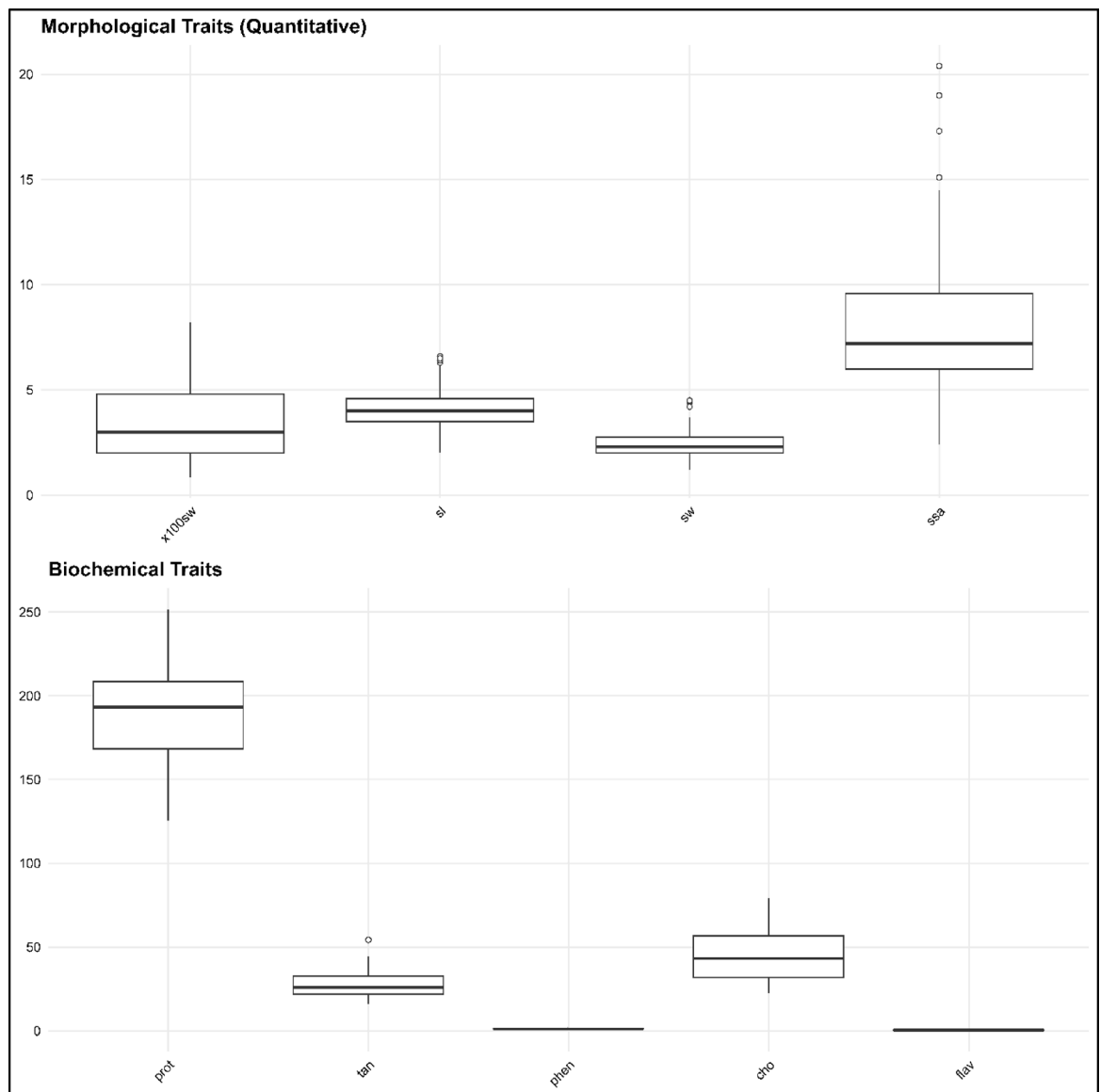
under CNC. The resistant accessions included *R. bracteata* (G26, G27, G60, G61) and *C. scarabaeoides* (G31–G42, G66, G67, G13, G12, G8, G9). However, SD revealed that *R. bracteata* (G26, G27, G60, G61) were the only highly resistant (HR) accessions across all test conditions, while *C. scarabaeoides* accessions (G31–42) were resistant in three conditions. Overall, SD confirmed the stability of *R. bracteata* as HR or R criteria and conditions, while *C. scarabaeoides* maintained HR to R responses, indicating broader resistance against *C. chinensis*. However, for *C. maculatus* (SI), resistance was most widespread, with 51 resistant accessions under FC, 48 under NC, and 22 under CNC. *R. bracteata* (G26, G27, G60, G61) and *C. scarabaeoides* (G31–G42, G66, G67, G13, G12, G8, G9) consistently showed resistance to highly resistant across conditions. SD confirmed this pattern, with *R. bracteata* maintaining HR across conditions, and *C. scarabaeoides* also showing HR or R responses in most cases.

**Variability of insect traits across conditions and species. Identification of key traits driving resistance**

*Did insect traits vary significantly across conditions within and between species, and which traits contributed?* Multivariate analyses (MANOVA) showed significant effects of both species and screening conditions on insect traits (Table 6 a&b, supplementary file 2). Within-species comparisons of FC vs. NC indicated significant differences for *C. analis* (Wilks’ Lambda=0.839, F=3.51, df=7128,  $p<0.05$ ) and *C. chinensis* ( $\Lambda=0.859$ , F=2.99, df=7128,  $p<0.05$ ), while *C. maculatus* exhibited a weaker but significant effect ( $\Lambda=0.955$ , F=0.86, df=7128,  $p<0.05$ ). Across species, the MANOVA revealed highly significant main effects of species ( $\Lambda=0.452$ , F=27.76, df=14,796,  $p<0.001$ ), confirming strong interspecific differences in insect traits, and smaller but significant effects of condition ( $\Lambda=0.935$ , F=3.97, df=7398,  $p<0.05$ ). The species  $\times$  condition interaction was also significant, indicating that the magnitude of condition effects differed among species (Table 6a). Follow-up univariate ANOVAs identified the traits contributing to these effects (Table 6b). In *C. analis*, seed damage (gd), adult density (ad), and numbers of males (m) and females (f) differed significantly between FC and NC. For *C. chinensis*, gd and mean developmental period (mdp) were most affected. In *C. maculatus*, condition effects were mainly reflected in adult emergence traits (ad, m, f). When pooled across species, seed damage, adult emergence, and developmental period were the primary contributors to the observed differences across screening conditions within each species and between species.

*What are the key traits driving accessions’ resistance as per SI and SD criteria?* Correlation funnel analysis revealed that between the two resistance outcomes (SD and SI), the influence of insect traits on resistance was significant over seed traits across species which was evident as they trended towards outcome variables (Fig. 5).

For *C. analis* (as per SD), the funnel showed that lower grain damage (gd) values strongly associated with resistance. In parallel, reduced adult density (ad), females (f), males (m), and adult survivability (as) aligned with resistant accessions. Conversely, higher values of these insect traits trended towards susceptibility. This pattern confirms that resistance to *C. analis* was largely due to restricted adult emergence and reproduction, thereby minimizing seed damage. The SI-based funnels highlighted a complementary picture: lower damage (gd) and higher development time (mdp) were major contributors towards resistance. Accessions with delayed adult development (high mdp) and minimal adult emergence (low ad, m, f, as) exhibited low SI values, reflecting stronger resistance. For *C. chinensis* (SD), resistance was again tied to lower gd and reduced adult emergence traits (ad, m, f, as). High mdp values also aligned with resistance. Accessions that delayed or suppressed emergence, maintained resistance across both FC and NC conditions, showing consistency in the outcome. The SI-based funnels revealed a strong negative correlation between resistance and adult emergence traits (ad, m, f, as). Higher



**Fig. 2.** Boxplots showing variation in seed morphological (68) and biochemical (17) traits across pigeonpea accessions. Each box displays the interquartile range (IQR), with the middle line indicating the median value. The upper and lower box edges represent the 75th and 25th percentiles, respectively. Whiskers extend to  $1.5 \times \text{IQR}$  from the quartiles, capturing the spread of most data points, while circles represent outliers lying beyond this range. This visualization highlights both central tendency and variability of traits among accessions. Abbreviations: x100sw, 100-seed weight; sl, seed length; sw, seed width; ssa, seed surface area; prot, protein; tan, tannins; phen, phenols; flav, flavonoids; cho, carbohydrates.

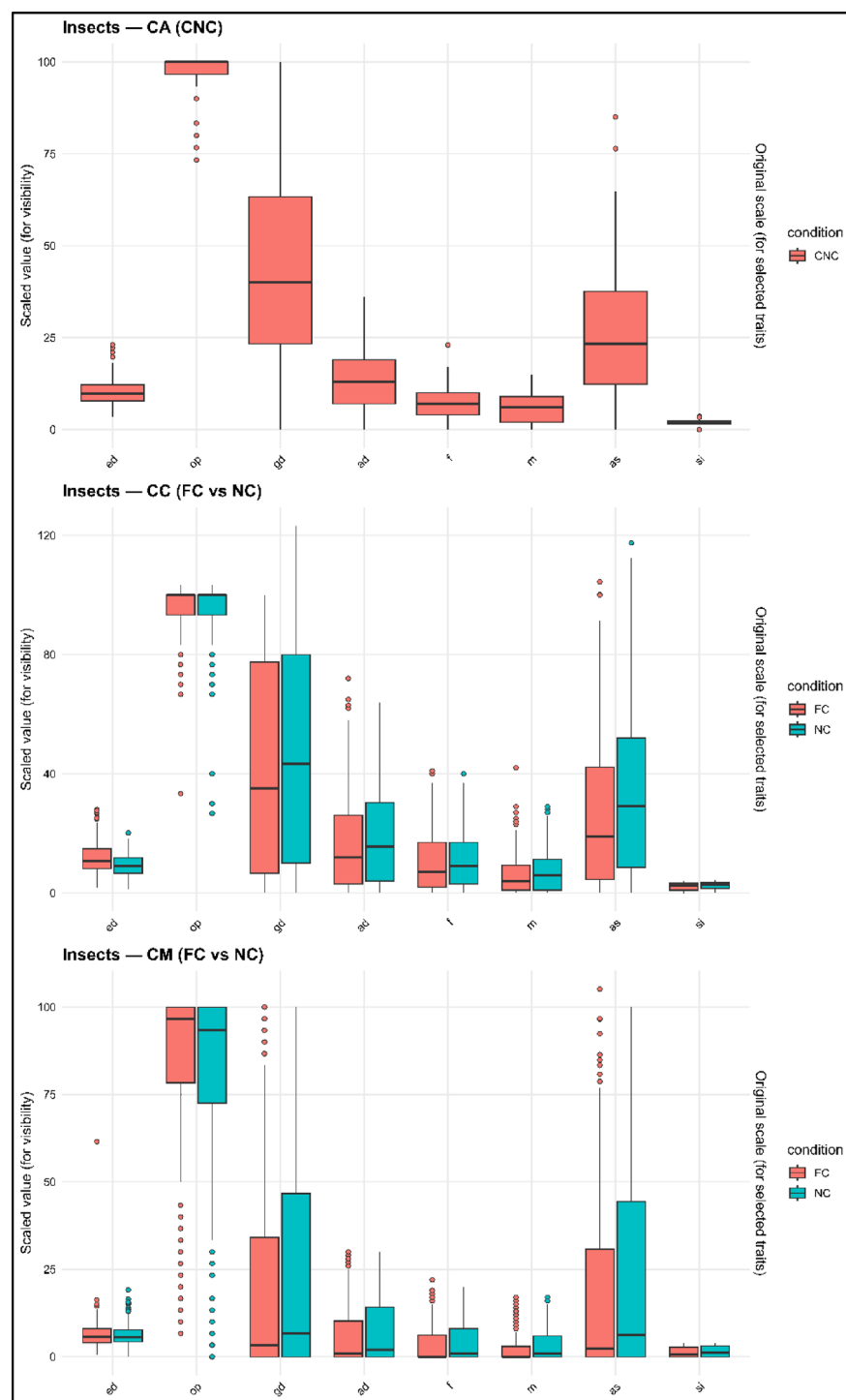
mdp reinforced this resistance trend. These results suggest that accessions resistant to *C. chinensis* prolong insect development while limiting adult establishment, thus reducing susceptibility. In *C. maculatus*, resistance in SD funnels was driven by lower gd, along with suppressed ad, m, f, and as. Interestingly, resistance was also tied more closely to seed morphological traits than in CA or CC, suggesting a larger role of antixenosis (smaller seed size and surface area). The SI funnel confirmed this pattern: resistant accessions consistently showed reduced egg density and oviposition. High mdp contributed to resistance, but the effect was less pronounced than in *C. analis* or *C. chinensis*, pointing towards morphological rather than purely antibiosis mechanisms in *C. maculatus* resistance.

