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Pod surface exudates of wild relatives of pigeonpea influence the feeding preference of the pod borer, *Helicoverpa armigera*

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Abstract Wild relatives of crops are an important source of resistance genes against insect pests. However, it is important to identify the accessions of wild relatives of crops with different mechanisms of resistance to broaden the basis and increase the levels of resistance to insect pests. Therefore, we studied the feeding behavior of pod borer, *Helicoverpa armigera,* which is the most damaging pest of pigeonpea, in relation to biochemical characteristics of the pod surface exudates in a diverse array of germplasm accessions belonging to 12 species of pigeonpea wild relatives. Feeding by *H. armigera* larvae was significantly lower on the unwashed or water-, methanol-, or hexane-washed pods of *Canajus sericeus*, *C. scarabaeoides*, *Flemingia bracteata*, *F. stricta*, and *Rhynchosia aurea* than those of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *D. ferruginea*, *P. scariosa*, *R. bracteata*, and the cultivated pigeonpea, *C. cajan* genotypes, ICPL 87, and ICPL 332, although there were a few exceptions. The methanol-washed pods of wild relatives were less preferred for feeding by the *H. armigera* larvae than the unwashed pods, but the hexane-washed pods were preferred more than the unwashed pods. The results suggested that methanol extracted the phagostimulants from the pod surface, while hexane removed the antifeedants. The high-performance liquid chromatography (HPLC) finger printing of methanol and hexane pod surface extracts showed qualitative and quantitative differences in compounds present on the pod surface of different wild relatives of pigeonpea. Some of the peaks in HPLC profiles were associated with feeding preference of the third-instar larvae of *H. armigera.* There was considerable diversity in wild relatives of pigeonpea as revealed by principal component analysis based on HPLC fingerprints of pod surface extracts in methanol and hexane, and *H. armigera* feeding on the pods. Wild pigeonpea accessions with low amounts of phagostimulants and high amounts of antifeedants may be used for introgression of resistance genes into the cultivated pigeonpea to develop varieties with broad-based resistance to *H. armigera.* There is considerable diversity among the wild relatives of pigeonpea, and the accessions with resistance to pod borer. These can be used to broaden the basis and increase the levels of resistance to *H. armigera*.

Keywords Wild relatives · Pigeonpea · Pod surface exudates · Feeding behavior · *Helicoverpa armigera*

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is grown in about 50 countries of Asia, Africa, and the Americas (Nene et al. 1990). In India, it is grown on nearly 3.8 million ha, which accounts for 85% of the world's area under pigeonpea cultivation. Although cultivars with a yield potential of more than 2,500 kg ha^{-1} have been developed, the average yields on peasant farms are only 750 kg ha^{-1} (Sharma et al. 2008). Several biotic and abiotic constraints limit pigeonpea production in farmers' fields, of which insect pests are the most important. More than 200 species of

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insects feed on pigeonpea, of which the pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is the most important pest worldwide (Shanower et al. 1999). It causes an estimated loss of US$317 million annually in pigeonpea in the semi-arid tropics (ICRISAT 1992) and over US$2 billion on other crops worldwide (Sharma 2005). Development of cultivars with resistance to *H. armigera* has considerable potential in minimizing the extent of losses due to this pest (Sharma et al. 2005). Screening of more than 14,000 accessions of cultivated pigeonpea for resistance to *H. armigera* has revealed low to moderate levels of resistance to this pest (Reed and Lateef 1990). However, high levels of resistance to *H. armigera* have been identified in wild relatives of pigeonpea such as *Cajanus scarabaeoides*, *C. sericeus*, and *C. acutifolius* (Sharma et al. 2001; Green et al. 2006). Pod surface exudates play an important role in host plant selection and feeding by the larvae of *H. armigera* in cultivated pigeonpea and the wild relative, *Cajanus scarabaeoides* (accession—ICPW 83) (Green et al. 2002, 2003). However, several other species of wild relatives of pigeonpea have also shown high levels of resistance to *H. armigera* under field conditions (Sharma et al. 2001).

