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RESEARCH ARTICLE

Understanding resistance mechanisms in crop wild relatives (CWRs) of pigeonpea (*Cajanus cajan* L.) against pod borer *Helicoverpa armigera* (Hub.)

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Abstract The pod borer, *Helicoverpa armigera* (Hübner), is a highly destructive pest of leguminous crops, particularly pigeonpea (*Cajanus cajan* L.). This crop suffers significant damage from *H. armigera*, with estimated yield losses ranging from 30 to 40% annually. Despite extensive screening of elite pigeonpea accessions from the primary gene pool for resistance, no stable and true resistant or tolerant accessions have been identified. In this study, we screened 96 pigeonpea accessions from diverse gene pools for resistance to *H. armigera* using larval (first and third instar larvae) antibiosis during *Rainy-2022*. Based on k-means clustering, 50% of these accessions were selected for further evaluation in *Rainy-2023* under field and laboratory conditions. Notably, accessions of *Cajanus scarabaeoides* from the secondary gene pool—specifically ICP 15716, ICP 15718, and ICP 15726—exhibited the lowest pod damage ratings (3.0–3.6), lower per cent larval survival (26–46%), and reduced per cent larval weight gain (27.0–35.18%) over two seasons. In addition, *Rhynchosia suaveolens* (ICP 15867) from the quaternary gene pool also exhibited minimal damage rating and low larval weight gain. The correlation of pod damage and oviposition with pod trichome density, pod length and pod width revealed that these morphological traits are key factors in conferring resistance against *H. armigera*. The Multi-trait Genotype Ideotype Index (MGIDI) identified seven superior accessions of *C. scarabaeoides*—ICP 15718, ICP 15716, ICP 15726, ICP 15730, ICP 15744, ICP 15732, and ICP 15703—as optimal candidate accessions for future breeding programs. This study highlights the critical role of host plant resistance in developing resilient pigeonpea cultivars resistant to *H. armigera* and emphasizing the potential of utilizing wild relatives in crop improvement strategies.

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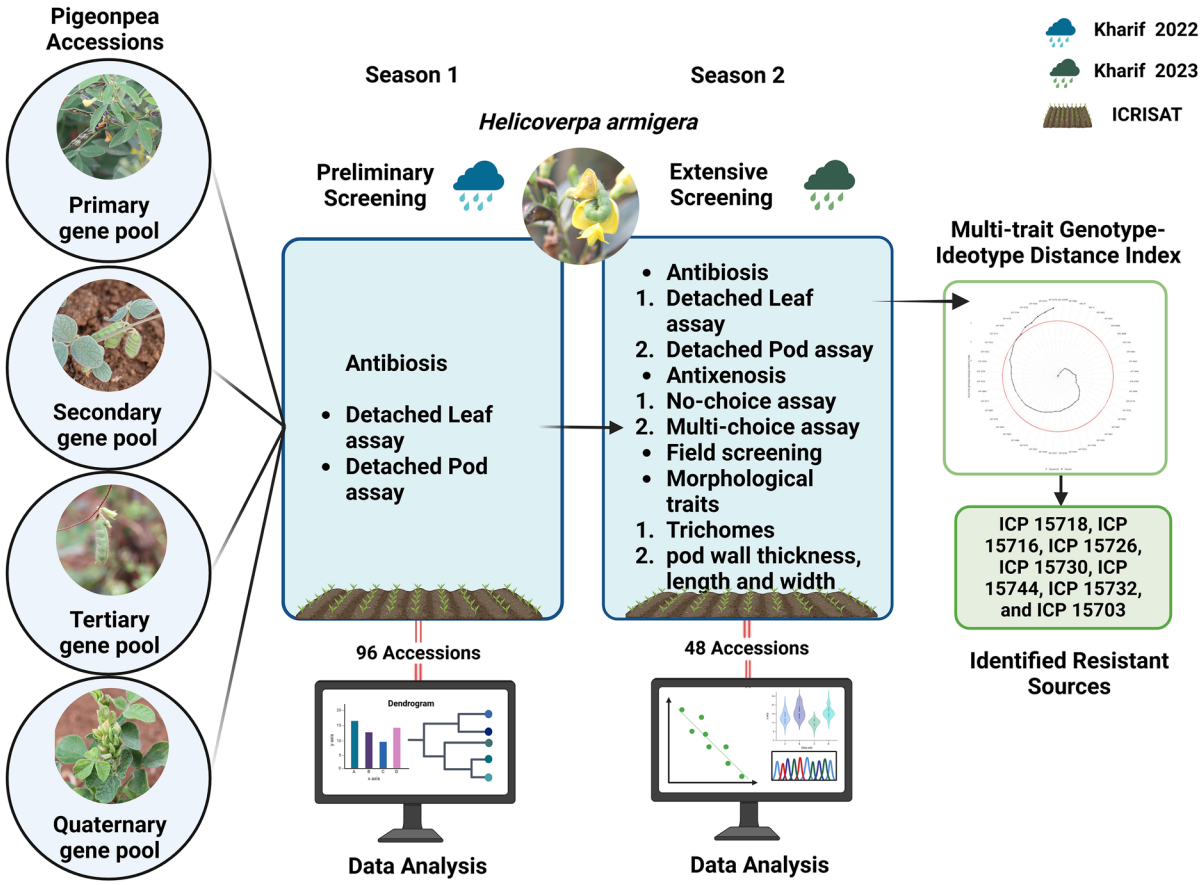
Graphical Abstract



Keywords Antibiosis · Antixenosis · *Cajanus cajan* · Crop wild relatives · *Helicoverpa armigera* · Host plant resistance

Introduction

Developing pest management strategies for highly polyphagous, insecticide-resistant pests can be a daunting challenge for any applied entomologist. Among the polyphagous pests, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera) is a pest that has been the focus of extensive research aimed at developing effective management strategies over several decades. It is the most notorious and destructive pest, highly difficult to control because of polymorphic populations, seasonal and temporal variability in occurrence, wide host range, migratory nature (Dua et al. 2005; Seethalam et al. 2021) and resistance to many insecticides (Kranthi et al. 2002; Sharma et al. 2009). *H. armigera* is responsible for significant economic losses in many crops, particularly pigeonpea (*Cajanus cajan* L.), where it is a key biotic constraint. Pigeonpea suffers enormous estimated yield losses of around 38 per cent due to *H. armigera* alone (Muchhadiya et al. 2024), with potential annual losses reaching USD 300 million worldwide (Sharma et al. 2022). A significant portion of farmer's expenditure for managing *H. armigera* is spent on indiscriminate insecticide usage, due to their easy availability and perceived short-term effectiveness (Dua et al. 2005). Due to its widespread distribution, the severity of damage that it inflicts, and insecticide resistance, H. armigera has been the focus of substantially more research than

the other insect pests (Volp et al. 2025). However, this practice has led to insecticide resistance, pest resurgence, and outbreaks of secondary pests (Sharma et al. 2022). As a solution to this challenge, utilizing host plant resistance offers a promising and sustainable approach for pigeonpea pest management.

Unfortunately, narrow genetic diversity intensifies the susceptibility of pigeonpea as there is only low to moderate resistance against *H. armigera* in the cultivated germplasm (Reed and Lateef 1990; Sharma et al. 2009). As a result, using cultivated germplasm to enhance resistance to pod borer through conventional breeding is largely ineffective (Volp et al. 2022). Wild relatives are vital for expanding the genetic base of cultivated lines, but their integration into breeding programs faces various challenges like linkage drag and cross incompatibility (Kashyap et al. 2022). Nevertheless, recent advances in multi-omics technologies have significantly improved the incorporation of crop wild relatives (CWRs) into breeding initiatives (Saxena et al. 2015; Mallikarjuna et al. 2017; Bohra et al. 2020). Previous research has evaluated only a limited number of CWRs for multiple traits associated with high levels of resistance in pigeonpea (Sharma et al. 2001b; Green et al. 2006; Sharma and Upadhyaya 2016). Notably, certain CWRs exhibit elevated proteinase inhibitor activity, which impacts key insect gut enzymes such as trypsin and chymotrypsin, ultimately reducing feeding and increasing insect mortality in early developmental stages (Parde et al. 2012; Jaba et al. 2021; Gujjarlapudi et al. 2023). Given their inherent resistance mechanisms, cross-compatible CWRs hold significant potential for incorporation into pigeonpea breeding programs to enhance pest resistance.

The CWRs of pigeonpea are classified into various gene pools according to their crossbreeding capabilities, with cultivated species falling within the primary gene pool. The secondary gene pool includes species such as *Cajanus scarabaeoides*, *C. sericeus*, *C. reticulatus*, *C. acutifolius*, and *C. cajanifolius*, which are resistant to pod borer and other insect pests (Sharma et al. 2003, 2022; Bohra et al. 2010). These species can be crossed with *C. cajan* (Pazhamala et al. 2015) to produce partially fertile hybrids (Upadhyaya et al. 2013a). Among them, *C. scarabaeoides*, native to India, possesses numerous beneficial traits (Upadhyaya 2006) such as short stature, increased branching and more pods per plant (Singh et al. 2020). Exploitation of the *C. scarabaeoides* is the primary focus of current research due to its strong resistance to *H. armigera* (Pundir and Singh 1987; Bohra et al. 2010; Sharma et al. 2022). Additionally, CWRs from tertiary and quaternary gene pools have also been identified as potential sources of resistance to *H. armigera* (Upadhyaya et al. 2013a) and can be utilized to widen the narrow genetic base of cultivated pigeonpea (Singh et al. 2022).

In this context, it is essential to explore maximum number of underutilized wild relatives of pigeonpea from various genera and species for their morphological and biochemical traits related to *H. armigera* resistance. However, making an informed selection among the diverse wild relatives requires thorough screening. In this study, we screened CWRs of pigeonpea from different gene pools to assess host plant resistance mechanisms and identify resistance to *H. armigera* in the closest relatives of *C. cajan.*

Material and methods:

Insect rearing:

The fourth and fifth instar larvae of *H. armigera* were collected from fields surrounding the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, to maintain insect culture for experimental use. These field-collected larvae were reared on the chickpea-based artificial diet (Armes et al. 1992) till pupation and adult emergence. They were then allowed to mate with six months old laboratory-reared moths to ensure genetic diversity and minimize inbreeding, which could otherwise reduce larval viability (Cacoyianni et al. 1995). The insect culture was sustained throughout the experimental period to meet the consistent need for neonates, third-instar larvae, and adult moths. Rearing was carried out under controlled laboratory conditions (27 ± 2 °C and 70–75% RH) at ICRISAT's insect-rearing facility. Subsequent generation larvae of *H. armigera* (beginning with the F_1 generation) were reared on a chickpea-based artificial diet and adult moths were provided with a 10% sucrose solution as a nectar supplement.