Across all six funnels, a consistent pattern emerged: wild pigeonpea resistance was primarily driven by traits that reduce insect multiplication (low ad, m, f, as) and extend development time (high mdp). Direct seed damage (gd) and susceptibility index (SI) served as reliable resistance discriminators, with SI integrating multiple biological determinants. Morphological traits, though minor, contributed species-specific resistance cues, particularly for *C. maculatus*. Thus, correlation funnel analysis provided a clear and ranked view of the key drivers of wild pigeonpea resistance, reinforcing the importance of insect biological parameters while also validating

| Descriptor \ Trait \ Condition | Adult density (ad) |        |        | Adult survivability (as) (%) |         |         | Egg density (ed) |       |       | Female density (f) |        |        | Seed damage (gd) (%) |         |         | Male density (m) |        |        | Oviposition (op) (%) |        |        | Susc. index (SI) |       |        |
|--------------------------------|--------------------|--------|--------|------------------------------|---------|---------|------------------|-------|-------|--------------------|--------|--------|----------------------|---------|---------|------------------|--------|--------|----------------------|--------|--------|------------------|-------|--------|
|                                | FC                 | NC     | CNC    | FC                           | NC      | CNC     | FC               | NC    | CNC   | FC                 | NC     | CNC    | FC                   | NC      | CNC     | FC               | NC     | CNC    | FC                   | NC     | CNC    | FC               | NC    | CNC    |
| <i>C. analis</i>               |                    |        |        |                              |         |         |                  |       |       |                    |        |        |                      |         |         |                  |        |        |                      |        |        |                  |       |        |
| Mean                           | 15.62              | 17.36  | 12.93  | 30.91                        | 41.88   | 26.01   | 1.97             | 1.67  | 2.05  | 8.86               | 10.94  | 7.10   | 51.91                | 57.68   | 42.42   | 6.76             | 6.42   | 5.83   | 96.93                | 91.03  | 97.10  | 0.04             | 0.05  | 0.04   |
| Min                            | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00    | 0.80             | 0.45  | 0.74  | 0.00               | 0.00   | 0.00   | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00   | 0.00   | 70.00                | 38.89  | 78.89  | 0.00             | 0.00  | 0.00   |
| Max                            | 29.00              | 30.00  | 32.00  | 83.79                        | 257.18  | 75.41   | 4.95             | 2.91  | 4.10  | 20.33              | 20.00  | 18.67  | 96.67                | 100.00  | 100.00  | 19.33            | 17.33  | 13.33  | 100.00               | 100.00 | 100.00 | 0.08             | 0.08  | 0.07   |
| SE(m)                          | 1.75               | 3.49   | 0.99   | 4.37                         | 18.03   | 3.41    | 0.14             | 0.35  | 0.20  | 1.54               | 2.54   | 0.97   | 5.89                 | 11.76   | 2.80    | 1.21             | 1.69   | 0.74   | 2.39                 | 11.16  | 1.96   | 0.00             | 0.01  | 0.00   |
| SE(d)                          | 2.48               | 4.94   | 1.40   | 6.18                         | 25.50   | 4.83    | 0.19             | 0.49  | 0.28  | 2.18               | 3.59   | 1.37   | 8.33                 | 16.63   | 3.97    | 1.72             | 2.39   | 1.05   | 3.39                 | 15.79  | 2.77   | 0.01             | 0.01  | 0.00   |
| CV                             | 68.18              | 48.65  | 67.78  | 76.52                        | 80.17   | 75.14   | 40.18            | 31.42 | 39.94 | 71.36              | 47.63  | 67.15  | 68.25                | 48.82   | 67.61   | 77.39            | 60.21  | 71.37  | 6.11                 | 16.17  | 5.71   | 56.28            | 36.09 | 52.75  |
| CD                             | 4.90               | 9.77   | 2.83   | 12.23                        | 50.44   | 9.73    | 0.38             | 0.98  | 0.57  | 4.31               | 7.10   | 2.77   | 16.47                | 32.89   | 7.99    | 3.40             | 4.73   | 2.12   | 6.70                 | 31.23  | 5.58   | 0.01             | 0.02  | 0.01   |
| Rep_DF                         | 2.00               | 2.00   | 2.00   | 2.00                         | 2.00    | 2.00    | 2.00             | 2.00  | 2.00  | 2.00               | 2.00   | 2.00   | 2.00                 | 2.00    | 2.00    | 2.00             | 2.00   | 2.00   | 2.00                 | 2.00   | 2.00   | 2.00             | 2.00  | 2.00   |
| Trt_DF                         | 67.00              | 67.00  | 22.00  | 67.00                        | 67.00   | 22.00   | 67.00            | 67.00 | 22.00 | 67.00              | 67.00  | 22.00  | 67.00                | 67.00   | 22.00   | 67.00            | 67.00  | 22.00  | 67.00                | 67.00  | 22.00  | 67.00            | 67.00 | 22.00  |
| MStrep                         | 1.10               | 29.43  | 2.28   | 0.66                         | 775.35  | 110.31  | 0.09             | 0.40  | 0.12  | 3.24               | 1.98   | 0.41   | 15.85                | 367.70  | 18.04   | 1.55             | 20.36  | 0.91   | 6.92                 | 76.63  | 10.14  | 0.00             | 0.00  | 0.00   |
| MStrrt                         | 340.18             | 213.97 | 230.36 | 1678.48                      | 3382.12 | 1146.24 | 1.89             | 0.83  | 2.00  | 119.86             | 81.39  | 68.23  | 3766.26              | 2378.74 | 2467.22 | 82.11            | 44.84  | 51.87  | 105.15               | 649.79 | 92.17  | 0.00             | 0.00  | 0.00   |
| MSError                        | 9.22               | 36.60  | 2.96   | 57.37                        | 975.75  | 34.96   | 0.06             | 0.37  | 0.12  | 7.12               | 19.34  | 2.83   | 104.02               | 414.85  | 23.59   | 4.42             | 8.59   | 1.66   | 17.20                | 373.93 | 11.49  | 0.00             | 0.00  | 0.00   |
| F-value                        | 36.89              | 5.85   | 77.90  | 29.26                        | 3.47    | 32.79   | 33.86            | 2.25  | 16.52 | 16.84              | 4.21   | 24.11  | 36.21                | 5.73    | 104.58  | 18.56            | 5.22   | 31.34  | 6.11                 | 1.74   | 8.02   | 38.83            | 6.65  | 111.28 |
| P-value                        | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00    | 0.00             | 0.00  | 0.00  | 0.00               | 0.00   | 0.00   | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00   | 0.00   | 0.00                 | 0.00   | 0.00   | 0.00             | 0.00  | 0.00   |
| <i>C. chinensis</i>            |                    |        |        |                              |         |         |                  |       |       |                    |        |        |                      |         |         |                  |        |        |                      |        |        |                  |       |        |
| Mean                           | 16.67              | 18.32  | 5.57   | 26.19                        | 34.13   | 7.43    | 2.38             | 1.89  | 2.52  | 10.35              | 10.84  | 3.32   | 42.32                | 46.01   | 16.28   | 6.31             | 7.49   | 2.23   | 96.09                | 94.90  | 99.03  | 0.04             | 0.05  | 0.02   |
| Min                            | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00    | 0.66             | 0.82  | 1.61  | 0.00               | 0.00   | 0.00   | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00   | 0.00   | 56.67                | 73.33  | 92.22  | 0.00             | 0.00  | 0.00   |
| Max                            | 54.67              | 49.00  | 33.33  | 96.22                        | 93.95   | 33.99   | 5.23             | 3.10  | 4.49  | 36.33              | 29.33  | 19.67  | 98.89                | 100.00  | 100.00  | 26.00            | 22.67  | 13.67  | 100.00               | 100.00 | 100.00 | 0.08             | 0.08  | 0.06   |
| SE(m)                          | 3.34               | 4.10   | 0.59   | 4.47                         | 8.86    | 1.41    | 0.23             | 0.35  | 0.20  | 2.46               | 2.74   | 0.42   | 6.50                 | 8.10    | 1.16    | 1.97             | 2.53   | 0.53   | 2.70                 | 5.83   | 1.06   | 0.00             | 0.00  | 0.00   |
| SE(d)                          | 4.72               | 5.80   | 0.83   | 6.33                         | 12.52   | 2.00    | 0.33             | 0.50  | 0.29  | 3.48               | 3.88   | 0.59   | 9.19                 | 11.46   | 1.64    | 2.78             | 3.58   | 0.76   | 3.82                 | 8.24   | 1.49   | 0.01             | 0.01  | 0.00   |
| CV                             | 93.39              | 77.37  | 140.98 | 93.29                        | 75.70   | 112.35  | 42.29            | 28.61 | 34.80 | 90.29              | 77.26  | 137.76 | 82.30                | 72.16   | 144.27  | 105.92           | 84.31  | 148.43 | 7.18                 | 6.96   | 2.33   | 57.27            | 48.91 | 73.65  |
| CD                             | 9.34               | 11.48  | 1.67   | 12.51                        | 24.77   | 4.03    | 0.65             | 0.99  | 0.58  | 6.88               | 7.68   | 1.20   | 18.18                | 22.67   | 3.30    | 5.50             | 7.08   | 1.52   | 7.56                 | 16.31  | 3.01   | 0.01             | 0.01  | 0.01   |
| Rep_DF                         | 2.00               | 2.00   | 2.00   | 2.00                         | 2.00    | 2.00    | 2.00             | 2.00  | 2.00  | 2.00               | 2.00   | 2.00   | 2.00                 | 2.00    | 2.00    | 2.00             | 2.00   | 2.00   | 2.00                 | 2.00   | 2.00   | 2.00             | 2.00  | 2.00   |
| Trt_DF                         | 67.00              | 67.00  | 22.00  | 67.00                        | 67.00   | 22.00   | 67.00            | 67.00 | 22.00 | 67.00              | 67.00  | 22.00  | 67.00                | 67.00   | 22.00   | 67.00            | 67.00  | 22.00  | 67.00                | 67.00  | 22.00  | 67.00            | 67.00 | 22.00  |
| MStrep                         | 6.36               | 51.81  | 2.26   | 13.22                        | 33.06   | 31.02   | 0.07             | 0.36  | 0.08  | 0.84               | 8.66   | 1.06   | 307.57               | 290.90  | 11.76   | 11.68            | 22.96  | 0.19   | 12.47                | 106.37 | 7.89   | 0.00             | 0.00  | 0.00   |
| MStrrt                         | 726.83             | 602.92 | 184.68 | 1791.01                      | 2002.53 | 208.94  | 3.03             | 0.88  | 2.31  | 262.13             | 210.33 | 62.71  | 3639.44              | 3307.38 | 1655.09 | 134.18           | 119.47 | 32.92  | 142.67               | 131.04 | 15.93  | 0.00             | 0.00  | 0.00   |
| MSError                        | 33.43              | 50.52  | 1.03   | 60.06                        | 235.26  | 5.99    | 0.16             | 0.38  | 0.12  | 18.17              | 22.60  | 0.53   | 126.70               | 197.04  | 4.01    | 11.60            | 19.24  | 0.86   | 21.93                | 101.95 | 3.35   | 0.00             | 0.00  | 0.00   |
| F-value                        | 21.74              | 11.93  | 178.68 | 29.82                        | 8.51    | 34.87   | 18.73            | 2.33  | 18.52 | 14.43              | 9.31   | 118.85 | 28.73                | 16.79   | 412.62  | 11.57            | 6.21   | 38.50  | 6.51                 | 1.29   | 4.76   | 33.07            | 23.86 | 45.47  |
| P-value                        | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00    | 0.00             | 0.00  | 0.00  | 0.00               | 0.00   | 0.00   | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00   | 0.00   | 0.00                 | 0.00   | 0.00   | 0.00             | 0.00  | 0.00   |
| <i>C. maculatus</i>            |                    |        |        |                              |         |         |                  |       |       |                    |        |        |                      |         |         |                  |        |        |                      |        |        |                  |       |        |
| Mean                           | 6.33               | 7.56   | 1.64   | 17.71                        | 22.10   | 2.65    | 1.32             | 1.15  | 1.55  | 3.70               | 4.40   | 0.83   | 21.19                | 25.05   | 5.46    | 2.63             | 3.17   | 0.81   | 82.96                | 76.99  | 98.07  | 0.03             | 0.03  | 0.01   |
| Min                            | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00    | 0.16             | 0.03  | 1.01  | 0.00               | 0.00   | 0.00   | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00   | 0.00   | 11.11                | 3.33   | 90.00  | 0.00             | 0.00  | 0.00   |
| Max                            | 30.00              | 30.00  | 30.00  | 83.52                        | 86.82   | 42.21   | 5.00             | 2.50  | 2.42  | 17.67              | 18.00  | 16.00  | 100.00               | 100.00  | 100.00  | 14.00            | 14.67  | 14.00  | 100.00               | 100.00 | 100.00 | 0.07             | 0.08  | 0.06   |
| SE(m)                          | 1.63               | 2.68   | 0.21   | 7.09                         | 8.02    | 1.07    | 0.46             | 0.28  | 0.15  | 1.03               | 1.73   | 0.34   | 5.67                 | 8.82    | 0.71    | 1.08             | 1.29   | 0.32   | 3.81                 | 12.62  | 1.87   | 0.01             | 0.01  | 0.00   |
| SE(d)                          | 2.30               | 3.79   | 0.30   | 10.02                        | 11.34   | 1.51    | 0.65             | 0.40  | 0.22  | 1.46               | 2.45   | 0.48   | 8.01                 | 12.47   | 1.00    | 1.53             | 1.82   | 0.45   | 5.38                 | 17.84  | 2.64   | 0.01             | 0.01  | 0.00   |
| CV                             | 143.51             | 121.60 | 380.14 | 132.20                       | 118.15  | 332.87  | 61.59            | 51.24 | 22.23 | 142.95             | 120.63 | 403.67 | 143.15               | 122.19  | 380.14  | 149.49           | 127.94 | 358.23 | 29.69                | 37.28  | 2.88   | 101.59           | 96.06 | 243.68 |
| CD                             | 4.56               | 7.49   | 0.61   | 19.82                        | 22.42   | 3.05    | 1.28             | 0.79  | 0.44  | 2.89               | 4.85   | 0.96   | 15.85                | 24.66   | 2.02    | 3.03             | 3.60   | 0.90   | 10.65                | 35.29  | 5.33   | 0.02             | 0.02  | 0.00   |
| Rep_DF                         | 2.00               | 2.00   | 2.00   | 2.00                         | 2.00    | 2.00    | 2.00             | 2.00  | 2.00  | 2.00               | 2.00   | 2.00   | 2.00                 | 2.00    | 2.00    | 2.00             | 2.00   | 2.00   | 2.00                 | 2.00   | 2.00   | 2.00             | 2.00  | 2.00   |
| Trt_DF                         | 67.00              | 67.00  | 22.00  | 67.00                        | 67.00   | 22.00   | 67.00            | 67.00 | 22.00 | 67.00              | 67.00  | 22.00  | 67.00                | 67.00   | 22.00   | 67.00            | 67.00  | 22.00  | 67.00                | 67.00  | 22.00  | 67.00            | 67.00 | 22.00  |
| Continued                      |                    |        |        |                              |         |         |                  |       |       |                    |        |        |                      |         |         |                  |        |        |                      |        |        |                  |       |        |