There are large differences in expression of antixenosis and antibiosis components of resistance to *H. armigera* among different species/accessions of pigeonpea wild relatives (Sujana et al. 2008). The feeding behavior of *H. armigera* is influenced by various chemicals in the trichome exudates (Green et al. 2003; Sharma et al. 2009). In the present studies, we examined the role of trichome exudates on the pod surface on feeding behavior of *H. armigera* in 29 accessions belonging to 12 species of wild relatives and two varieties of the cultivated pigeonpea to identify accessions with different mechanisms of resistance to this insect. This information will be useful for selecting accessions for introgression of resistance genes into the cultigen, and thus for increasing the levels and diversity of the basis of resistance to *H. armigera* in pigeonpea.

Materials and methods

Plant material

The feeding behavior of the larvae of *H. armigera* was studied on 29 accessions belonging to 12 species of wild relatives of pigeonpea and two genotypes of cultivated pigeonpea (ICPL 87—susceptible check, and ICPL 332—moderately resistant check). The seeds of the wild relatives of pigeonpea were obtained from the pigeonpea germplasm maintained by the Gene bank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The material was grown at the ICRISAT farm (altitude 545 m above mean sea level, latitude 17.53° N, and longitude 78.27° E) during the rainy season (June–February). The seeds were sown on ridges 75 cm apart, and each accession was planted in 2 rows, 2 m long. The plants were thinned to a spacing of 30 cm between the plants 15 days after seedling emergence. There were three replications in a randomized complete block design. The annual species and the cultivated pigeonpea accessions were planted twice at monthly intervals, while the perennial species were planted only once to have pods of all the accessions for bioassay at the same time. Standard agronomic practices were followed for raising the crop (basal fertilizer—N:P:K:100:60:40 kg ha^{-1}). Metalaxyl spray [1.0 kg *ai* (active ingredient) ha^{-1}) was applied to control *Fusarium* wilt during the seedling stage. The crop was raised under rainfed conditions between June and October and irrigated at monthly intervals between November and February. Wooden pegs (1.5 m high) were used to provide support for *C. scarabaeoides* and *C. platycarpus* accessions, which have a creeping habit. The pods of the same age (10 day old) were collected from different accessions during December–January for studies on feeding behavior of *H. armigera* and chemical analysis.

Insect culture

The *H. armigera* larvae for bioassays were obtained from insect culture maintained in the laboratory at ICRISAT, Patancheru, India. The laboratory culture was regularly supplemented with field-collected insects to maintain the representative character of the insect population. The larvae were reared on the chickpea-based artificial diet (Armes et al. 1992) at 27 ±_1°C, 65 ± 5% RH, and 12-h photoperiod. The adults were released in 30 cm × 30 cm × 30 cm cages, provided with nappy liners for oviposition, and fed on 10% sucrose solution in absorbent cotton. Eggs laid on the nappy liners were sterilized with 1% sodium hypochlorite solution and transferred into 200-ml plastic cups smeared with 2-mm-thick layer of artificial diet for rearing in groups of 200–250. After 5 days, the larvae were transferred to six cell-well plates (having 5–7 ml artificial diet in each cell well) and reared individually till pupation. Neonate and third-instar larvae were used for studying the feeding behavior of *H. armigera* on different accessions of wild relatives of pigeonpea.

Feeding behavior of *H. armigera* larvae on fresh pods of wild relatives of pigeonpea

Fresh pods of pigeonpea and its wild relatives were provided to the *H. armigera* larvae for feeding under no-choice conditions. The pods of each test genotype were kept in a petri dish (7.5 cm diameter), and a single third-instar larva was

released in each petri dish. To keep the test material afresh, a moistened filter paper (with 2 ml of water) was placed inside the lid of the petri dish, but did not touch the pod. There were twenty replicates for each accession. Observations on larval feeding were recorded visually on a 1–9 scale at 48 h after initiating the experiment [damage rating (DR); 1 = < 10% pod 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 = > 80% pod area damaged].