Table 1 List of selected pigeonpea accessions from different gene pools screened for resistance to pod borer *Helicoverpa armigera* during *Rainy-2023*

Accessions	Gene pool	Species type	No. of accessions	Name of the accessions
Cajanus scarabaeoides	Secondary	Wild	30	ICP 15683, ICP 15685, ICP 15686, ICP 15692, ICP 15694, ICP 15696, ICP 15703, ICP 15705, ICP 15711, ICP 15712, ICP 15713, ICP 15716, ICP 15718, ICP 15720, ICP 15721, ICP 15722, ICP 15723, ICP 15725, ICP 15726, ICP 15727, ICP 15728, ICP 15729, ICP 15730, ICP 15732, ICP 15733, ICP 15734, ICP 15736, ICP 15740, ICP 15744, ICP 15747
Cajanus platycarpus	Tertiary		3	ICP 15668, ICP 15669, ICP 15921
Rhynchosia densiflora	Quaternary		2	ICP 15827, ICP 15936
Rhynchosia suaveolens			1	ICP 15867
Rhynchosia sublobata			1	ICP 15868
Eriosema sp.			1	ICP 15887
Cajanus sp.	Unknown		1	ICP 16907
Cajanus cajan	Primary	Cultivated	9	ICPL332WR, ICPL 87091, ICPL 7035, ICPL 98008, ENT 11, ICPL 8863, ICPL 87119, ICPL 87, ICPL 84060

Plant material:

Seeds of pigeonpea CWRs were obtained from the Rajendra Singh Paroda Genebank-ICRISAT and those of cultivated lines were acquired from the Division of Entomology, ICRISAT, Patancheru, Hyderabad, India. Seeds of CWRs were scarified using a scalpel blade by making a small incision in the seed coat, they were then soaked in distilled water for 24 h for faster germination. Plants were raised under field conditions during *Rainy-2022* and *Rainy-2023* in randomized complete block design. The trial was planted with a spacing of 75 cm between the rows and 15 cm between plants in paired rows of 2-m length per replication in deep red soils with three replications. Normal agronomic practices were followed (Jaba et al. 2023) for raising the crop, but there was no insecticide application in the experimental plot.

Accessions obtained from different gene pools for the preliminary lab screening (detached leaf and pod bioassays) in *Rainy-2022* included *Cajanus scarabaeoides* (63 accessions), *C. platycarpus* (4), *C. sericeus* (1), *Rhynchosia densiflora* (2), *R. rothii* (1), *R. suaveolens* (1), *R. sublobata* (1), *R. americana* (1), *R. burkartii* (1), *R. edulis* (6), *Eriosema* spp. (2), *Cajanus* spp. (4) and *C. cajan* (9) (Table S1). In *Rainy-2023*, seeds of the selected accessions viz., *C. scarabaeoides* (30), *C. platycarpus* (3), *R. densiflora* (2), *R. suaeveolens* (1), *R. sublobata* (1), *Eriosema* spp. (1), *Cajanus* spp. (1) and cultivated checks (9) (moderately resistant and highly susceptible cultivars) (Table 1) were obtained from the previous season.

Season 1: preliminary screening based on leaf and pod bioassays

In *Rainy-2022* (July–December 2022), a total of 96 accessions (Table S1), comprising 87 CWRs and 9 cultivated checks, including the susceptible check ICPL 87 and the moderately resistant check ICPL 87119 (Asha Variety) were evaluated in a randomized block design at ICRISAT-Patancheru, Hyderabad. The accessions were subjected to antibiosis (leaf and pod bioassays) experiments in the laboratory (27 ± 2 °C and 70–75% RH). Observations included visual leaf damage rating (LDR) for leaf bioassay, where detached leaves were assessed for feeding damage by neonates on a 1–9 scale: 1 ($\leq$10% leaf area damaged), 2 (11–20%), 3 (21–30%), 4 (31–40%), 5 (41–50%), 6 (51–60%), 7 (61–70%), 8 (71–80%), and 9 ($\geq$80% leaf area damaged) (Sharma et al. 2005b), larval survival (LS) after the five-day feeding period was also recorded. For pod bioassay, the extent of pod damage by third-instar larvae after three days was assessed using the pod damage rating scale (1–9), where 1

indicates < 10% damage and 9 represents up to 80% damage (Golla et al. 2018; Vishal et al. 2023). Additionally, per cent weight gain (WG) by the larvae was calculated using the formula: per cent larval weight gain (%) = ((final larval weight—initial larval weight) / initial larval weight) × 100 (Sharma et al. 2005b; Sujana et al. 2008; Golla et al. 2018; Jaba et al. 2023). Based on the damage ratings (leaf and pod), larval survival and per cent larval weight gain data, a dendrogram was constructed using the R (*ggplot2*) package. Accessions from the different clusters exhibiting the lowest scores or highest levels of resistance were selected for further evaluation in *Rainy-2023*.

Season 2: Screening under laboratory and field conditions

In *Rainy-2023* (July-December 2023), the seeds of selected accessions from previous screening were planted at ICRISAT-Patancheru, Hyderabad, in a randomized block design in three replications. Experiments were conducted to explore the stable resistance among the 48 accessions of pigeonpea (39 CWRs and 9 cultivated checks- Table 1) by assessing different mechanisms of host plant resistance in the laboratory and field viz., antibiosis (pod and leaf bioassays), antixenosis (oviposition preference), morphological traits (trichomes, pod wall thickness, pod length, and pod width) and tolerance (pest incidence under field conditions).

Expression of antibiosis mechanisms of resistance to *H. armigera* in pigeonpea wild accessions using detached leaf and pod assay

Antibiosis experiments included detached leaf and pod assay (Fig. S1) conducted under laboratory conditions (27 ± 2 °C and 70–75% RH). In both experiments, 250 mL plastic cups were used. 15 mL of 3% agar was poured into each cup keeping the cups in an inclined position and left at room temperature until solidification (Sharma et al. 2005b). Terminal fully expanded leaves and tender pods were incised in the field using scissors and inserted into solidified agar. Ten neonates were released per replicate for the detached leaf assays and a single third instar larva was used per replicate for the detached pod assays (Sharma et al. 2005b) likewise, three replications were maintained for each accession. Experiments were terminated when more than eighty per cent of the leaf or pod area was damaged in the susceptible control within 3 days (pod bioassay) and 5 days (leaf bioassay) interval (Golla et al. 2018). Observations on Leaf Damage Rating (LDR), Larval Survival (LS), Pod Damage Rating (PDR) and Weight Gain percentage (WG) were recorded as described under 2.3.

Antixenosis mechanism of resistance to *H. armigera* in wild relatives of pigeonpea in *Rainy 2023*

Antixenosis for oviposition was studied under no-choice and multi-choice tests under laboratory conditions (27 ± 2 °C temperature, 65–75% RH, and photoperiod of 12 h) as described by (Vishal et al. 2023).

Screening under no-choice condition

Each accession was tested individually in a custom-made plastic container, equipped with a perforated mesh lid for ventilation and a front-side opening covered with cloth to facilitate the release of adult pairs, which was securely tied afterward (30 × 30 × 45 cm) (Fig. S2). Three to five inflorescences, each 30 cm long with three to five nodes, were collected from the field and carefully inspected with a hand lens to remove any eggs or larvae of pests. The inflorescences were then placed in a 50 mL conical flask filled with water (Kumari et al. 2006; Sujana et al. 2008). Two pairs of two days post-eclosion *H. armigera* moths were released inside the cage. The sucrose solution (10%) in a cotton swab served as food for the adults. After releasing the moths in the cages, the moths were allowed to oviposit for five nights on the test plants.

Screening under multi-choice condition

In the case of the multi-choice condition, the inflorescence of the test accessions were brought from the field and kept in a 50 mL conical flask filled with water. All forty-eight accessions were placed in a single large cage (80 × 70 × 60 cm) (Fig. S2), at room temperature in a completely randomized design. Twenty-five pairs of two-day post-eclosion adults were released inside the cage. Moths were provided with a 10% sucrose solution in a cotton swab as a

carbohydrate source. The moths were allowed to oviposit on the test entries for five consecutive nights. Observations on number of eggs laid were recorded 5 days after the release of adult pairs.

Evaluation of selected pigeonpea wild accessions for incidence of pest complex at different stages under field conditions

Under field conditions, all the accessions used for the experiment were screened for *H. armigera* resistance along with other key pests such as legume pod borer (*Maruca vitrata*), leafwebber (*Eucosma critica*), Leafhoppers (*Empoasca kerri*), aphids, stink bugs, plume moth, pod bugs and cow bugs at vegetative, flowering, and maturity stages. Data were recorded on the larval incidence, and oviposition of pod borer, *H. armigera* different stages (Jaba et al. 2023). The observations on the pest incidence of pigeonpea during the cropping period were recorded on three tagged plants per replication. The data were subjected to ANOVA to assess significant differences among the accessions.

Morphological traits associated with resistance

Trichome density on pods was recorded using an Axio Scope A1 upright wide-field microscope (Zeiss. Inc., Thornwood, NY) at 10× magnification. The trichome counts were expressed as the number of trichomes/10× microscopic field. Five pods were collected from each accession and each accession was replicated three times. All the pods were collected in 2:1 acetic acid and alcohol solution in 10 mL vials and kept for 24 h to remove chlorophyll contents (Jackai and Oghiakhe 1989). After 24 h, all the pods were transferred to 90% lactic acid, which acts as a preservative until trichome counting.