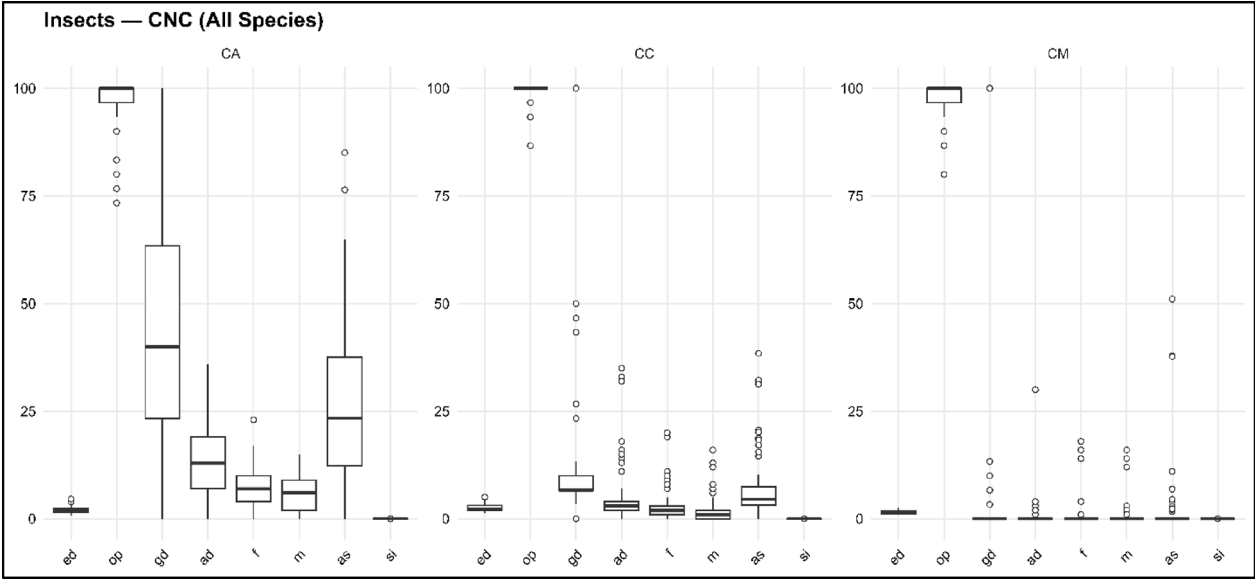
| Descriptor<br>Trait \<br>Condition | Adult density (ad) |        |        | Adult survivability (as) (%) |         |        | Egg density (ed) |      |      | Female density (f) |       |       | Seed damage (gd) (%) |         |         | Male density (m) |       |       | Oviposition (op) (%) |         |       | Susc. index (SI) |       |       |
|------------------------------------|--------------------|--------|--------|------------------------------|---------|--------|------------------|------|------|--------------------|-------|-------|----------------------|---------|---------|------------------|-------|-------|----------------------|---------|-------|------------------|-------|-------|
|                                    | FC                 | NC     | CNC    | FC                           | NC      | CNC    | FC               | NC   | CNC  | FC                 | NC    | CNC   | FC                   | NC      | CNC     | FC               | NC    | CNC   | FC                   | NC      | CNC   | FC               | NC    | CNC   |
| MSrep                              | 20.80              | 4.50   | 0.01   | 224.68                       | 296.93  | 3.95   | 0.81             | 0.75 | 0.42 | 5.03               | 0.72  | 0.52  | 242.86               | 56.05   | 0.16    | 6.62             | 1.86  | 0.71  | 38.29                | 1688.29 | 21.42 | 0.00             | 0.00  | 0.00  |
| MStrt                              | 247.46             | 253.77 | 116.27 | 1643.97                      | 2045.53 | 233.44 | 1.98             | 1.04 | 0.36 | 83.97              | 84.40 | 33.36 | 2761.12              | 2810.44 | 1291.89 | 46.28            | 49.24 | 25.36 | 1820.41              | 2470.98 | 23.98 | 0.00             | 0.00  | 0.00  |
| MSerror                            | 7.97               | 21.54  | 0.14   | 150.70                       | 192.79  | 3.43   | 0.62             | 0.24 | 0.07 | 3.20               | 9.02  | 0.34  | 96.32                | 233.16  | 1.51    | 3.52             | 4.97  | 0.30  | 43.49                | 477.62  | 10.47 | 0.00             | 0.00  | 0.00  |
| F-value                            | 31.06              | 11.78  | 856.79 | 10.91                        | 10.61   | 68.03  | 3.17             | 4.30 | 5.08 | 26.25              | 9.36  | 98.14 | 28.67                | 12.05   | 856.79  | 13.16            | 9.91  | 84.23 | 41.86                | 5.17    | 2.29  | 17.93            | 17.56 | 74.84 |
| P-value                            | 0.00               | 0.00   | 0.00   | 0.00                         | 0.00    | 0.00   | 0.00             | 0.00 | 0.00 | 0.00               | 0.00  | 0.00  | 0.00                 | 0.00    | 0.00    | 0.00             | 0.00  | 0.00  | 0.00                 | 0.00    | 0.00  | 0.00             | 0.00  | 0.00  |

**Table 3.** Statistical summary of insect parameters recorded on seeds (n = 30 seeds) of 68 wild pigeonpea accessions under three different screening conditions. Free-choice (FC), No-choice (NC), Confirmatory no-choice (CNC).



**Fig. 3.** Boxplots showing insect traits of pigeonpea genotypes under FC and NC screening conditions across three species. Boxes represent interquartile range (IQR), middle lines denote medians, whiskers extend to  $1.5 \times$  IQR, and circles indicate outliers. Abbreviations: ed, eggs density; op, oviposition; gd, seed damage; ad, adults density; m, males; f, females; mdp, mean development period; si, susceptibility index; as, adult survivability.

the roles of seed morpho-traits. Across species, the pattern of stronger link of resistance to seed damage, lower emergence and survivability (as), and prolonged mean development period (mdp) anticipate stronger antibiosis as a key mechanism. Species-specific differences were also evident. In *C. analis* and *C. chinensis*, resistance was largely driven by antibiosis, as indicated by lower damage, emergence and extended development times. While for *C. maculatus*, it was due to antixenosis on majority accessions except for few prominent ones.



**Fig. 4.** Boxplots showing insect traits of pigeonpea genotypes under CNC screening conditions across three species. Boxes represent interquartile range (IQR), middle lines denote medians, whiskers extend to  $1.5 \times$  IQR, and circles indicate outliers. Abbreviations: ed, eggs density; op, oviposition; gd, seed damage; ad, adults density; m, males; f, females; mdp, mean development period; si, susceptibility index; as, adult survivability.

| Species             | Condition | Susceptibility (rflag_SI)                                                                                                                                                                                                                                 |                                                                      |                                                                                         |                                                                                          |    |
|---------------------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----|
|                     |           | R                                                                                                                                                                                                                                                         | MR                                                                   | MS                                                                                      | S                                                                                        | HS |
| <i>C. analis</i>    | FC        | 30 (G13, G22, G26, G27, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, G50, G60, G61, G63, G65, G66, G67)                                                                                                 | 12 (G3, G8, G9, G10, G12, G25, G51, G52, G53, G59, G64, G68)         | 18 (G1, G2, G4, G5, G6, G7, G11, G14, G16, G17, G18, G19, G20, G21, G23, G24, G29, G58) | 08 (G15, G28, G30, G54, G55, G56, G57, G62)                                              | 0  |
|                     | NC        | 33 (G1, G6, G8, G9, G12, G13, G21, G26, G27, G31, G32, G33, G34, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G49, G50, G60, G61, G65, G66, G67, G68)                                                                                      | 13 (G11, G16, G17, G18, G19, G20, G22, G48, G53, G56, G58, G59, G63) | 16 (G2, G3, G4, G5, G7, G10, G14, G15, G24, G25, G35, G51, G52, G55, G57, G64)          | 06 (G23, G28, G29, G30, G54, G62)                                                        | 0  |
|                     | CNC       | 20 (G27, G60, G26, G61, G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G42, G66, G13, G9)                                                                                                                                                    | 02 (G8, G12)                                                         | 01 (G23)                                                                                | 0                                                                                        | 0  |
| <i>C. chinensis</i> | FC        | 36 (G4, G6, G8, G9, G10, G12, G13, G20, G22, G26, G27, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, G50, G57, G60, G61, G66, G67)                                                                       | 06 (G3, G5, G11, G18, G24, G65)                                      | 16 (G1, G2, G7, G14, G15, G16, G17, G19, G21, G30, G51, G52, G53, G56, G59, G63, G64)   | 08 (G25, G28, G29, G54, G55, G58, G62, G68)                                              | 0  |
|                     | NC        | 26 (G4, G8, G9, G22, G26, G27, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G46, G57, G60, G61, G65, G66, G67)                                                                                                                        | 11 (G3, G6, G10, G11, G12, G13, G45, G47, G48, G49, G50)             | 14 (G1, G2, G5, G7, G15, G17, G20, G21, G23, G24, G44, G51, G56, G63)                   | 17 (G14, G16, G18, G19, G25, G28, G29, G30, G52, G53, G54, G55, G58, G59, G62, G64, G68) | 0  |
|                     | CNC       | 22 (G27, G60, G26, G61, G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G42, G66, G13, G12, G8, G9)                                                                                                                                           | 01 (G23)                                                             | 0                                                                                       | 0                                                                                        | 0  |
| <i>C. maculatus</i> | FC        | 51 (G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G16, G18, G19, G20, G21, G22, G26, G27, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, G50, G57, G60, G61, G63, G65, G66, G67) | 08 (G24, G25, G51, G52, G53, G59, G64, G68)                          | 08 (G15, G17, G23, G28, G54, G55, G56, G58)                                             | 01 (G62)                                                                                 | 0  |
|                     | NC        | 48 (G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G16, G20, G21, G22, G24, G25, G26, G27, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G57, G59, G60, G61, G65, G66, G67)                | 04 (G14, G50, G53, G63)                                              | 12 (G15, G18, G19, G23, G49, G51, G52, G54, G56, G58, G64, G68)                         | 04 (G17, G28, G55, G62)                                                                  | 0  |
|                     | CNC       | 22 (G27, G60, G26, G61, G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G42, G66, G13, G12, G8, G9)                                                                                                                                           | 01 (G23)                                                             | 0                                                                                       | 0                                                                                        | 0  |