Influence of pod surface chemicals on feeding behavior of third-instar larvae of *H. armigera*

To study the role of trichome exudates and pod surface chemicals on feeding behavior of *H. armigera* larvae, the field-collected pods were washed with solvents of different polarity (water, methanol, and hexane) for 2–3 min to remove the pod surface chemicals by placing the pods in the respective solvents individually and stirring with a glass rod. The washed pods were air-dried for 3 h in the laboratory to evaporate the solvent from the surface of the pods. The pods were then offered to larvae to study their feeding behavior in terms of feeding on the pod under no-choice and dual-choice conditions. Under no-choice conditions, a single third-instar larva was released in a 7.5-cm petri dish arena with a washed or unwashed pod of the same accession. Third instars were used for feeding assays as they consumed sufficient amount of food over 2 days and displayed maximum sensitivity to the physico-chemical characteristics of the pods of different accessions. There were 20 replicates for each accession. Pod damage was recorded visually on a 1–9 scale at 48 h after initiating the experiment as described above. Under dual-choice conditions, the larvae were given a choice between the washed and unwashed pods of the same accession with a particular solvent (water, methanol, or hexane). There were 20 replicates for each accession. Observations were recorded on pod damage at 48 h after releasing the larvae.

The pod surface extracts of 10- to 12-day-old pods of *C. scarabaeoides* (ICPW 83) and the cultivated pigeonpea (ICPL 87 and ICPL 332) were also bioassayed using 3.44-mm-diameter glass fiber disks (Green et al. 2002). For this purpose, pods were extracted in water, methanol, or hexane. The pod surface extracts were roto-evaporated and re-dissolved in respective solvents at concentrations present on pods under natural conditions. The glass fiber disks were impregnated with 100 µl of solvent extract using a micropipette. Control disks were treated with respective solvents only. The disks were air-dried for 10 h and positioned 5 mm apart in an apposed manner on a thin wax layer in the center of a 7.5-cm-diameter petri dish. The wax layer was covered with a filter paper. Both the disks were moistened with 100 µl of distilled water as *H. armigera* larvae were less likely to feed on dry glass fiber disks. Bioassays were conducted with third-, fourth-, and fifth-instar larvae of *H. armigera* to measure the differences in feeding behavior of different instars. The larvae were deprived of food for 4 h prior to the bioassays. A single larva of known age was released in each petri dish, and the experiment was maintained at 27 ± 2°C. Twenty replicates were maintained for each treatment. After 24 h of initiating the experiment, the larvae were removed from the petri dishes, the disks were dried, and the disk area consumed by the larvae was measured on a leaf area meter.

HPLC fingerprints of pod surface extracts of wild relatives of pigeonpea

High-performance liquid chromatographic (HPLC) fingerprints were obtained for 14 accessions of the wild relatives and the two varieties (ICPL 87 and ICPL 332) of cultivated pigeonpea. Pods (125 g) of each accession/variety were extracted in 500 ml of methanol or hexane for 2 min at the room temperature. Extracts were then filtered through Whatman no. 1 filter paper, solvents evaporated under vacuum, and re-dissolved in 5 ml of respective solvents. Extracts from each accession (25 µl) were passed through Millipore filter (0.45 µm) and injected into a dual Shimadzu (Kyoto, Japan) HPLC unit with LC–10 ATVP high-pressure pumps, SIE–10ADVP automatic injector, SCL–10AVP integrated system controller, Symmetry® C^{18} reverse-phase analytical column (250 × 4.6 mm, RP-18, 5-µm particle size), and SPD-M 10 AVP diode array detector. The gradient elution schedule consisted of an initial 2-min run of 75 of 2% acetic acid and 25% methanol, followed by a linear gradient to 100% methanol for 55 min at a flow rate of 1 ml^{-1} min.