Observations on pod wall thickness, pod length, and pod width were measured using digital vernier calipers as mentioned in previous research (Ambidi et al. 2021; Jat et al. 2021; Tyagi et al. 2022). Randomly, 10 green pods per accession were collected at the podding stage for each replication. The experiment was done in triplicates. To obtain pod wall thickness, each pod was split open, and the pod wall thickness was measured. Three measurements for each pod were recorded and averaged to obtain data

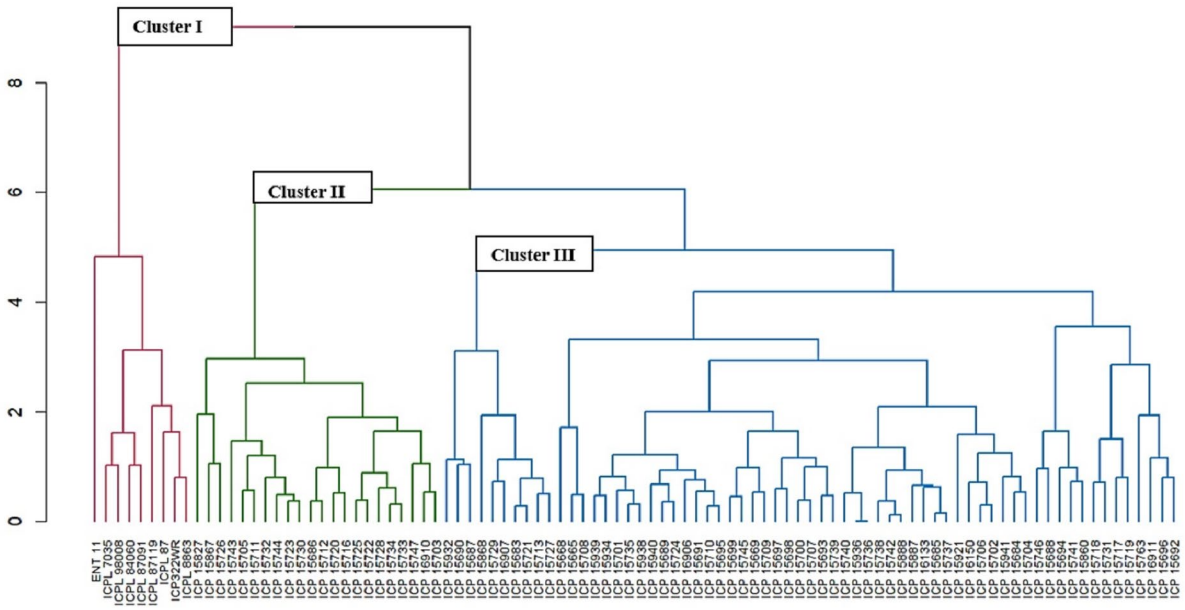


Fig. 1 Clustering of pigeonpea wild relatives based on their performance in antibiosis experiments using k-means clustering. The clustering was performed using damage-related traits to assess resistance to *Helicoverpa armigera*. The analysis resulted in three distinct clusters: Cluster 1 (red colour), representing cultivated lines; Cluster 2 (green colour), which included the wild relatives exhibiting resistance or tolerance to pod borer; and Cluster 3 (blue colour), which comprised accessions categorized as moderately resistant or susceptible

for a single pod. Hence, each replication included an average value of 10 such pods. Data on pod wall thickness, pod length, and pod width were represented in milli meter (mm).

Statistical analysis

Data analysis for the current study was conducted using R Software (version 2023.12.1), employing the *tidyr* package for data organization. Analysis of variance (ANOVA) was performed on all observations recorded during *Rainy-2022* and *2023* to assess significant differences between the accessions by 'F'-test, and the treatment means were compared by least significant difference (LSD) at $p < 0.05$ using GRAPES (Version 1.1.0) (Gopinath et al. 2020). Diversity analysis of the *Rainy-2022* data was conducted, and a dendrogram was constructed utilizing the *ggplot2* package to visualize clustering among accessions. Violin plots were generated to illustrate the distribution of observed variables across both seasons. Principal Component Analysis (PCA) was performed on the *Rainy-2023* data and PCA plots were created using the *PCAtools* package. Additionally, a correlation analysis between morphological traits and ovipositional incidence of *H. armigera* in no-choice and multi-choice assays was conducted using the *ggcorrplot* package. Population data were transformed using $\sqrt{x+0.5}$ before statistical analysis to stabilize variance and normalize the distribution for parametric testing.

Multi-trait genotype-ideotype distance index (MGIDI)

MGIDI was used to identify high-performance pigeonpea CWRs across various traits against *H. armigera* (Olivoto and Nardino 2021; Olivoto et al. 2022). For the MGID index *metan* and *tidyverse* packages were used in R software.

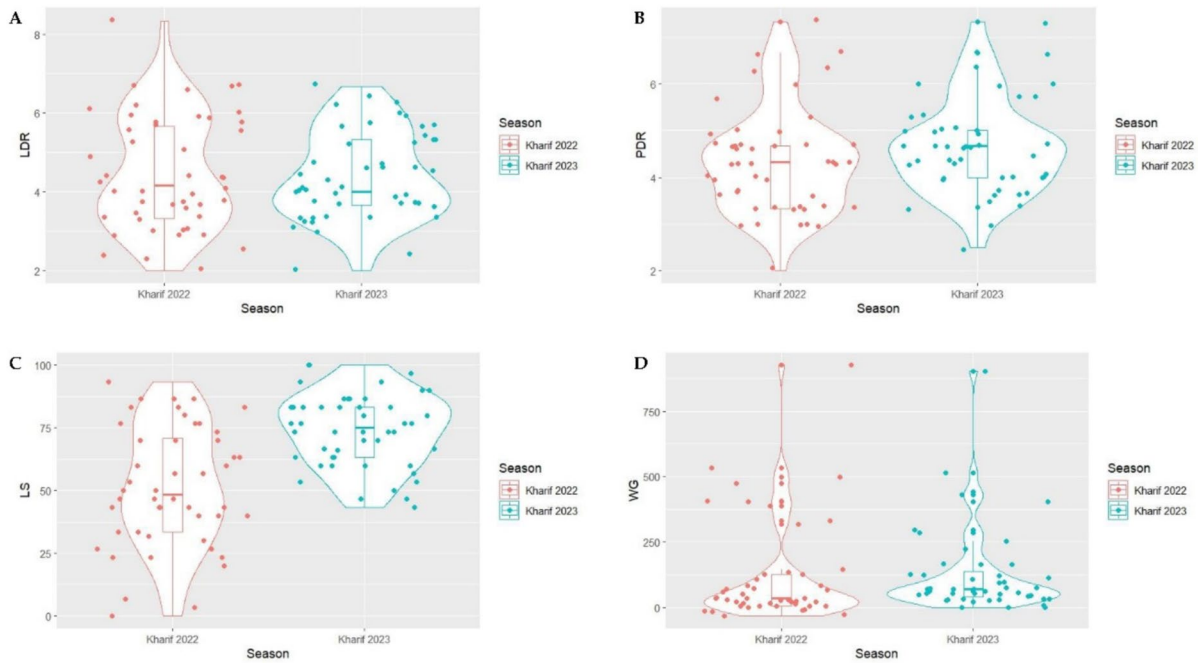


Fig. 2 Violin plots depicting the distribution of four observations—**A** Leaf Damage Rating (LDR), **B** Pod Damage Rating (PDR), **C** Larval Survival (LS) and **D** Percent larval Weight Gain (WG)—across two consecutive *Rainy* seasons (2022 and 2023) for 48 pigeonpea accessions. The plots were generated using the *ggplot2* package in R and provide a visual comparison of the trait distributions across seasons. The similar patterns observed in *Rainy-2022* and *2023* indicate stable environmental conditions, allowing for consistent expression of these observations across both seasons

Table 2 Expression of antibiosis mechanism of resistance in the crop wild relatives of pigeonpea against *Helicoverpa armigera* across two seasons