**Table 4.** Categorization of accessions’ reaction based on Susceptibility (rflag\_SI).

| Species             | Condition | Seed damage (rflag_SD) categories                                                                                                        |                                                                                    |                                                                                                                                                                                              |                                                                                                                                                              |
|---------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                     |           | HR                                                                                                                                       | R                                                                                  | MS                                                                                                                                                                                           | HS                                                                                                                                                           |
| <i>C. analis</i>    | FC        | 04 (G26, G27, G60, G61)                                                                                                                  | 12 (G31, G32, G33, G34, G35, G36, G37, G45, G46, G47, G48, G50)                    | 20 (G2, G3, G10, G11, G13, G22, G38, G39, G40, G41, G42, G43, G44, G49, G54, G55, G58, G65, G66, G67)                                                                                        | 32 (G1, G4, G5, G6, G7, G8, G9, G12, G14, G15, G16, G17, G18, G19, G20, G21, G23, G24, G25, G28, G29, G30, G51, G52, G53, G56, G57, G59, G62, G63, G64, G68) |
|                     | NC        | 04 (G26, G27, G60, G61)                                                                                                                  | 0                                                                                  | 38 (G1, G3, G6, G8, G9, G13, G21, G22, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, G50, G53, G54, G55, G56, G58, G59, G65, G66, G67, G68) | 26 (G2, G4, G5, G7, G10, G11, G12, G14, G15, G16, G17, G18, G19, G20, G23, G24, G25, G28, G29, G30, G51, G52, G57, G62, G63, G64)                            |
|                     | CNC       | 04 (G26, G27, G60, G61)                                                                                                                  | 0                                                                                  | 15 (G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G42, G66, G13)                                                                                                               | 04 (G12, G8, G9, G23)                                                                                                                                        |
| <i>C. chinensis</i> | FC        | 04 (G26, G27, G60, G61)                                                                                                                  | 16 (G8, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G48, G66, G67) | 28 (G1, G2, G3, G4, G5, G6, G7, G9, G10, G11, G12, G13, G16, G20, G21, G22, G24, G43, G44, G45, G46, G47, G49, G50, G55, G56, G57, G65)                                                      | 20 (G14, G15, G17, G18, G19, G23, G25, G28, G29, G30, G51, G52, G53, G54, G58, G59, G62, G63, G64, G68)                                                      |
|                     | NC        | 04 (G26, G27, G60, G61)                                                                                                                  | 13 (G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G67)               | 29 (G3, G4, G5, G6, G7, G8, G9, G11, G12, G13, G17, G20, G21, G22, G24, G43, G44, G45, G46, G47, G48, G49, G50, G53, G54, G56, G57, G65, G66)                                                | 22 (G1, G2, G10, G14, G15, G16, G18, G19, G23, G25, G28, G29, G30, G51, G52, G55, G58, G59, G62, G63, G64, G68)                                              |
|                     | CNC       | 04 (G26, G27, G60, G61)                                                                                                                  | 14 (G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G42, G66)          | 04 (G13, G12, G8, G9)                                                                                                                                                                        | 01 (G23)                                                                                                                                                     |
| <i>C. maculatus</i> | FC        | 28 (G1, G2, G3, G4, G5, G6, G7, G10, G11, G13, G26, G27, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G48, G60, G61, G66, G67) | 11 (G8, G9, G12, G16, G19, G30, G42, G45, G46, G47, G49)                           | 22 (G14, G15, G17, G18, G20, G21, G22, G24, G25, G28, G29, G43, G44, G50, G51, G54, G55, G56, G57, G58, G63, G65)                                                                            | 07 (G23, G52, G53, G59, G62, G64, G68)                                                                                                                       |
|                     | NC        | 25 (G1, G2, G3, G4, G5, G6, G7, G10, G11, G26, G27, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G60, G61, G66, G67)                | 09 (G8, G9, G12, G13, G25, G29, G30, G31, G42)                                     | 27 (G14, G16, G17, G18, G19, G20, G21, G22, G24, G28, G43, G44, G45, G46, G47, G48, G49, G50, G53, G56, G57, G58, G59, G62, G63, G64, G65)                                                   | 07 (G15, G23, G51, G52, G54, G55, G68)                                                                                                                       |
|                     | CNC       | 17 (G27, G60, G26, G61, G38, G41, G40, G37, G39, G33, G36, G31, G35, G67, G32, G34, G66)                                                 | 05 (G42, G13, G12, G8, G9)                                                         | 0                                                                                                                                                                                            | 01 (G23)                                                                                                                                                     |

**Table 5.** Categorization of accessions' reaction based on seed damage (rflag\_SD).*Validation and prediction of key traits driving bruchid resistance in wild pigeonpea accessions*

Random forest models demonstrated excellent performance in classifying pigeonpea accessions into resistant and susceptible categories based on seed traits. Across all datasets and targets, classification accuracy exceeded 99%, with areas under the ROC curve (AUC) approaching 1.0 (Table 7; Supplementary file 3). Models trained on the 68-accession dataset (insect + seed morpho traits) achieved near-perfect classification: for rflag\_sd, accuracy was 99.9%, sensitivity 1.0, and specificity 0.998 (AUC=0.999); for rflag\_si, accuracy was 99.8%, sensitivity 1.0, and specificity 0.996 (AUC=1.0). Similarly, the 17-accession dataset (insect + seed morphological + seed biochemical traits) yielded perfect discrimination, with both rflag\_sd and rflag\_si models achieving 100% accuracy and AUC=1.0, though these results should be interpreted cautiously due to the smaller sample size.

ROC curves confirmed excellent separation of resistant and susceptible classes for both resistance flags (Fig. 6). Variable importance rankings consistently identified seed damage (gd) and adult emergence traits (ad, m, f) as the strongest insect predictors. Among seed descriptors, 100-seed weight and seed surface area emerged as key morphological predictors, while phenolics and flavonoids contributed significantly within the biochemical subset (Fig. 7). Supplementary outputs (Supplementary file 4 and 5) provide additional transparency through full ranked importance scores, partial dependence plots, and confusion matrices.

**Response based grouping of accessions vis-à-vis insect and seed traits using FAMD and hierarchical clustering**

FAMD effectively captured the multivariate structure of resistance in pigeonpea accessions (Figs. 8, 9). For *C. analis*, the 68-accession FAMD plots showed clear separation of resistance groups. Under seed damage (SD), highly resistant (HR) and resistant (R) accessions formed distinct clusters on the negative side of Dim1, while highly susceptible (HS) accessions grouped on the positive axis. Moderate categories (MS, MR) overlapped partially, reflecting intermediate responses. For susceptibility index (SI), clustering was less distinct, but resistant categories still trended towards separation. In the 17-accession plots (insects + seed biochemical), HR and R categories again grouped separately from HS, confirming that biochemical traits reinforced resistance categorization. For *C. chinensis*, the 68-accessions FAMD revealed similar structuring, with HR and R accessions clustered tightly apart from HS on the positive Dim1 axis. SI-based grouping separated MR and S categories more clearly, with resistant groups localized towards negative coordinates. In the 17-accession analysis, biochemical traits improved discrimination: resistant accessions formed a compact cluster, while the lone HS accession (G23) appeared isolated, confirming its distinct susceptibility.

| Scope                                      | Species              | Model                                 | Effect                          | Wilks_Lambda | Approx_F                                                  | num_df | den_df | p_value |
|--------------------------------------------|----------------------|---------------------------------------|---------------------------------|--------------|-----------------------------------------------------------|--------|--------|---------|
| a. Summar of multivariate (MONOVA) results |                      |                                       |                                 |              |                                                           |        |        |         |
| Within-species                             | CA                   | condition                             | condition                       | 1            | 0.84                                                      | 3.5    | 7.0    | 128.0   |
| Within-species                             | CA                   | condition                             | Residuals                       | 134          |                                                           |        |        |         |
| Within-species                             | CC                   | condition                             | condition                       | 1            | 0.86                                                      | 2.99   | 7.0    | 128.0   |
| Within-species                             | CC                   | condition                             | Residuals                       | 134          |                                                           |        |        |         |
| Within-species                             | CM                   | condition                             | condition                       | 1            | 0.95                                                      | 0.86   | 7.0    | 128.0   |
| Within-species                             | CM                   | condition                             | Residuals                       | 134          |                                                           |        |        |         |
| Across-species                             | All                  | species * condition                   | species                         | 2            | 0.45                                                      | 27.76  | 14.0   | 796.0   |
| Across-species                             | All                  | species * condition                   | condition                       | 1            | 0.93                                                      | 3.97   | 7.0    | 398.0   |
| Across-species                             | All                  | species * condition                   | Residuals                       | 404          |                                                           |        |        |         |
| Test                                       | Effect               | Wilks Lambda (p)                      | Significant Traits (Univariate) |              | Interpretation                                            |        |        |         |
| b. Summary of univariate ANOVA results     |                      |                                       |                                 |              |                                                           |        |        |         |
| CA ( <i>C. analis</i> )                    | Condition (FC vs NC) | < 0.05                                | gd, ad, m, f                    |              | Some traits differ significantly between FC and NC        |        |        |         |
| CC ( <i>C. chinensis</i> )                 | Condition (FC vs NC) | < 0.05                                | gd, mdp                         |              | Differences observed, driven by damage-related traits     |        |        |         |
| CM ( <i>C. maculatus</i> )                 | Condition (FC vs NC) | < 0.05                                | ad, m, f                        |              | Significant condition effect, mainly in emergence traits  |        |        |         |
| Across Species                             | Species & Condition  | < 0.001 (species), < 0.05 (condition) | gd, ad, m, f, mdp               |              | Strong species differences; moderate FC vs NC differences |        |        |         |

**Table 6.** (a) MANOVA results for within- and across-species comparisons of insect traits under different screening conditions. (b) Summary of univariate ANOVA results identifying insect traits contributing to significant MANOVA effects. Condition = Screening conditions (FC, Free-choice, NC, No-choice, CNC, Confirmatory no-choice), CA, *Callosobruchus analis*, CC, *C. chinensis*, CM, *C. maculatus*, Wilks\_Lambda, Wilks' Lambda statistic, Approx\_F, Approximate F statistic, num\_df, Numerator degrees of freedom, den\_df, Denominator degrees of freedom, p\_value, Probability value, gd, Seed damage, ad, Adult density, m, Males, f, Females, mdp, Mean developmental period.