Statistical analysis

Data on larval feeding on the pods under no-choice conditions were checked for normal distribution and homogeneity and subjected to analysis of variance by using Genstat Release 8.2 (Genstat 2008). The significance of differences between the genotypes was judged by F test, and the treatment means were compared by least significant difference (LSD) at $P \leq 0.05$. Significance of differences between the genotypes in dual-choice tests was judged by paired t test at $P \leq 0.05$. Associations of pod surface biochemical components and *H. armigera* resistance were determined by correlation analysis. Principle component analysis based on feeding preference of *H. armigera* larvae toward wild relatives of pigeonpea, and HPLC profiles of methanol and hexane extracts was used to determine the genotypic diversity among wild relatives of pigeonpea with different levels of resistance/susceptibility to the pod borer, *H. armigera*.

Results

Feeding behavior of third-instar larvae of *H. armigera* on pods of wild relatives of pigeonpea under no-choice conditions

There were significant differences in feeding by the third-instar larvae of *H. armigera* on pods of wild relatives of pigeonpea (Table 1). Feeding by *H. armigera* larvae was significantly lower on the pods of *C. sericeus* (except ICPW 159 pods washed with hexane), *C. scarabaeoides* (except the unwashed pods of ICPW 94; water-washed pods of ICPW 116, ICPW 141, and ICPW 152; and hexane-washed pods of ICPW 181), *F. bracteata*, *F. stricta*, and *R. aurea* than on the unwashed and water-, methanol-, and hexane-washed pods of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *D. ferruginea*, *P. scariosa*, *R. bracteata*, and the cultivated pigeonpea genotypes, ICPL 87 and ICPL 332.

Feeding behavior of third-instar larvae of *H. armigera* on unwashed and washed pods of wild relatives of pigeonpea under dual-choice conditions

Under dual-choice conditions (when the larvae were given a choice between the water-, methanol-, or hexane-washed pods and unwashed pods of the same accession), there were no significant differences in larval feeding between