Accession	Season 1 (*Rainy-2022*)				Season 2 (*Rainy-2023*)			
	LDR	LS (%)	PDR	WG (%)	LDR	LS (%)	PDR	WG (%)
ICP 15668	5.67^{bcd}	70.00^{a-g}	4.33^{f-i}	108.65^{f-i}	6.67^{a}	60^{d-i}	5.00^{c-g}	127.08^{e-i}
ICP 15669	6.67^{b}	73.33^{a-f}	4.67^{e-h}	126.08^{fgh}	6.33^{ab}	100^{a}	6.33^{abc}	223.66^{d-h}
ICP 15683	4.00^{e-h}	56.67^{c-j}	3.67^{hij}	22.11^{i-l}	3.67^{efg}	76.67^{a-h}	4.33^{e-i}	46.68^{hi}
ICP 15685	5.67^{bcd}	50.00^{e-l}	4.33^{f-i}	35.15^{h-l}	4.00^{efg}	76.67^{a-h}	5.33^{b-f}	62.88^{ghi}
ICP 15686	2.67^{hij}	50.00^{e-l}	4.00^{g-j}	36.82^{h-l}	3.33^{fgh}	86.67^{a-e}	4.67^{d-h}	76.28^{ghi}
ICP 15692	6.67^{b}	80.00^{a-d}	6.33^{abc}	145.46^{f}	3.67^{efg}	96.67^{ab}	5.33^{b-f}	49.64^{hi}
ICP 15694	6.33^{bc}	33.33^{i-n}	4.33^{f-i}	84.01^{f-j}	3.00^{ghi}	73.33^{a-i}	5.00^{c-g}	127.13^{e-i}
ICP 15696	6.00^{bc}	83.33^{abc}	5.67^{b-e}	135.13^{fg}	3.67^{efg}	93.33^{abc}	5.00c^{-g}	113.99^{e-i}
ICP 15703	5.00^{c-f}	31.67^{j-n}	3.67^{hij}	73.65^{f-k}	3.67^{efg}	70^{a-i}	4.00^{f-j}	30.41^{hi}
ICP 15705	2.33^{ij}	23.33^{l-p}	4.67^{e-h}	70.42^{f-k}	4.00^{efg}	76.67^{a-h}	5.00^{c-g}	97.73^{f-i}
ICP 15711	3.00^{ghi}	20.00^{m-p}	5.00^{d-g}	83.99^{f-j}	2.33^{hi}	53.33^{f-i}	6.00^{a-d}	165.26^{e-i}
ICP 15712	3.00^{ghi}	43.33^{g-m}	4.00^{g-j}	30.31^{i-l}	4.67^{cde}	83.33^{a-f}	5.00^{c-g}	63.08^{ghi}
ICP 15713	3.33^{ghi}	60.00^{b-i}	3.67^{hij}	35.56^{h-l}	4.33^{def}	60^{d-i}	3.67^{g-j}	27.65^{hi}
ICP 15716	3.33^{ghi}	33.33^{i-n}	4.33^{f-i}	-7.15^{jkl}	3.33^{fgh}	53.33^{f-i}	4.33^{e-i}	-31.17^{i}
ICP 15718	3.67^{f-i}	83.33^{abc}	4.33^{f-i}	24.07^{i-l}	3.00^{ghi}	46.67^{hi}	3.00^{ij}	27.30^{hi}
ICP 15720	3.00^{ghi}	40.00^{h-m}	4.67^{e-h}	12.33^{jkl}	3.33^{fgh}	76.67^{a-h}	2.67^{j}	113.00^{e-i}
ICP 15721	4.33^{d-g}	53.33^{d-k}	3.67^{hij}	5.70^{jkl}	4.67^{cde}	83.33^{a-f}	4.67^{d-h}	44.22^{hi}
ICP 15722	3.00^{ghi}	46.67^{f-m}	3.33^{ij}	-11.27^{kl}	5.33^{bcd}	86.67^{a-e}	4.67^{d-h}	124.84^{e-i}
ICP 15723	3.33^{ghi}	30.00^{j-o}	4.67^{e-h}	22.37^{i-l}	3.33^{fgh}	46.67^{hi}	3.67^{g-}j	-19.07^{i}
ICP 15725	3.00^{ghi}	43.33^{g-m}	3.00^{jk}	-27.59^{l}	3.33^{fgh}	73.33^{a-i}	4.33^{e-i}	32.92^{hi}
ICP 15726	3.67^{f-i}	6.67^{nop}	4.67^{e-h}	35.18^{h-l}	3.33^{fgh}	50^{ghi}	4.67^{d-h}	73.64^{ghi}
ICP 15727	3.33^{ghi}	60.00^{b-i}	3.33^{ij}	-17.62^{kl}	5.33^{bcd}	76.67^{a-h}	3.67^{g-j}	30.72^{hi}
ICP 15728	4.33^{d-g}	43.33^{g-m}	3.00^{ijk}	-13.96^{kl}	4.67^{cde}	83.33^{a-f}	4.00^{f-j}	94.94^{f-i}
ICP 15729	4.00^{e-h}	63.33^{b-h}	3.00^{ijk}	5.66^{jkl}	4.33^{def}	70^{a-i}	3.67^{g-j}	55.75^{ghi}
ICP 15730	3.67^{f-i}	26.67^{k-p}	4.67^{e-h}	60.37^{f-l}	4.00^{efg}	60^{d-i}	4.00^{f-j}	101.32^{e-i}
ICP 15732	3.67^{f-i}	23.33^{l-p}	4.00^{g-j}	16.75^{i-l}	4.00^{efg}	43.33^{i}	3.33^{hij}	53.93^{hi}
ICP 15733	4.00^{e-h}	43.33^{g-m}	3.33^{ij}	-0.91^{jkl}	4.00^{efg}	63.33^{c-i}	4.00^{f-j}	32.59^{hi}
ICP 15734	3.67^{f-i}	46.67^{f-m}	3.33^{ij}	25.99^{i-l}	4.67^{cde}	83.33^{a-f}	4.00^{f-j}	54.55^{ghi}
ICP 15736	6.00^{bc}	70.00^{a-g}	5.33^{c-f}	125.51^{fgh}	5.33^{bcd}	66.67^{b-i}	5.67^{b-e}	96.60^{f-i}
ICP 15740	6.67^{b}	40.00^{h-m}	3.33^{ij}	17.31^{i-l}	5.67^{abc}	73.33^{a-i}	5.00^{c-g}	108.01^{e-i}
ICP 15744	3.00^{ghi}	23.33^{l-p}	4.67^{e-h}	30.55^{i-l}	3.67^{efg}	60^{d-i}	3.67^{g-j}	69.78^{ghi}
ICP 15747	4.00^{e-h}	26.67^{k-p}	3.00^{ijk}	5.70^{jkl}	4.00^{efg}	90^{a-d}	4.33^{e-i}	74.07^{ghi}
ICP 15827	2.33^{ij}	3.33^{op}	3.00^{ijk}	-32.00^{l}	4.00^{efg}	86.67^{a-e}	4.00^{f-j}	61.08^{ghi}
ICP 15867	2.00^{j}	0.00^{p}	4.67^{e-h}	20.94^{i-l}	2.00^{i}	56.67^{e-i}	4.00^{f-j}	69.81^{ghi}
ICP 15868	3.67^{f-i}	70.00^{a-g}	2.00^{k}	51.33^{g-l}	6.33^{ab}	83.33^{a-f}	4.33^{e-i}	164.38^{e-i}
ICP 15887	6.00^{bc}	43.33^{g-m}	4.00^{g-j}	3.95^{jkl}	3.33^{fgh}	60^{d-i}	3.33^{hij}	53.33^{hi}
ICP 15921	8.33^{a}	40.00^{h-m}	4.33^{f-i}	66.56^{f-k}	6.33^{ab}	83.33^{a-f}	4.67^{d-h}	113.69^{e-i}
ICP 15936	6.00^{bc}	46.67^{f-m}	3.33^{ij}	5.05^{jkl}	3.67^{efg}	80^{a-g}	3.33^{hij}	1.04^{i}
ICP 16907	4.33^{d-g}	76.67^{a-e}	3.33^{ij}	4.73^{jkl}	4.67^{cde}	63.33^{c-i}	4.67^{d-h}	122.29^{e-i}
ICPL332WR	5.00^{c-f}	86.67^{ab}	6.67^{ab}	407.41^{cde}	5.67^{abc}	90^{a-d}	6.67^{ab}	404.36^{bcd}
ICPL 87091	4.33^{d-g}	83.33^{abc}	4.67^{e-h}	330.14^{e}	4.67^{cde}	83.33^{a-f}	4.67^{d-h}	251.76^{c-g}
ICPL 7035	5.33^{b-e}	76.67^{a-e}	4.33^{f-i}	532.61^{b}	5.33^{bcd}	73.33^{a-i}	4.33^{e-i}	580.59^{b}
ICPL 98008	5.67^{bcd}	63.33^{b-h}	5.00^{d-g}	473.17^{bcd}	5.33^{bcd}	73.33^{a-i}	6.00^{a-d}	441.36^{bc}
ENT 11	6.67^{b}	93.33^{a}	6.67^{ab}	925.52^{a}	5.67^{abc}	86.67^{a-e}	6.00^{a-d}	901.74^{a}
ICPL 8863	5.67^{bcd}	86.67^{ab}	6.00^{bcd}	388.24^{de}	6.00^{ab}	100^{a}	5.67^{b-e}	297.44^{cde}

Table 2 (continued)

Accession	Season 1 (*Rainy-2022*)				Season 2 (*Rainy-2023*)			
	LDR	LS (%)	PDR	WG (%)	LDR	LS (%)	PDR	WG (%)
ICPL 87119	4.33^{d-g}	56.67^{c-j}	6.33^{abc}	317.72^{e}	4.00^{efg}	63.33^{c-i}	6.67^{ab}	284.39^{c-f}
ICPL 87	6.00^{bc}	76.67^{a-e}	7.33^{a}	497.84^{bc}	6.00^{ab}	66.67^{b-i}	7.33^{a}	431.17^{bc}
ICPL 84060	5.67^{bcd}	86.67^{ab}	5.00^{d-g}	404.67^{cde}	5.67^{abc}	93.33^{abc}	4.67^{d-h}	372.20^{cd}
F	8.01	6.22	7.79	33.76	7.98	1.57	3.15	5.96
Sem	0.51	9.63	0.4	33.28	0.39	11.68	0.57	70.46
CV (%)	19.83	32.44	16.03	48.6	15.6	27.47	21.21	84.9
LSD	1.44	27.04	1.13	93.44	1.11	32.81	1.59	197.8

LDR Leaf Damage Rating, *LS* Larval Survival, *PDR* Pod Damage Rating, *WG* Weight Gain
Superscripts following the mean values represent groupings based on statistical comparisons
Values followed by same alphabet did not differ significantly @ $P < 0.05$ (LSD)

Results

Analysis of variance for antibiosis experiments during *Rainy-2022* and *Rainy-2023*

ANOVA for LDR, LS, PDR, and WG during *Rainy-2022* (96 accessions) and *Rainy-2023* (48 accessions) showed significant differences. In 2022, F values for LDR, LS, PDR, and WG were 8.00, 4.63, 5.45, and 19.75 ($p = < 0.05$; Df: 95). In 2023, F values were 7.99, 1.57, 3.15, and 5.97 ($p = < 0.05$; Df: 47) (Tables S2 and S3). These results confirm significant variability across accessions over two seasons.

Diversity among the different accessions for antibiosis mechanism of resistance against pod borer

A dendrogram was created to illustrate the damage caused by *H. armigera* larvae in the bioassay experiments conducted during preliminary screening for

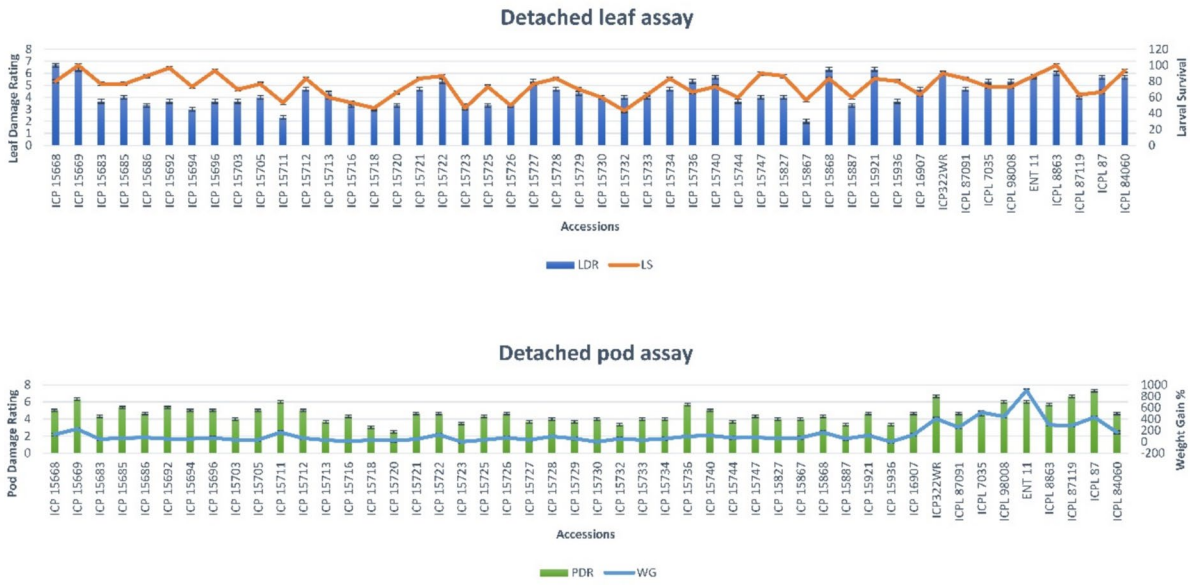


Fig. 3 Performance of pigeonpea wild accessions in the antibiosis mechanism of resistance against *Helicoverpa armigera*. The detached leaf assay (top) displays Leaf Damage Rating (LDR) as blue bars and Larval Survival (LS) as an orange line, highlighting variation in leaf-based resistance across accessions. The detached pod assay (bottom) presents Pod Damage Rating (PDR) as green bars and larval Weight Gain (WG) as a blue line, assessing pod-based resistance

Rainy-2022 (Fig. 1). This dendrogram grouped all accessions into three distinct clusters, with cultivated lines placed in cluster 1 and wild accessions exhibiting higher and lower values in clusters 2 and 3, respectively (Vishal et al. 2023). Accessions from clusters 2 and 3 were selected based on bioassay results, ensuring representation from all three gene pools (secondary, tertiary, and quaternary), in addition to all cultivated accessions from cluster 1 (primary gene pool). In total, 48 accessions were chosen for further evaluation in *Rainy-2023*, comprising 39 wild relatives and 9 cultivated lines (Table 1).