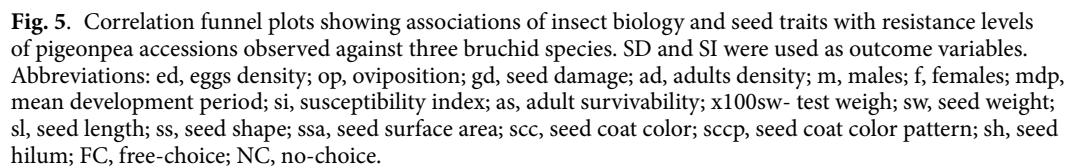
However, for *C. maculatus*, resistance patterns were stronger than other species. In the 68-accessions plots, HR and R accessions grouped tightly on the left-hand side of Dim1, whereas HS and S clustered distinctly on the right. SI plots highlighted antixenosis-driven resistance, as resistant accessions overlapped but remained distinct from susceptible categories. The 17-accession plots further underscored this: HR and R accessions formed a cohesive group influenced by biochemical defenses, while susceptible accessions were widely dispersed. The clear separation supports the interpretation that *C. maculatus* resistance is mediated both by oviposition deterrence and biochemical composition. Overall, across species, the FAMD plots confirmed that resistance categories derived from insect traits aligned well with multivariate clustering. Inclusion of biochemical traits in the 17-accession subset provided further resolution, especially for distinguishing intermediate categories.

Hierarchical clustering revealed clear but species-specific accession groupings (Figs. 10 and 11). When insect resistance traits were combined with seed morphological traits across the 68 accessions, three to four distinct clusters were consistently observed per species (Fig. 10). In *C. analis*, clusters differentiated resistant accessions (e.g., G26, G27, G60, G61) from susceptible accessions (e.g., G23, G34, G35), with seed damage and adult emergence traits contributing strongly to separation. *C. chinensis* displayed broader divergence, with one large cluster grouping mostly susceptible accessions (e.g., G59, G52, G55) showing high seed damage and emergence, and a smaller cluster comprising resistant accessions with low oviposition and damage. In *C. maculatus*, clusters were less sharply separated, but resistant accessions (G26, G27, G60, G61) consistently grouped together, highlighting shared defense responses.

When biochemical traits were incorporated for the 17 selected accessions, discrimination improved further. Clustering separated resistant accessions (e.g., G26, G27, G60, G61) from highly susceptible accessions (e.g., G23), with biochemical traits such as phenols, tannins, and flavonoids contributing significantly. In *C. analis* and *C. chinensis*, biochemical profiles reinforced resistance groupings previously identified by insect traits alone, while in *C. maculatus*, inclusion of biochemical data revealed more refined subgrouping among moderately resistant accessions. Notably, susceptible accessions exhibited consistently low levels of phenolic compounds, aligning with higher insect survival and seed damage. These results indicate that biochemical defense traits complement insect and morphological data, improving discrimination of resistance categories across species.

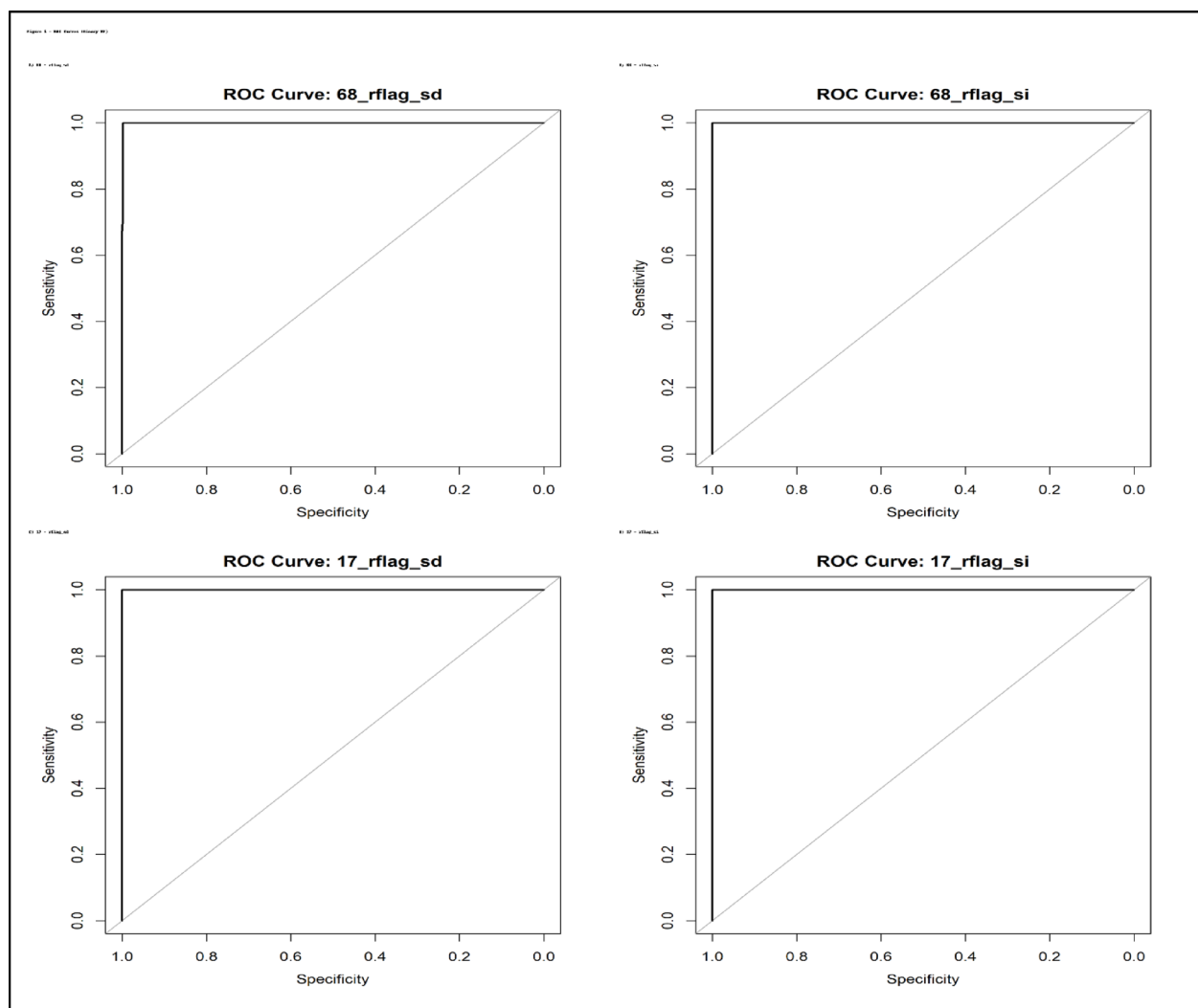
Role of antibiosis in accessions' resistance against bruchid species

Correlation analysis revealed distinct associations between biochemical seed traits and insect developmental parameters across the three *Callosobruchus* species (Fig. 12). In *C. chinensis*, strong positive correlations were observed among insect traits such as grain damage, adult emergence, and susceptibility index ( $r > 0.85^{***}$ ), confirming their reliability as indicators of susceptibility. Phenols and tannins displayed significant negative correlations with grain damage and adult emergence, indicating their role in resistance. Protein content showed



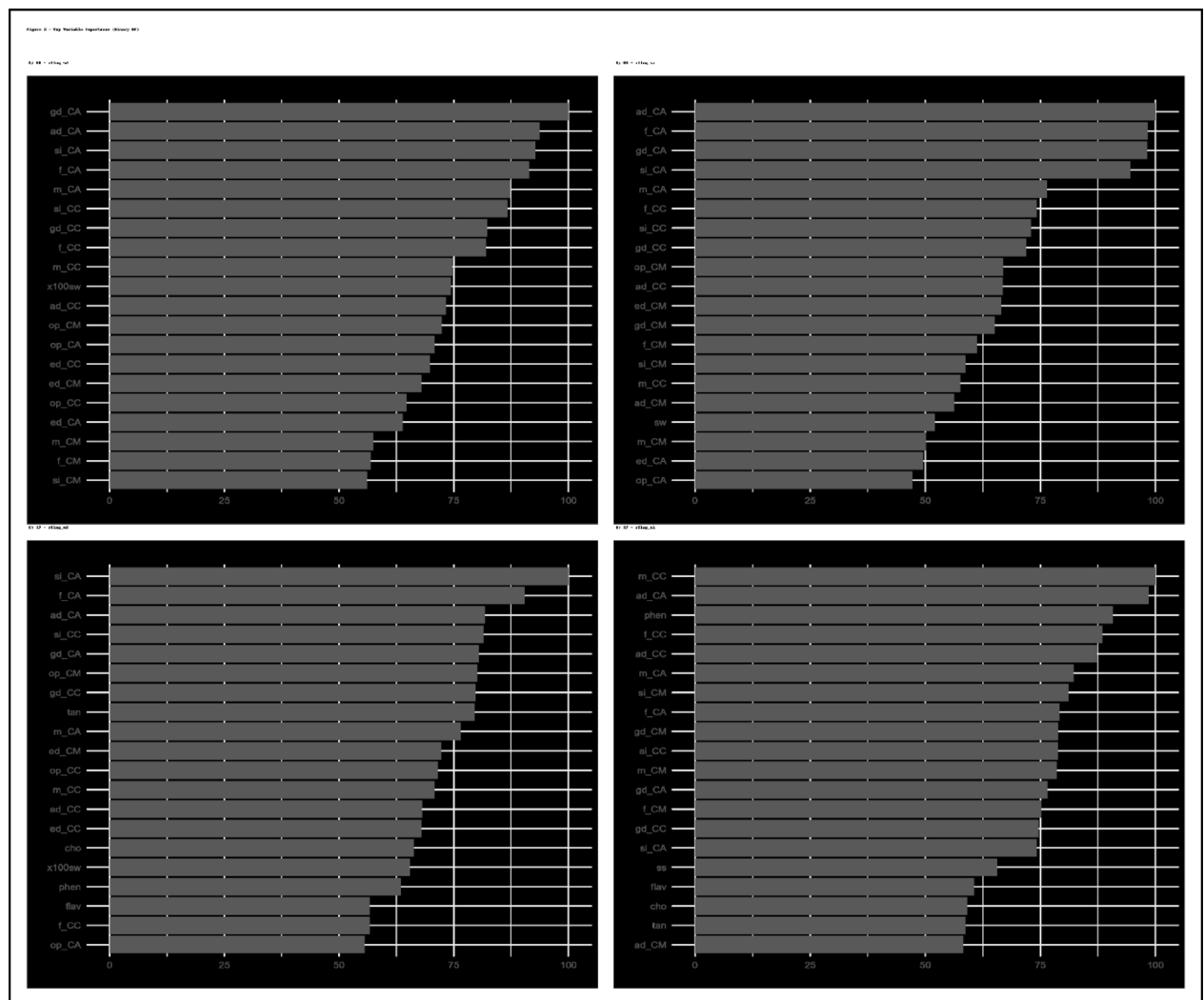
| Dataset     | AUC   | Accuracy | Sensitivity | Specificity |
|-------------|-------|----------|-------------|-------------|
| 68_rflag_sd | 0.997 | 0.95     | 0.96        | 0.94        |
| 68_rflag_si | 0.999 | 0.96     | 0.97        | 0.95        |
| 17_rflag_sd | 0.999 | 0.94     | 0.95        | 0.93        |
| 17_rflag_si | 0.999 | 0.93     | 0.94        | 0.92        |

**Table 7.** Classification performance of random forest models predicting resistance categories (rflag\_sd and rflag\_si) under Dataset A (68 accessions; insect + morpho traits) and Dataset B (17 accessions; insect + morpho + biochem traits). Metrics (Accuracy, Sensitivity, Specificity, and AUC) were compiled from model outputs for clarity.



**Fig. 6.** Receiver operating characteristic (ROC) curves showing model performance for rflag\_sd and rflag\_si predictions (Area under the curve (AUC) values are indicated in Table 6).

moderate positive correlations with oviposition and egg density, particularly under free-choice conditions, suggesting its influence on insect fecundity. For *C. analis*, a similar trend was noted, with insect traits remaining strongly interrelated and Phenols and tannins negatively associated with infestation parameters. Carbohydrates and flavonoids exhibited weak or inconsistent correlations, reflecting limited involvement in resistance. In *C. maculatus*, grain damage and adult emergence were again tightly correlated ( $r > 0.9^{***}$ ), while phenolic compounds consistently showed negative correlations with susceptibility traits. Overall, across species and conditions, phenols as major followed by tannins, emerged as key biochemical defenses against bruchids, while protein contributed variably and carbohydrates and flavonoids appeared less influential (Fig. 13).