Table 1 Pod damage by third-instar larvae of *H. armigera* on unwashed and water-, methanol-, and hexane-washed pods of wild relatives of pigeonpea under no-choice conditions (ICRISAT, Patancheru, India)

Species	Accession	Unwashed pods	Water-washed pods	Methanol-washed pods	Hexane-washed pods
Cajanus acutifolius	ICPW 1	3.2^{fg}	2.9^{de}	1.7^{cd}	3.7^{e}
C. acutifolius	ICPW 2	2.9^{efg}	2.0^{c}	0.7^{a}	3.7^{e}
C. albicans	ICPW 13	5.3^{h}	4.8^{g}	2.0^{d}	6.4^{g}
C. albicans	ICPW 14	3.5^{gh}	3.6^{ef}	0.9^{abc}	4.9^{f}
C. cajanifolius	ICPW 28	4.4^{h}	4.0^{fg}	1.9^{d}	4.9^{f}
C. cajanifolius	ICPW 29	1.9^{cd}	1.4^{ac}	1.0^{a}	1.6^{abc}
C. lineatus	ICPW40	2.8^{efg}	2.1^{c}	1.4^{cd}	2.4^{cd}
C. lineatus	ICPW 41	1.6^{bcd}	1.7^{bc}	1.4^{cd}	2.1^{b}
C. sericeus	ICPW 159	1.3^{abc}	1.1^{ab}	0.8^{ab}	1.7^{abc}
C. sericeus	ICPW 160	1.6^{bcd}	1.2^{ab}	1.1^{abc}	1.9^{bc}
C. platycarpus	ICPW 68	2.0^{cd}	2.2^{cd}	1.2^{bcd}	2.1^{b}
C. scarabaeoides	ICPW 83	0.8^{ab}	0.7^{a}	1.0^{abc}	1.8^{abc}
C. scarabaeoides	ICPW 90	0.6^{a}	0.9^{ab}	0.8^{ab}	2.2^{bc}
C. scarabaeoides	ICPW 94	2.0^{cde}	1.4^{ac}	0.9^{abc}	0.7^{a}
C. scarabaeoides	ICPW116	1.3^{abc}	2.2^{cd}	0.4^{a}	1.4^{abc}
C. scarabaeoides	ICPW 125	0.9^{ab}	0.7^{a}	0.5^{a}	1.2^{ab}
C. scarabaeoides	ICPW 130	1.1^{abc}	1.1^{ab}	0.3^{a}	1.2^{ab}
C. scarabaeoides	ICPW 137	0.8^{ab}	1.8^{bc}	0.3^{a}	1.5^{abc}
C. scarabaeoides	ICPW 141	1.4^{abc}	1.9^{c}	0.6^{a}	1.4^{abc}
C. scarabaeoides	ICPW 152	1.1^{abc}	1.9^{c}	0.7^{a}	1.6^{abc}
C. scarabaeoides	ICPW278	1.6^{bcd}	1.0^{ab}	0.7^{a}	2.0^{b}
C. scarabaeoides	ICPW 280	1.5^{abcd}	1.0^{ab}	0.5^{a}	1.5^{abc}
C. scarabaeoides	ICPW 281	1.3^{abc}	0.9^{ab}	0.4^{a}	2.4^{cd}
Dunbaria ferruginea	ICPW 178	2.4^{def}	1.9^{c}	1.3^{bcd}	3.4^{cde}
Flemingia bracteata	ICPW 192	1.1^{abc}	1.1^{ab}	0.8^{ab}	1.9^{bc}
F. stricta	ICPW 202	0.9^{ab}	0.7^{a}	0.4^{a}	1.2^{ab}
Paracalyx scariosa	ICPW 207	1.9^{cd}	2.0^{c}	0.8^{ab}	2.0^{b}
Rhyncosia aurea	ICPW 210	1.9^{cd}	1.3^{ab}	0.9^{abc}	1.6^{abc}
R. bracteata	ICPW 214	3.2^{fg}	3.0^{de}	0.9^{abc}	3.4^{de}
C. cajan (S)	ICPL 87	3.3^{fg}	2.5^{cd}	1.5^{cd}	3.6^{e}
C. cajan (MR)	ICPL 332	2.9^{efg}	2.8^{de}	1.2^{bcd}	3.5^{de}
SE±		0.33	0.31	0.30	0.40
LSD at *P* 0.05 (*df* = 30, 62)		0.93**	0.87**	0.84**	1.13**