Violin plots-data distribution of the same accessions in season 1 and season 2

In *Rainy-2022* and *Rainy-2023*, the violin plots of all the traits (Fig. 2) show a consistent distribution pattern, indicating that the performance of the accessions remained largely similar between the two seasons. Additionally, the embedded box plots indicate that the median values for these traits are stable across both seasons, with interquartile ranges (IQR) and outliers being comparable, suggesting minimal seasonal effects on the performance of the accessions.

Expression of antibiosis mechanism of resistance to *H. armigera* in pigeonpea wild accessions using detached leaf assay

The lowest LDR was observed in the *R. suaveolens* (ICP 15867) (2.00) and *C. scarabaeoides* (ICP 15711) (2.33) followed by *C. scarabaeoides* (ICP 15718) (3.00) (Table 2 and Fig. 3). The maximum LDR (6.67 and 6.33) was recorded in *C. platycarpus*, ICP 15668 and ICP 15669, respectively followed by *R. sublobata*, ICP 15868 (6.33) among the CWRs. The highest and lowest ratings were observed among the cultivated lines of *C. cajan* in ICPL 87 (6.00) and ICPL 87119 (4.00). Similarly, per cent larval survival also differed significantly among the accessions. The highest per cent larval survival was recorded in *C. platycarpus* (ICP 15669) (100%) and the lowest survival in *C. scarabaeoides* (ICP 15732) (43.33%).

Expression of antibiosis mechanisms of resistance to *H. armigera* in pigeonpea wild accessions using detached pod assay

The lowest PDR was observed in the accessions of *C. scarabaeoides* (ICP 15720- 2.67 and ICP 15718-3.00) followed by *R. densiflora,* ICP 15936 (3.33) (Table 2 and Fig. 3). The highest PDR (6.33) was recorded in *C. platycarpus,* ICP 15669 followed by *C. scarabaeoides,* ICP 15711 (6.00) among the CWRs. The highest and lowest pod damage ratings were observed in ICPL 87 (7.33) and ICPL 87119 (6.67) of *C. cajan* among the cultivated lines. Similarly, the per cent larval weight gain differed significantly among the accessions. The largest per cent larval weight gain was observed on *C. platycarpus*, ICP 15669 (223.66%) and the lowest larval weight gain (-31.17%) was recorded on *C. scarabaeoides* (ICP 15716). Among the cultivated lines of *C. cajan*, the highest and lowest per cent larval weight gain was observed in ENT 11 (901.74%) and ICPL 87091 (251.76%), respectively.

Antixenosis mechanism of resistance to *H. armigera* in wild relatives of pigeonpea in *Rainy-2023*

No-choice condition

There were significant differences in oviposition by the *H. armigera* females on different wild relatives of pigeonpea under no-choice conditions. Among the CWRs tested, the highest oviposition was observed on *Cajanus* spp. (ICP 16907) (466.67 eggs/accession), which was significantly ($P<0.05$) different from susceptible cultivated line ICPL 87 (358.67 eggs/accession) (Table S4). The least preference for *H. armigera* moth was observed on *R. densiflora* (ICP 15936) (12 eggs/accession) followed by *C. scarabaeoides* (ICP 15740) (47 eggs/ accession).

Multi-choice condition

When all the wild accessions were given as a choice to the adult *H. armigera* in a large cage, the preference varied significantly ($P<0.05$) among the accessions. The highest number of eggs were laid on ICPL 87 (154 eggs/accession), followed by ICPL 98008 (134 eggs/accession). Among the cultivated accessions of *C. cajan*, the least oviposition was recorded

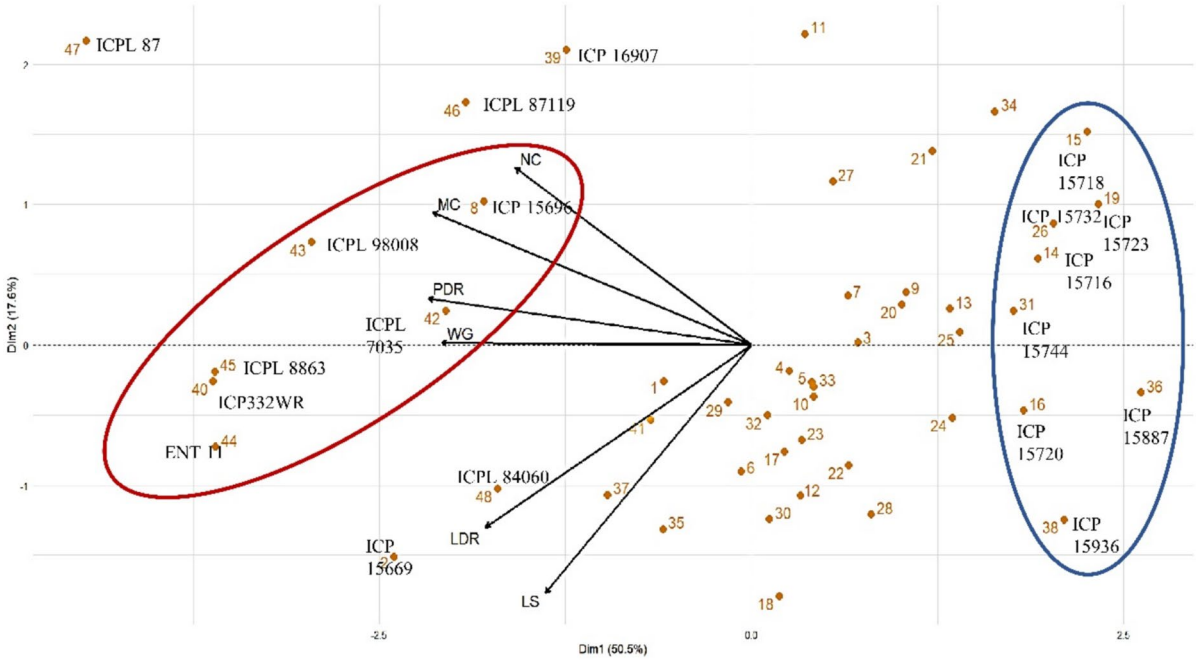


Fig. 4 Principal Component Analysis (PCA) of pigeonpea wild relatives based on resistance traits from antibiosis (LDR–Leaf Damage Rating, LS–Percent Larval Survival, PDR–Pod Damage Rating and WG–Percent Weight Gain). Antixenosis (NC–No-choice, and MC–Multi-choice) assays against *Helicoverpa armigera*, generated using the *PCAtools* package in R. Dimension1 (50.5%) and Dimension2 (17.6%) capture the majority of trait variation among accessions. The red ellipse groups susceptible accessions, while the blue ellipse highlights accessions with stronger resistance, reflected by lower LDR, LS, PDR, WG, NC and MC values. Trait vectors indicate their contribution, with resistant accessions positioned further from the origin

in ICPL 84060 (31.33 eggs/accession). Among the CWRs, the most eggs were deposited on *C. scarabaeoides* (ICP 15696) with 136 eggs/accession, followed by *Cajanus* spp. (ICP 16907) with 58.67 eggs/accession. *Helicoverpa armigera* showed the least preference for *R. densiflora* accessions ICP 15827 and ICP 15936, which had 5.33 and 6.67 eggs/accession, respectively. *C. scarabaeoides* (ICP 15744) also recorded fewer eggs (6.67 eggs/accession).

Principal component analysis (PCA)

PCA revealed that there were associations among different observations and their contribution towards *H. armigera* resistance, as demonstrated in the biplot (Fig. 4). The biplot was characterized by two principal components, Dimension 1 (50.5%) and Dimension 2 (17.6%), which accounted for 68.1% of the total variance. The vectors (arrows) in the biplot represent the traits analyzed, namely LDR, LS, PDR, WG, Multi-choice, and No-choice. The length and direction of each arrow indicate the strength and contribution of each trait to the principal components. Traits closer to an accession indicate stronger association. The accessions aligned with the vectors exhibit higher trait values for that vector, while those in the opposite direction exhibit lower values. The accessions outside and within the red ellipse in the direction of vectors including major cultigens and few CWRs (ICPL 87, ICP 15696, ICPL 98008, ICPL 7035, ICPL 8863, ICP 15669) had higher values with damage-related traits, making them susceptible candidates. In contrast, accessions within the blue ellipse (ICP 15718, ICP 15732, ICP 15723, ICP 15716, ICP 15744, ICP 15720, ICP 15887, ICP 15936) were characterized by lower values for traits related to resistance, making them suitable for pod borer resistance breeding.

Evaluation of selected pigeonpea wild accessions on the incidence of pest complex at different stages under field conditions

Vegetative stage

The incidence of various pests of pigeonpea observed during the vegetative stage varied significantly ($P \leq 0.05$) among the accessions (Table S5). The incidence of pod borer, *H. armigera* (eggs and larvae) did not show significant variation among the CWRs. In contrast, the incidence on cultivars varied from 0.707 to 1.551 eggs/plant and 0.707 to 1.248 larvae/plant. Among all the accessions, the maximum number of eggs and larvae of pod borer were recorded on ENT 11 and ICPL 87 respectively. The incidence of spotted pod borer (*M. vitrata*) was higher on ICPL 87091 (0.844 larvae/plant) followed by ENT 11 (0.775 larvae/plant). The highest number of leafwebber (*E. critica*) larvae were recorded on ICPL 87 (1.377 larvae/ plant) followed by ICPL 87091 (1.372 larvae/ plant) and ICPL 98008 (1.052 larvae/plant). Leafhoppers (*E. kerri*) were maximum on ICPL 87119 (1.732 nymphs/plant) followed by ICPL 7035 (1.717 nymphs/plant). Other pests including aphids, stink bugs, plume moth and cow bugs were recorded during the observation. Statistically, there were no significant differences in their incidence among all the accessions.