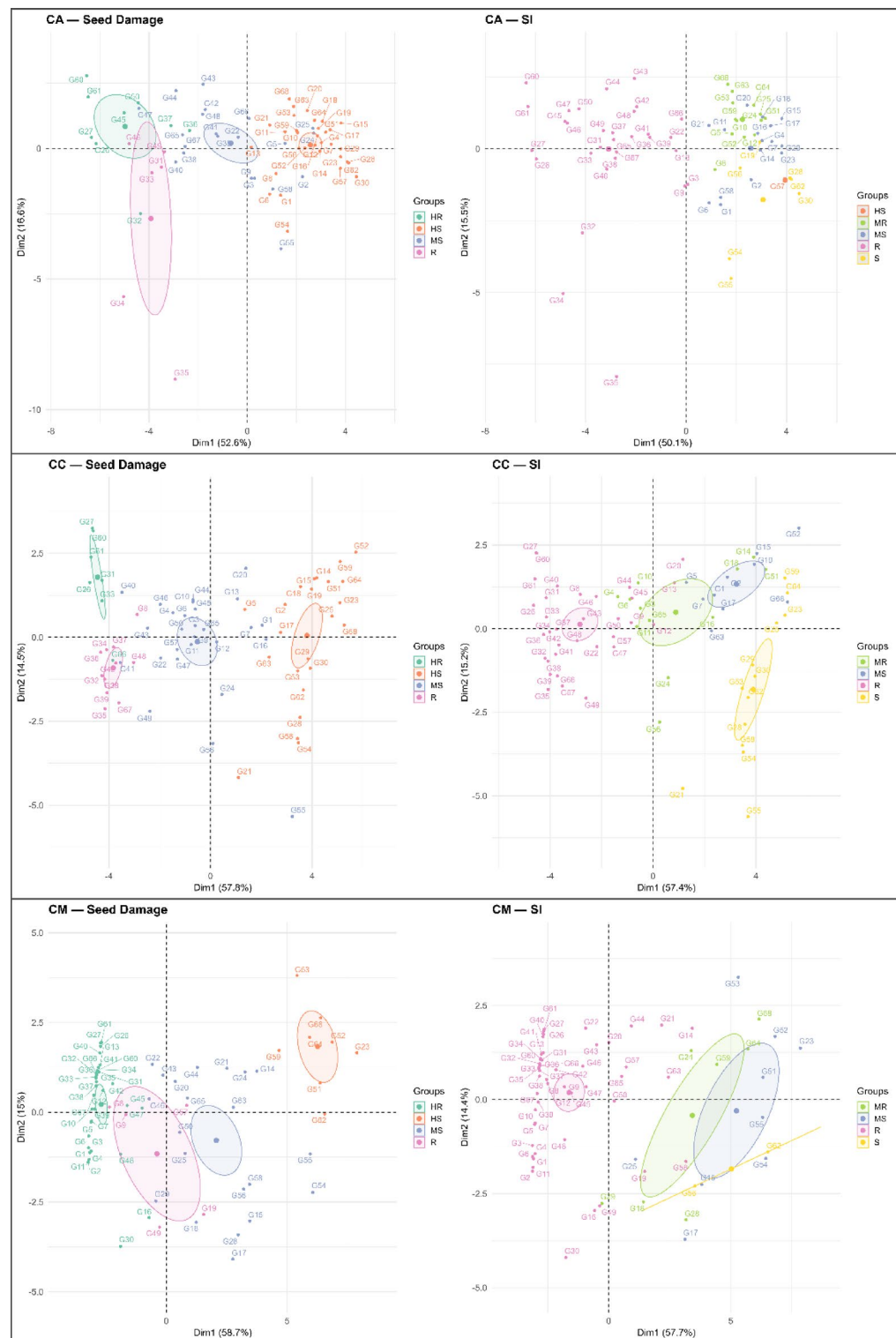
**Fig. 7.** Random forest variable importance scores for predictors of resistance, highlighting the top-ranked morphological, biochemical, and insect traits contributing to *rflag\_sd* and *rflag\_si* classification.

### Does sex ratio is influenced by germplasms resistance levels?

The sex ratios of three bruchid species recorded on all the accessions across resistance classes in different screening conditions were consistently female-biased (mean < 0.5). In both seed damage-based and susceptibility index-based classifications, the average proportion of males remained below 0.5, indicating predominance of females among emerged adults. Across resistance classes, resistant accessions (HR, R, MR) generally exhibited stronger female bias, with lower sex ratios than moderately susceptible or highly susceptible accessions. In contrast, susceptible categories (MS, S, HS) displayed slightly elevated sex ratios, approaching but not exceeding 0.5, suggesting a relative increase in male emergence under higher susceptibility but without reversing the overall female dominance. Condition-specific comparisons showed that NC assays tended to yield higher sex ratios than FC assays, implying that less favorable screening conditions may reduce the degree of female bias. Species-level differences were also observed: *C. analis* consistently showed stronger female bias than other two species, though all three species followed the same broad trend of female dominance across resistance classes. Mixed-effects models supported these observations. For both resistance classes, species and condition effects were statistically significant ( $p < 0.05$ ), while resistance class effects were weaker but retained significance in interaction with species. Post-hoc contrasts indicated that differences between resistant and highly susceptible classes were significant in several species–condition combinations. Diagnostic checks confirmed adequate model fit, and beta regression provided consistent results. A concise summary of statistical outcomes is provided in supplementary file 6. Full model summaries, ANOVA outputs, Tukey comparisons or pairwise contrasts, and diagnostic plots are retained in the supplementary file 7 for transparency and reproducibility.

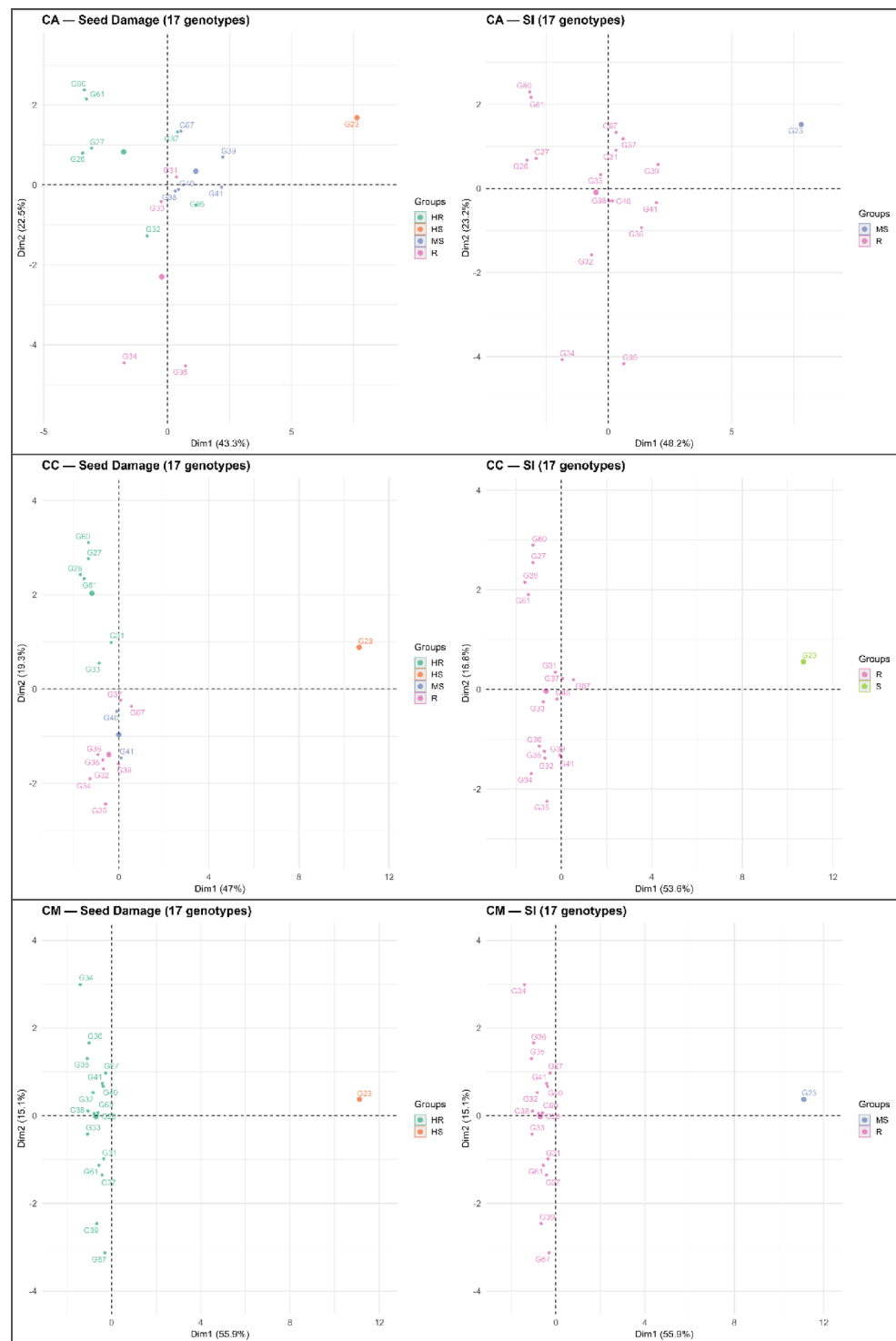
### Discussion

Wild pigeonpea relatives are known to contribute valuable traits for multiple biotic and abiotic stresses, and some also serve as cytoplasmic male sterility sources (Table 1). Although earlier studies evaluated a few wild species



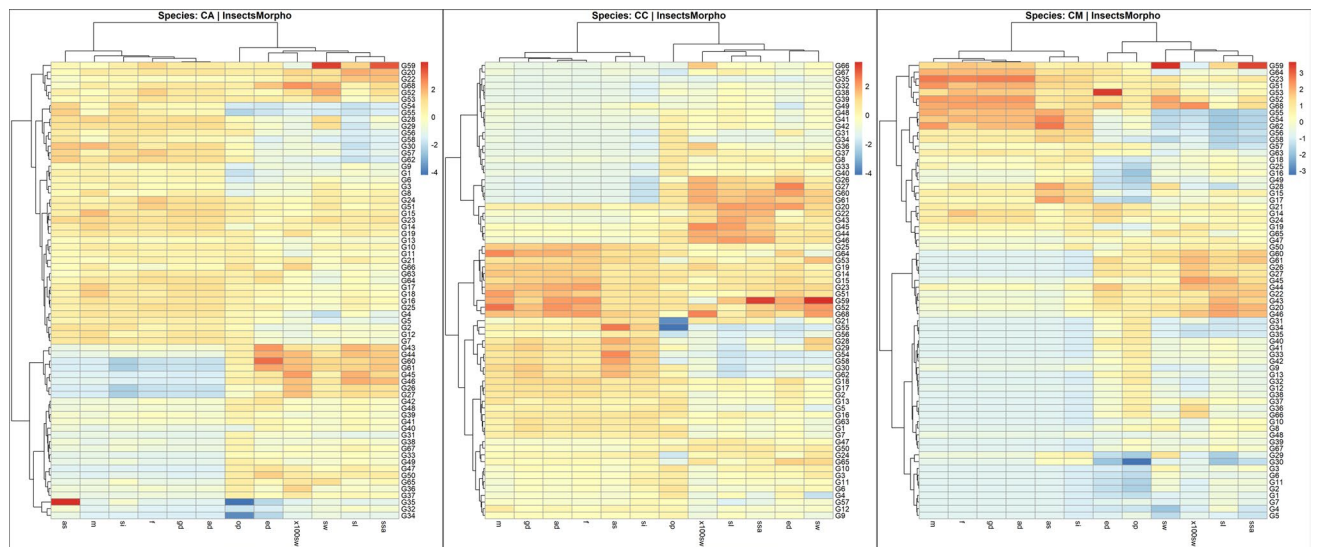
**Fig. 8.** FAMD plots showing the grouping of pigeonpea 68 genotypes based on insect traits and resistance categories. Each figure presents three paired plots (*Callosobruchus* species- CA, CC, CM) for seed damage (SD, left) and susceptibility index (SI, right). Genotypes are colored by resistance categories, with 95% confidence ellipses drawn to illustrate group clustering.

against one or two bruchids<sup>22–24</sup>, the present work<sup>22–24</sup> identifies and validates bruchid-resistance donors with broader applicability using high throughput screening against multiple *Callosobruchus* species. Notably, it provides the first empirical evidence of convergent high in *R. bracteata*, underscoring its potential as a durable resistance donor.