Pod damage rating (1 = <10%, 9 = >80% pod damage)

Figures followed by the same letter within a column are not significantly different at $P \leq 0.05$

S susceptible, *MR* moderately resistant.

** *F* test significant at $P \leq 0.05$

Table 2 Feeding preference of third-instar larvae of *H. armigera* on water-, methanol-, and hexane-washed versus unwashed pods of wild relatives of pigeonpea under dual-choice conditions (ICRISAT, Patancheru, India)

Species	Accession	Water		Methanol		Hexane	
		Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
C. acutifolius	ICPW 1	2.3[a]	1.7[b]	3.0[a]	1.0[b]	1.4[a]	4.1[b]
C. acutifolius	ICPW 2	1.7[a]	1.9[a]	2.0[a]	0.5[b]	1.3[a]	4.5[b]
C. albicans	ICPW 13	2.2[a]	3.7[b]	3.5[a]	0.9[b]	1.0[a]	3.0[b]
C. albicans	ICPW 14	2.4[a]	2.8[a]	3.7[a]	0.9[b]	1.1[a]	3.0[b]
C. cajanifolius	ICPW 28	2.8[a]	2.3[a]	2.4[a]	1.1[b]	0.6[a]	1.2[b]
C. cajanifolius	ICPW 29	1.3[a]	1.1[a]	1.1[a]	0.6[b]	1.3[a]	1.8[b]
C. lineatus	ICPW40	1.5[a]	1.2[b]	1.6[a]	0.5[b]	0.8[a]	3.8[b]
C. lineatus	ICPW 41	0.9[a]	1.2[b*]	1.6[a]	0.7[b]	1.0[a]	1.8[b]
C. sericeus	ICPW 159	1.2[a]	1.4[a]	1.5[a]	0.5[b]	0.9[a]	1.7[b]
C. sericeus	ICPW 160	1.2[a]	1.5[a]	1.4[a]	0.7[b]	0.6[a]	1.5[b]
C. platycarpus	ICPW 68	1.2[a]	1.4[a]	1.5[a]	0.6[b]	0.7[a]	1.8[b]
C. scarabaeoides	ICPW 83	0.9[a]	0.7[a]	1.0[a]	0.3[b]	0.6[a]	0.9[b]
C. scarabaeoides	ICPW 90	0.9[a]	0.5[b]	0.2[a]	0.6[b]	0.8[a]	1.0[a]
C. scarabaeoides	ICPW 94	0.8[a]	0.8[a]	1.2[a]	0.4[b]	0.8[a]	1.3[b]
C. scarabaeoides	ICPW116	0.8[a]	1.0[a]	1.0[a]	0.5[b]	0.9[a]	1.3[b]
C. scarabaeoides	ICPW 125	0.8[a]	1.1[b]	0.6[a]	0.4[a]	0.5[a]	0.9[b]
C. scarabaeoides	ICPW 130	1.1[a]	0.7[b]	1.2[a]	0.4[b]	0.4[a]	0.7[b]
C. scarabaeoides	ICPW 137	0.9[a]	0.7[a]	0.8[a]	0.5[a]	0.8[a]	1.6[b]
C. scarabaeoides	ICPW 141	1.1[a]	1.2[a]	1.0[a]	0.4[b]	0.7[a]	1.3[b]
C. scarabaeoides	ICPW 152	1.2[a]	1.1[a]	1.0[a]	1.1[a]	0.8[a]	2.0[b]
C. scarabaeoides	ICPW278	1.4[a]	1.3[a]	1.4[a]	0.6[b]	0.5[a]	1.5[b]
C. scarabaeoides	ICPW 280	1.5[a]	1.5[a]	1.2[a]	1.3[a]	0.8[a]	1.6[b]
C. scarabaeoides	ICPW 281	1.4[a]	1.3[a]	1.3[a]	0.4[b]	2.0[a]	1.8[a]
D. ferruginea	ICPW 178	2.1[a]	0.9[b]	1.0[a]	0.9[a]	0.9[a]	1.0[a]
F. bracteata	ICPW 192	0.8[a]	0.9[a]	1.1[a]	0.4[b]	0.7[a]	1.5[b]
F. stricta	ICPW 202	0.6[a]	0.9[b]	0.8[a]	0.2[b]	0.7[a]	1.4[b]
P. scariosa	ICPW 207	1.4[a]	1.0[b]	1.3[a]	0.2[b]	1.0[a]	1.2[b]
R. aurea	ICPW 210	1.1[a]	1.0[b]	1.7[a]	0.4[b]	0.8[a]	1.6[b]
R. bracteata	ICPW 214	1.7[a]	2.1[a]	1.7[a]	1.2[b]	1.4[a]	2.2[b]
C. cajan (S)	ICPL 87	2.7[a]	1.8[b]	2.9[a]	1.0[b]	2.0[a]	3.2[b]
C. cajan (MR)	ICPL 332	2.1[a]	1.8[a]	2.5[a]	0.9[b]	1.8[a]	2.5[b]

Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged)

S susceptible, *MR* moderately resistant

Figures marked with the same letter within a column are not significantly different at $P \leq 0.05$ ($df = 18$)

unwashed and water-washed pods of 19 accessions (Table 2). However, significant differences in larval feeding were observed between water-washed and unwashed pods of 13 accessions [*C. acutifolius* (ICPW 2), *C. albicans* (ICPW 13), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. lineatus* (ICPW 40 and ICPW 41), *C. scarabaeoides* (ICPW 90, ICPW 125, and ICPW 130), *D. ferruginea* (ICPW 178), *F. stricta* (ICPW 202), *P. scariosa* (ICPW 207), *P. aurea* (ICPW 210), and the cultivated pigeonpea genotype, ICPL 87. Pod damage on unwashed pods of ICPL 87 was greater than that on pods washed with water, indicating that water-soluble components acted as feeding stimulants for the larvae of *H. armigera*.