Flowering stage

During the flowering stage, the maximum number of *H. armigera* eggs and larvae were found on ICPL 87091 (1.984 eggs/plant) and ICPL 98008 (2.945 larvae/plant) respectively (Table S6), whereas the least number of eggs and larval counts were observed in ICPL 84060 (1.095 eggs/plant) and ENT 11 (1.839 larvae/plant). Among the CWRs, the highest larval incidence was observed on *C. platycarpus* (ICP 15668) (0.879 larvae/plant) followed by *R. sublobata* (ICP 15868) (0.831 larvae/plant). The incidence of spotted pod borer was observed only on ICPL 87 (0.775 larvae/plant) and no significant difference was observed among the rest of the accessions.

Table 3 Variation in trichome density (per 10× microscopic field) among crop wild relatives of pigeonpea exhibiting different levels of resistance to *Helicoverpa armigera*

Accession	Trichomes/10× microscopic field			
	Glandular		Non-glandular	
	A	B	C	D
ICP 15668	7.33^{fgh}	0.00^{f}	5.00^{st}	10.67^{k-o}
ICP 15669	5.67^{gh}	4.00^{c-f}	10.00^{rst}	4.00^{mno}
ICP 15683	0.00^{h}	7.67^{a-e}	114.33^{j-n}	28.00^{d-k}
ICP 15685	0.00^{h}	0.00^{f}	187.00^{ef}	27.33^{d-k}
ICP 15686	0.00^{h}	0.00^{f}	121.67^{h-m}	33.67^{b-f}
ICP 15692	0.00^{h}	10.67^{abc}	128.33^{g-m}	32.67^{b-g}
ICP 15694	0.00^{h}	12.67^{a}	117.67^{i-n}	12.67^{i-o}
ICP 15696	0.00^{h}	11.33^{ab}	239.67^{cd}	48.33^{bc}
ICP 15703	0.00^{h}	6.67^{a-f}	259.67^{bcd}	44.33^{bcd}
ICP 15705	0.00^{h}	8.00^{a-e}	315.00^{a}	33.67^{b-f}
ICP 15711	0.00^{h}	4.33^{b-f}	127.00^{h-m}	14.67^{g-o}
ICP 15712	0.00^{h}	4.00^{c-f}	266.00^{bcd}	30.67^{c-i}
ICP 15713	0.00^{h}	5.33^{b-f}	143.00^{g-k}	35.67^{b-e}
ICP 15716	0.00^{h}	6.67^{a-f}	277.67^{abc}	20.67^{e-n}
ICP 15718	0.00^{h}	5.00^{b-f}	272.33^{bcd}	37.00^{b-e}
ICP 15720	0.00^{h}	4.67^{b-f}	102.33^{l-o}	29.00^{d-i}
ICP 15721	0.00^{h}	2.00^{ef}	105.33^{k-o}	37.67^{b-e}
ICP 15722	0.00^{h}	8.33^{a-e}	113.33^{j-n}	14.33^{h-o}
ICP 15723	0.00^{h}	4.00^{c-f}	101.33^{l-o}	10.33^{k-o}
ICP 15725	0.00^{h}	8.67^{a-e}	186.33^{ef}	37.33^{b-e}
ICP 15726	0.00^{h}	4.67^{b-f}	237.67^{d}	49.67^{b}
ICP 15727	0.00^{h}	4.00^{c-f}	135.67^{g-l}	25.33^{e-l}
ICP 15728	0.00^{h}	7.67^{a-e}	145.33^{g-j}	36.33^{b-e}
ICP 15729	0.00^{h}	2.67^{def}	186.00^{ef}	25.67^{e-l}
ICP 15730	0.00^{h}	2.33^{def}	286.67^{ab}	21.33^{e-m}
ICP 15732	0.00^{h}	4.33^{b-f}	145.33^{g-j}	20.00^{e-n}
ICP 15733	0.00^{h}	2.33^{def}	123.00^{h-m}	22.00^{e-m}
ICP 15734	0.00^{h}	5.33^{b-f}	133.00^{g-m}	44.00^{bcd}
ICP 15736	0.00^{h}	6.67^{a-f}	104.33^{l-o}	17.00^{f-o}
ICP 15740	0.00^{h}	5.33^{b-f}	166.67^{efg}	12.33^{j-o}
ICP 15744	0.00^{h}	5.33^{b-f}	188.33^{e}	29.33^{d-j}
ICP 15747	0.00^{h}	2.33^{def}	153.00^{e-i}	32.00^{b-h}
ICP 15827	11.33^{efg}	0.00^{f}	124.67^{h-m}	6.33^{mno}
ICP 15867	10.67^{efg}	0.00^{f}	149.67^{f-j}	0.00^{o}
ICP 15868	0.00^{h}	0.00^{f}	156.67^{e-h}	0.00^{o}
ICP 15887	0.00^{h}	0.00^{f}	0.00^{t}	0.00^{o}
ICP 15921	8.67^{fg}	3.00^{def}	6.00^{st}	3.00^{no}
ICP 15936	0.00^{h}	0.00^{f}	27.67^{rst}	68.67^{a}
ICP 16907	13.67^{d-g}	0.00^{f}	23.00^{rst}	0.00^{o}
ICPL332WR	27.33^{b}	11.33^{ab}	105.33^{k-o}	6.67^{mno}
ICPL 87091	44.33^{a}	7.67^{a-e}	70.00^{opq}	4.33^{mno}

Table 3 (continued)

Accession	Trichomes/10 × microscopic field			
	Glandular		Non-glandular	
	A	B	C	D
ICPL 7035	26.33^{bc}	9.33^{a-d}	33.00^{rst}	3.00^{no}
ICPL 98008	14.67^{def}	5.67^{a-f}	41.67^{p-s}	0.00^{o}
ENT 11	28.33^{b}	6.33^{a-f}	79.67^{nop}	13.33^{i-o}
ICPL 8863	18.67^{cde}	8.00^{a-e}	104.67^{k-o}	8.33^{l-o}
ICPL 87119	27.67^{b}	3.67^{c-f}	94.67^{mno}	10.33^{k-o}
ICPL 87	20.67^{bcd}	4.00^{c-f}	45.00^{pqr}	5.00^{mno}
ICPL 84060	17.00^{de}	0.00^{f}	105.00^{k-o}	5.33^{mno}

Superscripts following the mean values represent groupings based on statistical comparisons

Values followed by same alphabet did not differ significantly @ $P < 0.05$ (LSD)

Maturity stage

All the accessions differed significantly for the incidence of *H. armigera* eggs and larvae during the maturity stage (Table S7). Among the CWRs 0.88 and 1.032 eggs/plant were observed in ICP 15747 and ICP 15734 accessions of *C. scarabaeoides* respectively. Similarly, *C. platycarpus* (ICP 15921) recorded 0.831 eggs/plant. Among the cultivated lines, the highest number of eggs and larvae were found on ENT 11 (1.290 eggs/plant) and ICPL 87 (1.740 larvae/ plant). In contrast, ICPL 332WR (0.707 eggs/plant) and ICPL 87091 (1.024 larvae/ plant) recorded the lowest numbers among the cultivated lines. The incidence of spotted pod borer was not significantly different among the accessions. The highest and lowest incidence of leaf webber was found in ICPL 87091 (1.004 larvae/plant) and ICPL 332WR (0.707 larvae/plant). The incidence of other minor pests including stem fly, pod bugs, cow bugs, leafhoppers and mealybugs was minimal during the maturity stage.

Morphological traits associated with *H. armigera* resistance

Pigeonpea accessions were characterized by two different types of trichomes viz., glandular and non-glandular and classified into four different types A and B (glandular), C and D (non-glandular) (Table 3 and Fig. 5). There were significant differences among the accessions for all the types of trichomes on pod surface. Maximum number of type A trichomes were found on ICPL 87091 (43.33 trichomes/10 × microscopic field), ENT 11 (28.33 trichomes/10 × microscopic field) and ICPL 87119 (27.67 trichomes/10 × microscopic field). However, they were absent in almost all the wild accessions belonging to *C. scarabaeoides* (Table 3). Interestingly, type B trichomes were found in large numbers in *C. scarabaeoides* (ICP 15694) (12.67 trichomes/10 × microscopic field) and *C. scarabaeoides* (ICP 15696) (11.33 trichomes/10 × microscopic field), followed by ICPL 332WR (11.33 trichomes/10 × microscopic field). Type B trichomes were absent in ICPL 84060, *Cajanus* spp. (ICP 16907), *R. densiflora* (ICP 15936) and *R. sublobata* (ICP 15868). Non-glandular trichomes (type C and type D) were densely distributed among the CWRs and varied significantly. The highest number of type

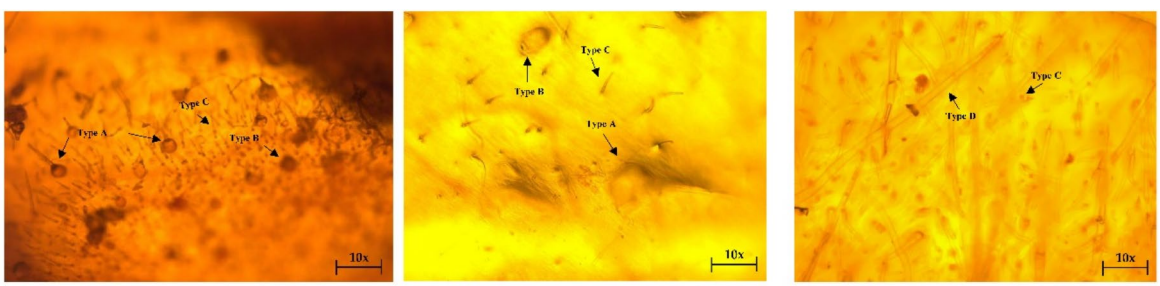


Fig. 5 Types and distribution of trichomes observed on pigeonpea cultivated lines and wild relatives. Type A and Type B represent glandular trichomes, while Type C and Type D are non-glandular