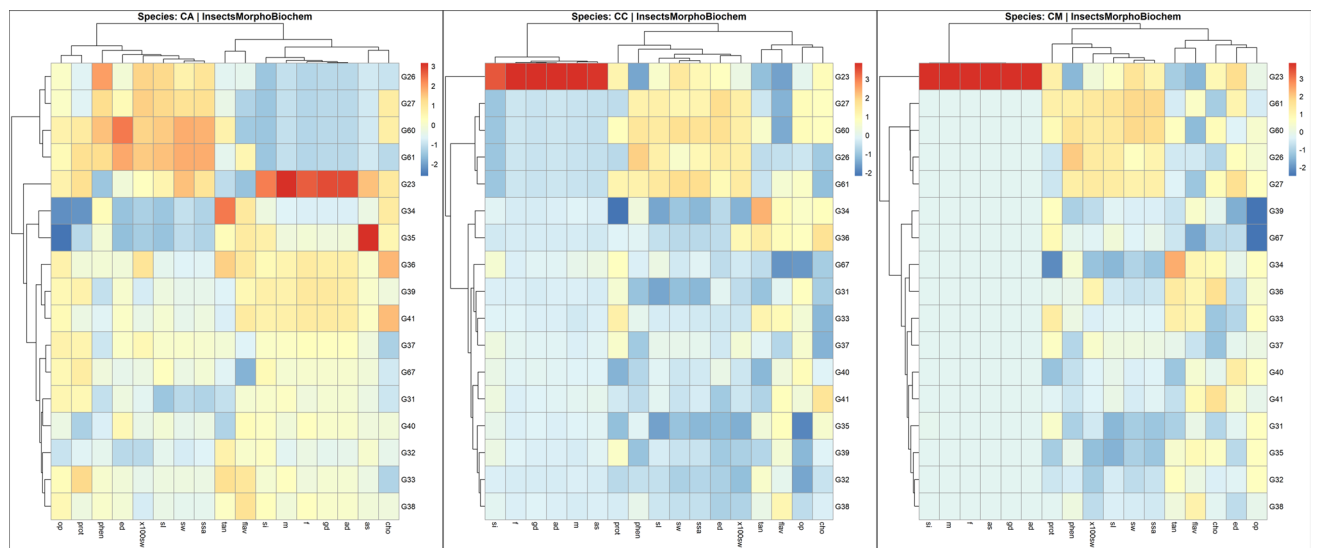


**Fig. 9.** FAMD plots showing the grouping of pigeonpea 68 genotypes based on insect and seed biochemical traits and resistance categories. Each figure presents three paired plots (*Callosobruchus* species- CA, CC, CM) for seed damage (SD, left) and susceptibility index (SI, right).

Earlier literature only broadly recognized *Rhynchosia* spp. as wild relatives with potential sources of tolerance to pod borer (*Helicoverpa armigera*), pod fly (*Melanagromyza obtusa*), and SMD, and abiotic stress adaptation such as drought tolerance<sup>16,56,58,64,68</sup>. However, no accession-level evaluations had been conducted, and their donor potential remained largely theoretical due to severe crossability barriers with cultivated pigeonpea. *R. bracteata* accessions (G26, G27, G60, G61) exhibited consistence and convergent high resistance across *C. analis*, *C. chinensis*, and *C. maculatus* under FC, NC, and CNC, establishing them as robust multi-species bruchid-resistant donors.

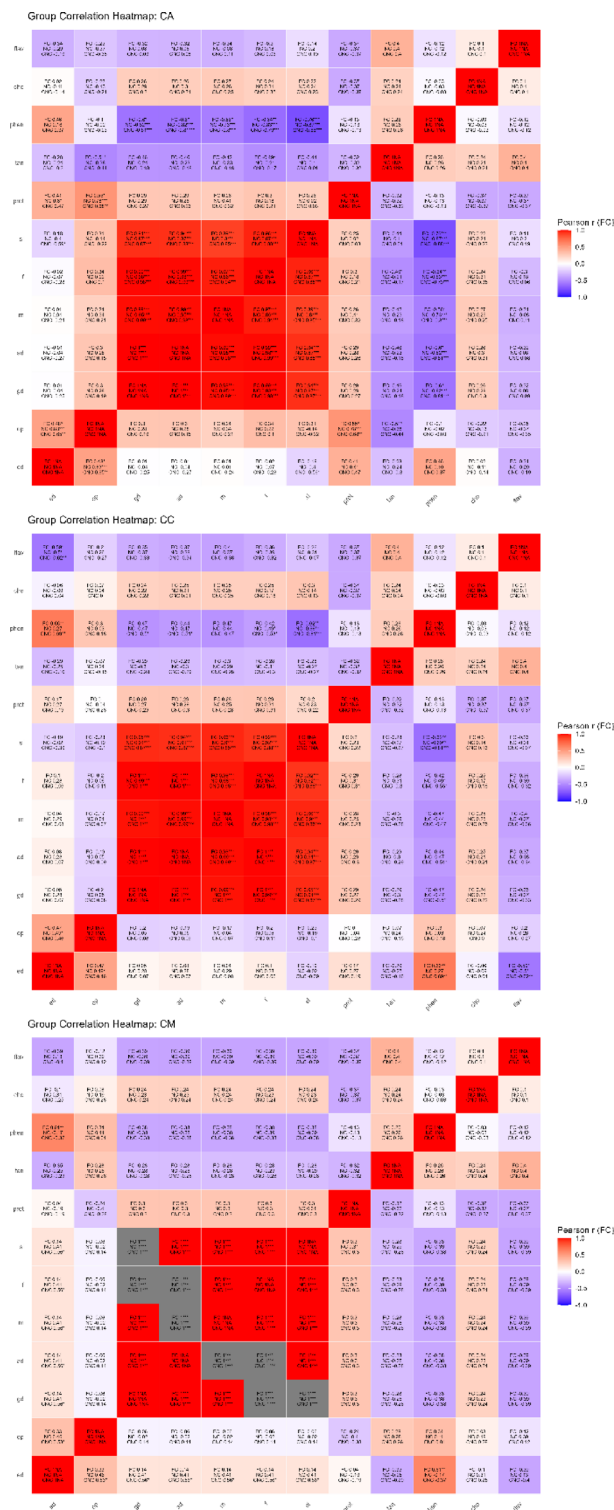


**Fig. 10.** Hierarchical clustering heatmaps of 68 wild pigeonpea genotypes screened against three species using insect bioassay and seed morphological traits. Clustering was performed with Ward's minimum variance method based on standardized (Z-score) data. Warmer colors indicate higher trait values, cooler colors lower values. Abbreviations: *Callosobruchus analis* (CA); *C. chinensis* (CC); and *C. maculatus* (CM); ed, egg density; op, oviposition; gd, seed damage; ad, adult density; m, males; f, females; mdp, mean development period; as, adult survivability; si, susceptibility index; x100sw, 100-seed weight; sl, seed length; sw, seed width; ssa, seed surface area.

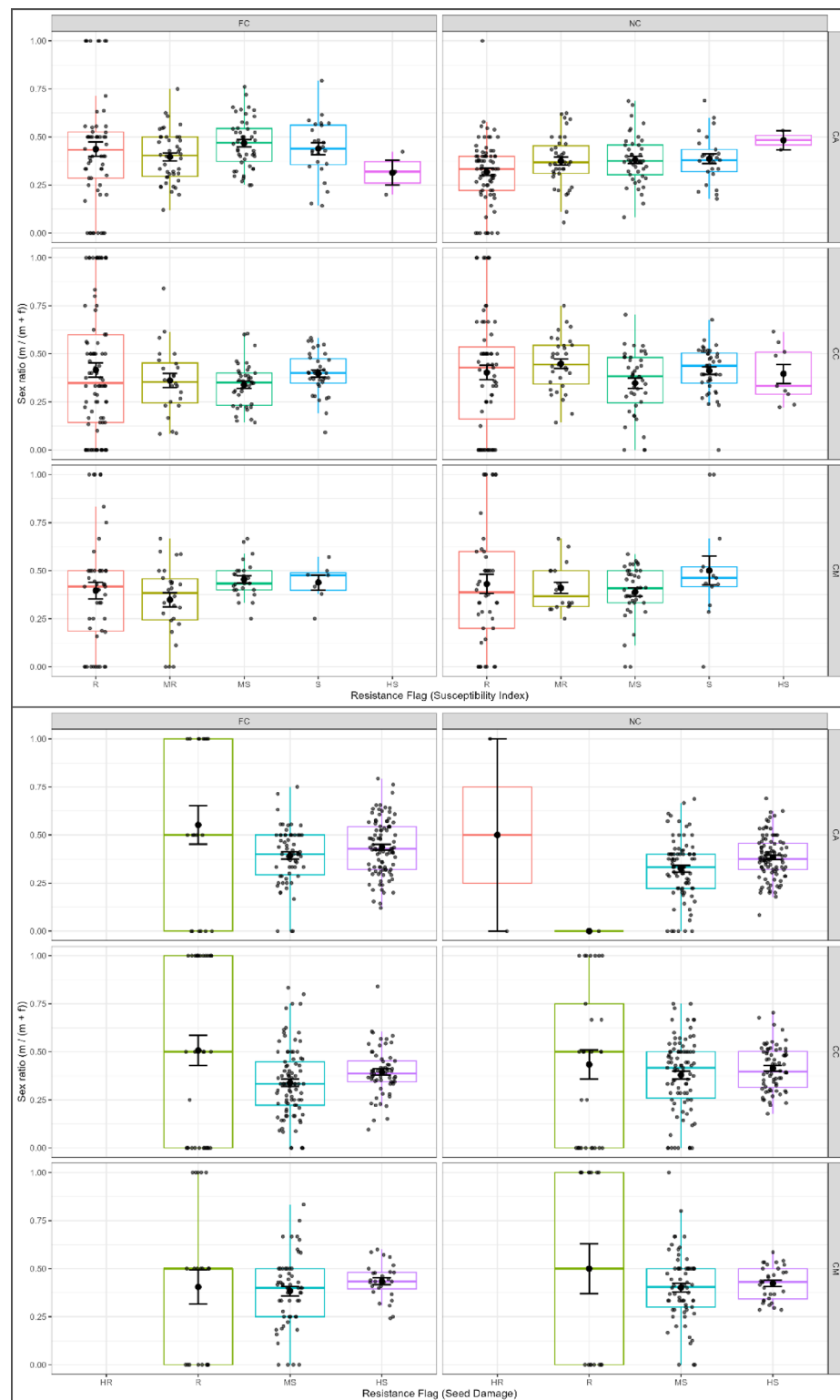


**Fig. 11.** Hierarchical clustering heatmaps of 17 wild pigeonpea genotypes incorporating insect, seed morphological and biochemical traits for three species. Warmer colors indicate higher trait values, cooler colors lower values. Abbreviations: CA, *Callosobruchus analis*; CC, *C. chinensis*; CM, *C. maculatus*; ed, egg density; op, oviposition; gd, seed damage; ad, adult density; m, males; f, females; mdp, mean development period; as, adult survivability; si, susceptibility index; x100sw, 100-seed weight; sl, seed length; sw, seed width; ssa, seed surface area.

*C. scarabaeoides* has been frequently reported as a donor for many biotic (including *C. maculatus*, pod borer, pod fly, pod wasp, Fusarium, SMD, and nematode) and abiotic (draught, salinity and aluminium toxicity tolerance) stresses<sup>16,22,56,67,69</sup>. Similarly, *C. platycarpus* (accession ICPW-130) was previously reported to be tolerant to *C. maculatus* with 16% seed damage<sup>23</sup>. In this study, multiple accessions of *C. scarabaeoides* (G31–G42, G66, G67, G13, G12, G8, G9) showed resistant to highly resistant reactions, especially against *C. chinensis* and *C. maculatus*. However, their convergence across screening conditions occurred only as per SI and not as per SD. Likewise, accessions of *C. platycarpus* (G20–G22, G43–G47, G50) displayed resistance to the same species,



**Fig. 12.** Heatmaps showing Pearson correlation coefficients (r) between seed biochemical traits and insect biology parameters across three bruchid species under three screening conditions. Positive and negative correlations are represented by red and blue gradients, respectively, with significance levels indicated (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ). Abbreviations: CA, *Callosobruchus analis*; CC, *C. chinensis*; CM, *C. maculatus*; FC, free-choice; NC, no-choice; CNC, confirmatory no-choice; op, oviposition; ed, egg density; gd, seed damage; ad, adult density; m, male density; f, female density; mdp, mean developmental period; as, adult survivability; si, susceptibility index; x100sw, 100-seed weight; sl, seed length; sw, seed width; ssa, seed surface area; prot, protein; tan, tannins; phen, phenols; cho, carbohydrates; flav, flavonoids.