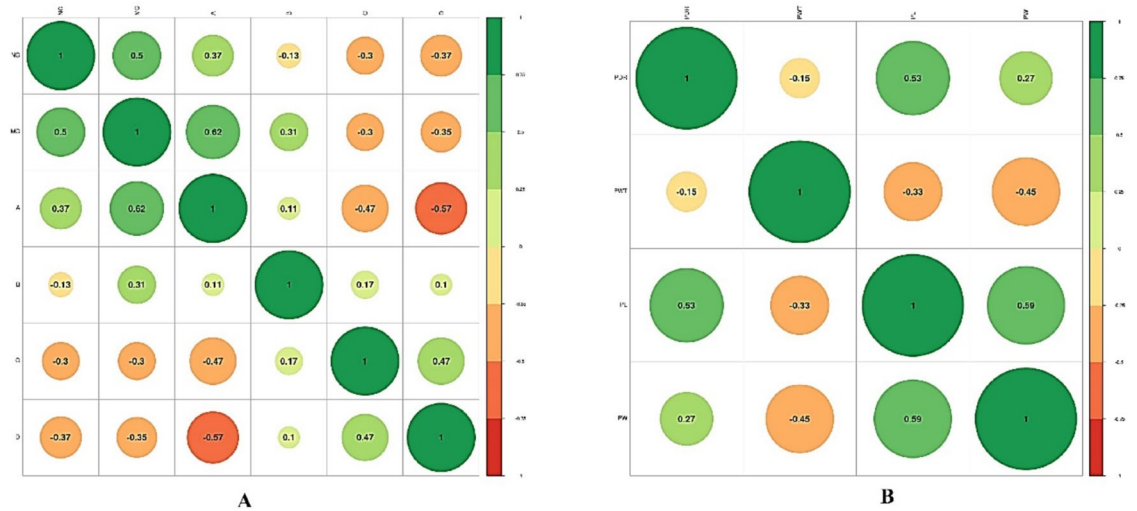


Fig. 6 Correlation analysis of morphological traits with insect resistance parameters in pigeonpea. **A** Correlation between trichome density and oviposition behavior of *Helicoverpa armigera*, illustrating the influence of trichome types on pest egg-laying preference. **B** Relationship between pod characteristics (including pod length and width) and the extent of pod damage inflicted by *H. armigera*, highlighting the role of pod morphology in modulating resistance to pod borer infestation

C trichomes were found in *C. scarabaeoides* (ICP 15705) (315 trichomes/10 × microscopic field), followed by *C. scarabaeoides* (ICP 15730) (286.67 trichomes/10 × microscopic field). The least number of type C trichomes were observed in *C. platycarpus* (ICP 15668, ICP 15921, ICP 15669) and *Cajanus* spp. (ICP 16907). The high density of type D trichomes were observed in *R. densiflora* (ICP 15936) (68.67 trichomes/10 × microscopic field), *C. scarabaeoides* (ICP 15726) (49.67 trichomes/10 × microscopic field), while the least were recorded on ICPL 98008 and *Cajanus* spp. (ICP 16907).

Association of trichome density and pod dimensions with H. armigera oviposition preference and pod damage in wild relatives of pigeonpea

Under no-choice and multi-choice experimental setups, the preference of *H. armigera* varied significantly towards accessions having glandular and non-glandular trichomes (Fig. 6A). Moth preference for egg laying showed a moderate (0.37) and strong (0.61) positive correlation with type A glandular trichomes under no-choice and multi-choice conditions respectively. A weak and negative correlation (−0.092) in no-choice and a moderate positive correlation (0.306) in multi-choice experiment was observed towards type B trichomes. In contrast, *H. armigera* showed a weak to moderate negative (−0.217 to −0.286) correlation towards type C and type D (non-glandular) trichomes. A strong positive correlation (0.514) existed between oviposition preference in multichoice and no-choice experiments. Various pod parameters were correlated with the PDR (Fig. 6B) to understand their influence on the extent of damage caused by the *H. armigera* larvae. The association between PDR and pod wall thickness showed a weak negative correlation (r = −0.16), while there was a moderate positive correlation with pod length (r = 0.53) and a weak positive correlation with pod width (r = 0.27).

Multi-trait genotype-ideotype distance index (MGIDI)

MGIDI focuses on the simultaneous selection of genotypes based on multiple traits (Olivoto and Nardino 2021). In the current study MGIDI selection index (Fig. 7) was used to identify the superior CWRs of pigeonpea showing high resistance towards *H. armigera.* The red circle represents the cut point according to the selection pressure and the accessions closer and outer to the cut point were selected as resistant based on the MGID index. Selected CWRs based on the MGID index in the current study included ICP 15718, ICP 15716, ICP 15726, ICP 15730, ICP 15744,

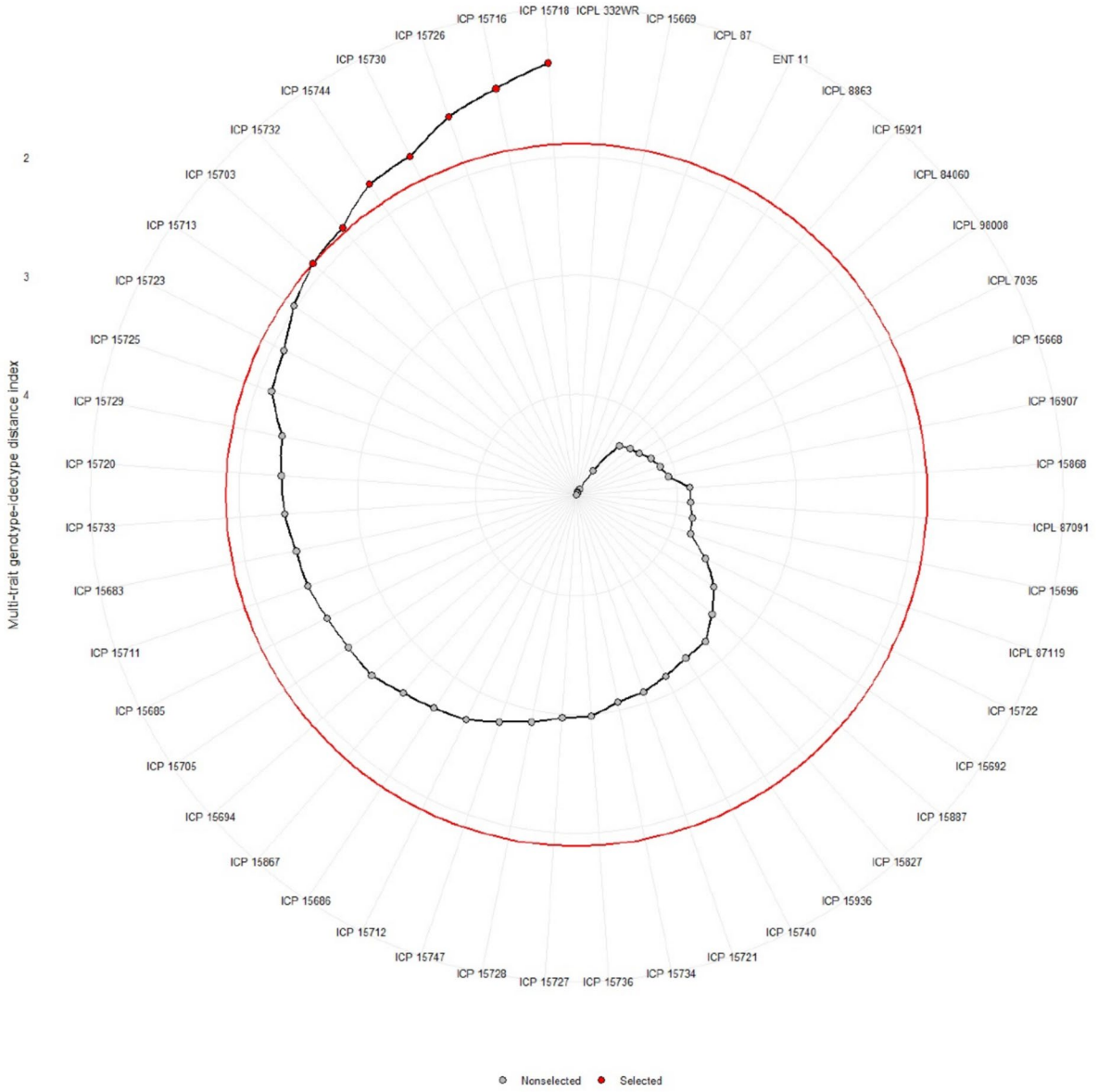


Fig. 7 Ranking of crop wild relatives of pigeonpea based on the Multi-Trait Genotype-Ideotype Distance Index (MGIDI). The accessions are arranged in ascending order of their MGIDI values, which reflect their overall performance across multiple traits. The red line indicates the selection threshold, with accessions positioned above this line are considered superior based on the desired traits. Accessions highlighted in red represent those selected as the most promising candidates for further breeding or resistance research. Non-selected accessions are shown in black, indicating those falling below the selection cutpoint

ICP 15732, ICP 15703 and ICP 15713 having index values of 1.20, 1.36, 1.48, 1.67, 1.70, 1.85, 1.89 and 1.98 respectively (Table 4). Meanwhile, the accessions far from the ideal cut point were ICPL 332WR, *C. platycarpus* (ICP 15669), ICPL 87 and ENT 11 having index values of 4.85, 4.83, 4.83, and 4.79 respectively.

Table 4 Multi-trait genotype-ideotype distance index (MGIDI) values of pigeonpea crop wild relatives based on antibiosis, antixenosis, and trichome characteristics

S.No	Accessions	MGID Index
1	ICP 15718	1.2004
2	ICP 15716	1.3576
3	ICP 15726	1.4840
4	ICP 15730	1.6686
5	ICP 15744	1.6986
6	ICP 15732	1.8544
7	ICP 15703	1.8913
8	ICP 15713	1.9838
9	ICP 15723	2.0993
10	ICP 15725	2.1337
11	ICP 15729	2.3160
12	ICP 15720	2.3520
13	ICP 15733	2.3802
14	ICP 15683	2.4374
15	ICP 15711	2.4578
16	ICP 15685	2.5014
17	ICP 15705	2.5377
18	ICP 15694	2.5461
19	ICP 15867	2.6297
20	ICP 15686	2.6877
21	ICP 15712	2.7436
22	ICP 15747	2.8292
23	ICP 15728	2.9030
24	ICP 15727	2.9697
25	ICP 15736	2.9851
26	ICP 15734	3.0739
27	ICP 15721	3.0983
28	ICP 15740	3.1447
29	ICP 15936	3.1948
30	ICP 15827	3.2027
31	ICP 15887	3.3266
32	ICP 15692	3.4568
33	ICP 15722	3.6419
34	ICPL 87119	3.8320
35	ICP 15696	3.8544
36	ICPL 87091	3.8883
37	ICP 15868	3.8934
38	ICP 16907	4.0602
39	ICP 15668	4.1096
40	ICPL 7035	4.1531
41	ICPL 98008	4.2141
42	ICPL 84060	4.2527
43	ICP 15921	4.3010
44	ICPL 8863	4.6093
45	ENT 11	4.7979
46	ICPL 87	4.8306
47	ICP 15669	4.8338
48	ICPL332WR	4.8516

Discussion

Pigeonpea is an important pulse crop in India that is gaining global attention for its significant contributions towards food and nutritional security. However, *H. armigera* causes devastating yield losses making it a major hindrance in pigeonpea cultivation (Sharma et al. 2009, 2022). Despite extensive breeding efforts, developing resistant cultivars has remained challenging due to the complex inheritance of resistance traits and limited genetic variability in cultivated germplasm (Sison and Shanower 1994; Anitha Kumari et al. 2010; Volp et al. 2022). While transgenic approaches are promising, regulatory hurdles and public acceptance issues in India have hindered their deployment (Rakesh and Ghosh 2024). The exploration of pigeonpea germplasm in primary gene pool and its CWRs offers a promising alternative, as several *Cajanus* species have demonstrated higher levels of resistance to *H. armigera* (Green et al. 2006; Kumari et al. 2006; Sharma et al. 2009; Anitha Kumari et al. 2010).