**Fig. 13.** Boxplots with mean  $\pm$  SE bars showing sex ratio ( $m / [m + f]$ ) across seed damage and susceptibility index resistance categories, faceted by species and condition. Values  $< 0.5$  indicate female-biased emergence.

in agreement with earlier reports<sup>22–24</sup>, but did not exhibit full convergence across SI and SD like *R. bracteata*, indicating species-specific rather than broad-spectrum resistance.

*C. crassus* and *C. cajanifolius* have been reported to harbor CMS sources and disease resistance, and two accessions (ICPW-30, ICPW-31) of *C. cajanifolius* were also reported as resistance against *C. maculatus* and *C. chinensis*<sup>32</sup>. However, in this study none of the accessions of *C. crassus* or *C. cajanifolius* showed notable bruchid

resistance. Three underexplored taxa (*C. mollis*, *R. minima*, *R. rothii*) were evaluated for the first time for bruchid resistance. *R. rothii* was previously noted mainly for drought tolerance<sup>60</sup>. Similarly, *R. minima* has rarely studied for pest resistance. No accessions from these species showed bruchid resistance. Collectively, these findings expand the evidence base for lesser-studied wild relatives in pigeonpea improvement.

The convergence in resistance across species, conditions and indices (SI and SD) for prominent accessions confirms their status as robust, multi-species resistant donors. Importantly, SI did not classify any accession as highly susceptible under any species or condition, highlighting a baseline tolerance in wild relatives. The distinction between true convergent high resistance (*R. bracteata*) and partial resistance (*C. scarabaeoides*) provides clear pre-breeding guidance: the former are ideal for immediate deployment in durable resistance breeding, while latter serve as species-specific resistance sources. These accessions belong to secondary to quaternary gene pools, which face significant transfer challenges due to crossability barriers, linkage drag, and sterility<sup>14,58,67,70</sup>. Advances in embryo rescue, tissue culture, molecular breeding, and genome editing<sup>14</sup> offer new avenues to harness these resources effectively.

Regarding criteria used for resistance categorization, the seed damage (SD) scoring emerged as the most reliable indicator of stable bruchid resistance. Although susceptibility index (SI) reflects host suitability for insect development<sup>35,37</sup>, its reliance on mean developmental period (MDP) can lead to misclassification, particularly where delayed development reduces SI despite substantial seed damage<sup>25,36</sup>. Correlation funnel, MANOVA, Random Forest, FAMD, and clustering consistently identified seed damage and adult emergence as key predictors, validating SD-based HR and R accessions as the most stable donors.

Seed size plays a critical role in supporting bruchid larval development. Female bruchids carefully assess seeds for adequate resources, influencing oviposition behavior. Among the species studied, *C. maculatus* is the largest<sup>5</sup>; it laid fewer eggs on smaller seeds, resulting in lower seed damage, adult densities, and survivability, leading many accessions to appear resistant primarily due to non-preference. In contrast, *C. chinensis* (3–4 smaller than *C. maculatus*), being much smaller, deposited more eggs and survived on many accessions except those expressing strong antibiosis. *C. analis* (~1.2–1.5 times smaller than *C. maculatus*) exhibited greater adaptability, surviving on small-seeded accessions by reducing F1 body size and persisting even on accessions resistant to the other two species. This species tolerated a broader biochemical spectrum, making it the most challenging species.

Most pigeonpea accessions displayed strong resistance to *C. maculatus* due to antixenosis. However, some accessions of *R. bracteata* and *C. scarabaeoides* received higher egg loads from *C. maculatus* yet remained resistant, attributable to elevated phenolic content, indicating antibiosis. Seed morphology (100-seed weight, surface area) contributed to non-preference, while phenols and tannin provided effective antibiosis. Similer findings were reported by Hamzei et al.<sup>71</sup>, who showed that biochemical (phenol, tannin and anthocyanin) and physical traits determined seed susceptibility or resistance to *C. maculatus*. Species-specific responses revealed that *C. chinensis* and *C. maculatus* were most suppressed by high-phenol, high-tannin accessions, whereas *C. analis* survived wider biochemical range, rendering many accessions that resisted the other two species only moderately resistant or susceptible. Overall, accessions with minimal seed damage, low adult densities, reduced survivability, and prolonged MDP showed stable resistance.

Across species and conditions, all traits showed highly significant genotypic variation, confirming the existence of resistant and susceptible accessions. Between species, *C. analis* was the most aggressive pest, *C. chinensis* intermediate, and *C. maculatus* the least damaging. Across conditions, CNC validated resistance accessions by putting them under maximum pressure with higher oviposition and egg density. Further, lower infestation traits and higher mdp, in CNC than FC and NC validated the consistent convergence of resistance accessions.

Multivariate (MANOVA) and univariate (ANOVA) analyses showed that bruchid resistance in wild pigeonpea is shaped by both species identity and experimental conditions. Significant inter-specific variation on accessions reflected biological differences among *C. analis*, *C. chinensis*, and *C. maculatus*, matching earlier reports<sup>24,25</sup>. SD- and SI-based resistance classifications were generally consistent, but trait sensitivities reflected distinct mechanisms: in *C. analis* and *C. chinensis*, antibiosis dominated, with reduced seed damage, suppressed emergence, and delayed development which previously recognized as strong indicators of resistance<sup>35,37</sup>. In contrast, resistance to *C. maculatus* was associated mainly with seed morphology, indicating antixenosis as a major mechanism<sup>72</sup>.

The correlation funnel approach effectively highlighted the biological and morphological traits driving bruchid resistance in pigeonpea. Traits influenced by antibiosis consistently defined resistance across species, consistent with earlier findings in legumes<sup>73,74</sup>. These patterns parallel Aidbhavi et al.<sup>25</sup>, where resistance in wild *Vigna* was almost exclusively explained by lower seed damage, adult survivability, and egg density, with minimal role of seed morphology, reinforcing antibiosis as the central mechanism. In pigeonpea, however, seed morphological traits contributed for antixenosis against *C. maculatus*. Some accessions of *R. bracteata* and *C. scarabaeoides* received higher oviposition yet remained highly resistant indicating a biochemical defense. Thus, accessions categorized as resistant solely due to small seed size may not be reliable donors, whereas biochemically mediated resistance provides more durable resistance sources. Collectively, these findings indicate that antibiosis is the dominant mechanism, with antixenosis providing a minor, species-specific layer.

Machine learning approaches, particularly Random Forest have been widely used for pests detection, prediction and forecasting, and crop yield performance modelling<sup>75–77</sup>. Recently, Random Forest has been employed for disease-resistance prediction using multi-omics data<sup>78,79</sup>. This study is the first to apply Random Forest to predict key bruchid-resistance governing traits. Grain damage, insect emergence, and seed weight consistently emerged as the strongest predictors, highlighting contributions from both host and insect traits. These findings corroborate reports identifying insect emergence traits as accurate classifiers of bruchid-resistance<sup>26,80</sup>. Stronger model performance in the 68-accession dataset underscores the importance of adequate

sample size for machine-learning stability<sup>81,82</sup>, while the smaller biochemical dataset (17 accessions) yielded exploratory but less stable predictions, emphasizing that biochemical or omics traits require larger sampling scales for robust inference. These results reinforce the feasibility of integrating machine-learning tools with phenotypic and biochemical screening to enhance bruchid-resistance breeding pipelines in pigeonpea.

The FAMD analysis demonstrated that resistance against *Callosobruchus* species is a multivariate trait, jointly shaped by insect bioassay outcomes and biochemical factors. Previous studies<sup>73,83,84</sup> emphasized the roles of antixenosis and antibiosis, particularly seed coat and chemical traits. The present results support this: resistant accessions clustered distinctly in FAMD plots, especially when biochemical traits were included. FAMD captures both quantitative and qualitative dimensions, enabling accession groupings that mirror biological resistance patterns. Aïdhavi et al.<sup>25</sup> highlighted the utility of FAMD for grouping accessions based on trait interactions; here, FAMD extends these insights by visualizing how traits jointly structure accession groupings and reinforce predictor traits identified using other tools. These results confirm that SD and SI resistance flags reflect integrated trait interactions, not arbitrary thresholds.

The clustering analyses further support the fact that insect resistance in pigeonpea is shaped by both insect and seed biochemical traits. The strong separation of resistant versus susceptible accessions aligns with previous reports showing that biochemical defenses play central roles in limiting bruchid infestation<sup>73,74,85</sup>. Similar multivariate clustering approaches have been effective in identifying key bruchid-resistance traits in pigeonpea and other legumes<sup>32,86</sup>. Importantly, biochemical traits enhanced resolution among moderately resistant lines, suggesting that integrating these enhances accuracy in breeding for durable resistance.

The group correlation patterns confirm that seed biochemicals, especially phenols and tannins, are key determinants of resistance. Their consistent negative association with grain damage and adult emergence supports the role of seed antibiosis, where chemical defenses limit insect development. Similar effects of phenolics and tannins have been reported in other legumes<sup>26,73,74,86</sup>. Conversely, protein showed positive correlations with oviposition and egg density, suggesting its role as a nutritional cue, though responses varied across conditions. Carbohydrates and flavonoids exhibited weak or inconsistent relationships, implying a minor role in resistance. Some specific chemicals and antinutritional compounds were reported to influence the legumes resistance against pod fly and borers<sup>68,69,83,84,87</sup>. Thus, investigating the role of these factors in bruchid-resistance would be valuable.

Sex ratio analysis explored whether resistance levels influenced male–female patterns, based on hypothesis that the insect development (emergence, MDP) reflects host suitability. Results demonstrated slightly female-biased (sex ratio < 0.5) across accessions, resistance classes, species, and conditions. Female dominance was stronger on across accessions irrespective of resistance classes, with only slight male-bias in susceptible accessions. These patterns agree with ecological evidence showing that sex-specific survival differences arise under variation in host quality and stress<sup>88</sup>. Previous studies report female bias ratios under restricted or stressful environments<sup>89,90</sup>. However, in haplodiploid sawflies<sup>91</sup> and parasitoid wasps<sup>92</sup>, sex ratios shift adaptively depending on host quality, with high-quality hosts producing female-biased offspring. Understanding bruchid sex allocation under differing host types may require integrating host quantity (seed density) with host quality parameters for better clarity in adaptive patterns.

## Summary and conclusion

This study represents the most comprehensive evaluation of bruchid resistance in wild pigeonpea, involving 68 accessions from multiple gene pools screened against three destructive species namely *C. analis*, *C. chinensis*, and *C. maculatus*. Notably, this is the first systematic screening including *C. analis*, a neglected yet highly destructive species. Seed damage (SD)- resistance categorization proved more reliable than susceptibility index (SI) for identifying true resistant donors. Across three screening conditions resistance varied significantly, with *Rhynchosia bracteata* (IC15815, IC15816, IC15817 and IC618509) consistently exhibiting stable, convergent highly resistant reactions across all species and conditions. Several *C. scarabaeoides* accessions also resistance, especially to *C. chinensis* and *C. maculatus*, though reactions were less stable against *C. analis*.

Advanced analyses confirmed that seed damage, adult emergence, and developmental period as the strongest predictors of resistance. MANOVA, ANOVA, correlation funnels, and machine learning consistently highlighted antibiosis as the dominant mechanism, with antixenosis contributing mainly against *C. maculatus*. FAMD and clustering further confirmed biologically meaningful grouping of accessions, strengthened by biochemical traits such as phenols and tannins which showed strong negative correlations with susceptibility. Collectively, this study identifies *R. bracteata* as a robust multi-species resistance donor and *C. scarabaeoides* as a species-specific source, establishing both as invaluable resources for developing bruchid-resistant cultivars. It also highlights integrating traditional screening with advanced analytical tools strengthens donor identification pipelines and accelerates targeted breeding for durable resistance in pigeonpea.

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## Data availability

Data is provided within the manuscript or supplementary information files.

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## Author contributions

RS conceptualized the idea, conducted experiment, data collection, analyses and writing manuscript. SNSJ, DD, AsP, AB involved in collection, maintenance and multiplication seeds required for the study. SNSJ and AsP recorded seed morphological traits. SMB assisted in screening experiments and analyses. JJ and PIG estimated biochemical analyses of contrasting accessions. MA, AdP and GPD guided during the entire study.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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