Unfortunately, screening of around fourteen thousand pigeonpea accessions against *H. armigera* indicated that the majority of cultivated genotypes exhibited only low to moderate levels of resistance (Reed and Lateef 1990; Sharma et al. 2009). In contrast, certain accessions from the wild relatives of pigeonpea have exhibited strong resistance to *H. armigera* (Sharma et al. 2001a, 2009; Green et al. 2006). These wild relatives present a valuable genetic resource for enhancing resistance in cultivated germplasm. In this study, we screened 87 CWRs and 9 checks (cultivated lines), making a total of 96 accessions belonging to different gene pools. The pigeonpea CWRs are resistant sources of *H. armigera* (Mallikarjuna et al. 2007; Sharma et al. 2009; Bohra et al. 2010). Screening of these accessions was carried out using the detached

leaf assay (Sharma et al. 2005a; Gopal Swamy et al. 2007; Sharma 2016) and detached pod assay (Sharma et al. 2005b; Sujana et al. 2012) and 50% of accessions from *Rainy-2022*, were selected for further evaluation based on a dendrogram clustering (Kahraman et al. 2014). Among the 87 CWRs screened in *Rainy-2022*, the maximum number of accessions chosen for further experimentation belonged to *C. scarabaeoides,* as this species is a potential candidate to develop resistance against *H. armigera* (Njaci et al. 2020; Ngugi-Dawit et al. 2021; Sharma et al. 2022).

In *Rainy-2023*, the selected accessions were tested and screened for different mechanisms of host plant resistance (Painter 1951; Sharma 2016) to assess stable resistance. The data from antibiosis experiments over two consecutive seasons (*Rainy-2022* and *Rainy-2023*) demonstrated consistency, indicating stable environmental conditions. In these experiments, most accessions of *C. scarabaeoides* exhibited the lowest damage ratings and per cent larval survival compared to cultivated checks and other CWRs. Other research has documented higher antibiosis and antixenosis properties in these species (Shanower et al. 1997; Green et al. 2002; Ngugi-Dawit et al. 2020). Notably, ICP 15669 of *C. platycarpus* displayed the highest damage ratings, elevated per cent larval survival, and the largest per cent larval weight gain among the CWRs. Previous research reported (Shanower et al. 1997; Sharma et al. 2009; Sujana et al. 2012) that the damage caused by *H. armigera* was significantly greater in *C. platycarpus* accessions.

The survival instincts of *H. armigera* are notably strong, leading adult female moths to oviposit even on inert substrates when in captivity (Sujana et al. 2008). However, when provided with a choice between a single accession (no-choice) or multiple accessions (multi-choice), oviposition is observed across all accessions, albeit at varying levels (Kumari et al. 2006). This preference tends to increase as the host plant reaches the flowering stage (Firempong and Zalucki 1989). Consequently, all tested accessions against *H. armigera* were at the flowering stage during the experimental trials. Under no-choice conditions, *Cajanus* spp. (ICP 16907) recorded a higher number of eggs than the susceptible check ICPL 87, possibly due to glandular secretions or chemical properties that attract *H. armigera* (Hartlieb and Rembold 1996). Nevertheless, ICPL 87 emerged as the most preferred host for *H. armigera* after ICP 16907, corroborating findings from previous research (Kumari et al. 2006; Volp et al. 2022). Additionally, oviposition was noted on the resistant accessions of *C. scarabaeoides, R. suaveolens*, and *R. densiflora*, as previously reported (Sujana et al. 2008; Sharma et al. 2009). Significantly, this study identifies *R. suaveolens* (ICP 15867) from the quaternary gene pool as a novel resistant source against *H. armigera*. The least preference was recorded for *R. suaveolens* by both *H. armigera* larvae and adults during antibiosis and antixenosis experiments.

Under field conditions, all accessions were monitored for pest infestations at three intervals: the vegetative stage, flowering stage, and maturity stage. Notably, no natural infestation of any pod borer complex pests were observed among the CWRs. Low to medium levels of sap-sucking insects, such as leafhoppers, aphids, and mealybugs, were noted, consistent with earlier reports (Durairaj et al. 2009). There was no incidence of pod fly (*Melanogromyza obtusa*), the second most damaging pest of pigeonpea after *H. armigera* (Sharma et al. 2022), and it has been reported that CWRs of pigeonpea are resistant to pod fly (Sharma et al. 2003; Mallikarjuna et al. 2007). Pod wasp (*Tanaostigmodes cajaninae* La Salle) incidence was observed in *C. scarabaeoides* accessions at the maturity stage, which are negatively correlated with *H. armigera* infestations (Sharma et al. 2003). Pest incidence varied significantly among cultivated pigeonpea accessions, with pod borer populations peaking at the maturity stage in the susceptible check (ICPL 87) (Night and Ogenga-Latigo 1993; Anitha Kumari et al. 2010; Rathod et al. 2014; Patil and Dadmal 2016). Our observations of pest incidence on CWRs during the three stages—vegetative, flowering, and maturity—under unsprayed field conditions indicated that CWRs were less preferred by the pigeonpea pest complex, likely due to their morphological, biochemical, and inherent genetic traits.

Morphological traits such as the presence of trichomes on the pod surface, pod toughness, and pod wall structure have been identified as resistance factors against *H. armigera* (Shanower et al. 1997). Trichomes are distinctive features on plant surfaces that play various roles in defending against insect pests (Levin 1973; Peter et al. 1995). Pigeonpea and its wild relatives are broadly characterized by two types of trichomes: glandular (type A, type B, type E) and

non-glandular (type C and type D) (Romeis et al. 1999). Type E trichomes are often excluded from research due to their rarity and likely minimal role in pest resistance (Romeis et al. 1999; Sharma et al. 2009). In this study, cultivated accessions exhibited a higher number of type A trichomes, which may explain their greater susceptibility to *H. armigera* compared to CWRs (Hartlieb and Rembold 1996; Green et al. 2002). Notably, type A trichomes were completely absent in *C. scarabaeoides* (Aruna et al. 2005), while type D trichomes were absent in *R. suaveolens* (ICP 15867) and *R. sublobata* (ICP 15868). In contrast, *C. scarabaeoides* had a high density of type C trichomes, which serve as a physical barrier against *H. armigera* (Shanower et al. 1999; Romeis et al. 1999; Sharma et al. 2009; Upadhyaya et al. 2013b). The density of type A glandular trichomes demonstrated a significant positive correlation with oviposition under both no-choice (r = 0.368*) and multi-choice (r = 0.608***) conditions. Conversely, a negative correlation was found between non-glandular trichomes (C and D) and oviposition under no-choice (r = 0.368*) and multi-choice (r = 0.608***) conditions (Sharma et al. 2009). Thus, type A, C and D trichomes significantly mitigate damage by *H. armigera*, while type B has not been shown to influence oviposition preference (Sharma et al. 2009). The correlation analysis indicated that type A trichomes attract *H. armigera* moths significantly, particularly in multi-choice conditions, while types C and D seem to deter oviposition to varying extents.

Pod characteristics such as pod wall thickness, length, and width significantly influence the extent of damage caused by pod borer (Nahdy 1995; Ambidi et al. 2021). *Cajanus scarabaeoides* accessions ICP 15720 and ICP 15718 exhibited the lowest PDR (2.50 and 3.00), with pod wall thicknesses of 0.30 mm and 0.37 mm, lengths of 22.17 mm and 23.02 mm, and widths of 7.70 mm and 6.65 mm, respectively. In contrast, *C. platycarpus* accessions ICP 15668 and ICP 15669 had higher pod damage ratings (5.00 and 6.00), characterized by thinner pod walls (0.17 mm and 0.20 mm), longer (31.48 mm and 34.26 mm), and wider pods (12.10 mm and 11.95 mm), respectively. Previous research has indicated that increased pod wall thickness is associated with resistance, while greater pod length and width correlate with susceptibility (Jagtap et al. 2014; Ambidi et al. 2021; Tyagi et al. 2022).

The MGID index indicated the difference between the ideal and observed values for each accession across multiple traits. Lower MGID index values suggest a higher preference, as they reflect accessions that are closer to the ideal cut-off, making them more appealing for selection. The accessions with the lowest index values (1.20 and 1.36) found in this study were *C. scarabaeoides* accessions ICP 15718 and ICP 15716, respectively. Previous research noted *C. scarabaeoides* (ICP 15726) as resistant to *H. armigera* (Green et al. 2006) with an MGID index of 1.48 in this study. The findings suggest that selected *C. scarabaeoides* accessions could serve as valuable sources of resistance to *H. armigera* in future breeding programs.

Conclusion

This study investigates the potential of pigeonpea CWRs that exhibit resistance to *H. armigera* through host plant resistance mechanisms. The CWRs such as *C. scarabaeoides* (ICP 15716, ICP 15718, and ICP 15726) from the secondary gene pool, along with *R. suaveolens* (ICP 15867) from the quaternary gene pool, possess a range of genetic traits that provide resistance to this pest, making them valuable resources for breeding programs aimed at developing pest-resistant pigeonpea cultivars. By utilizing the genetic diversity in these wild species, researchers can identify and integrate resistance traits into cultivated pigeonpea, enhancing its resilience to biotic stresses like *H. armigera*. Incorporating wild relatives into breeding programs addresses current pest issues and promotes sustainable agricultural practices by decreasing dependence on chemical pesticides. CWRs are promising candidates for improving pod borer resistance in cultivated pigeonpea through gene pyramiding, gene editing, multi-omics approaches and breeding initiatives. They can offer novel and diverse sources of resistance that can be leveraged in large-scale breeding programs to develop new pigeonpea cultivars with enhanced pod borer resistance.

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Data availability Data is provided within the manuscript and also in supplementary information files.

Declarations

Conflict of interest The authors declare no competing interests.

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