When the larvae were provided a choice between methanol-washed and unwashed pods of the same accession, the larvae preferred to feed on the unwashed pods, suggesting that methanol removed the feeding stimulants from the pods surface of pigeonpea and its wild relatives (Table 2). However, the differences were non-significant in case of four accessions belonging to *C. scarabaeoides* (ICPW 125, ICPW 137, ICPW 152, and ICPW 280) and one accession of *D. ferruginea* (ICPW 178). On the contrary, the *H. armigera* larvae preferred to feed on the hexane-washed pods, rather than on the unwashed pods of the respective accessions of pigeonpea and its wild relatives, indicating that hexane removed the antifeedant compounds from the pod surface (except in two accessions of *C. scarabaeoides,* ICPW 90 and ICPW 281, and one accession of *D. ferruginea*, ICPW 178) (Table 2). In general, methanol removed the feeding stimulants, while hexane removed the antifeedants from the pod surface of pigeonpea and its wild relatives.

Feeding by different instars of *H. armigera* on glass fiber disks treated with extracts from pod surface of pigeonpea and its wild relatives

Bioassays with third-, fourth-, and fifth-instar *H. armigera* larvae indicated that, in general, larvae preferred to feed more on glass fiber disks treated with methanol extract than on control disks. However, significant increases in feeding by fourth- and fifth-instar *H. armigera* larvae on glass fiber disks were observed only with methanol extracts of ICPL 87 (0.044 vs. 0.130, and 0.018 vs. 0.129 cm^{-2}, for fourth- and fifth-instar larvae, respectively). Feeding by fourth-instar *H. armigera* larvae was greater on disks treated with methanol pod surface extract of ICPL 87 (0.130 cm^{-2}) than the disks treated with the pod surface extracts of ICPL 332 (0.009 cm^{-2}) and the pods of the wild relative, *C. scarabaeoides* ICPW 83 (0.013 cm^{-2}).

HPLC fingerprints of pod surfaces exudates of wild relatives of pigeonpea

There were substantial differences in the number of peaks for methanol and hexane pod surface extracts of different species of wild relatives of pigeonpea. There were both qualitative and quantitative differences in the compounds present on the pod surfaces of different accessions of wild relatives of pigeonpea. The number of peaks observed in the methanol extract was greater compared to the number of peaks in the hexane extract in all the accessions, except in ICPW 2, ICPW 160, ICPW 83, ICPW 178, ICPW 192, and ICPW 207. Lowest number of peaks in methanol extract were observed in ICPW 207, while the cultivated pigeonpea (ICPL 87 and ICPL 332) and *R. bracteata* (ICPW 214) had more peaks than the rest of the wild accessions tested. In the hexane extract, ICPW 125 (*C. scarabaeoides*) and ICPW 210 (*R. aurea*) had eight peaks compared to 18 peaks in the moderately resistant cultivated pigeonpea variety, ICPL 332.

Compounds in methanol extracts with retention times 6.27 and 11.50 min were negatively associated with susceptibility to *H. armigera* [egg and larval numbers per 5 inflorescences, and pod damage in the field (Sharma et al. 2009)] and with larval feeding on the young pods under laboratory conditions (Table 3). The peaks at retention times 11.24, 16.46, 25.49, 27.444, and 32.0 min were associated with susceptibility to *H. armigera,* although some of the correlation coefficients are non-significant. In the hexane extract, compounds with retention times of 20.41, 22.29, 23.39, 26.80, 30.09, 35.34, and 36.74 min were associated with resistance to *H. armigera,* while the compounds at retention times 11.92, 14.49, 15.50, 16.35, and 17.74 min were associated with susceptibility to this insect (Table 4). However, some of the correlation coefficients were non-significant.

Table 3 Correlation of HPLC peaks in methanol extracts of pod surfaces of wild relatives of pigeonpea with resistance/susceptibility to pod borer, *H. armigera* (ICRISAT, Patancheru, India)

Retention time (min.)	Eggs per 5 inflorescences	Larvae per 5 inflorescences	Pod damage under field conditions	Feeding on unwashed pods in no-choice tests
6.272	−0.20	−0.20	−0.24	−0.44
11.238	0.56*	0.45	0.14	0.23
11.499	−0.18	−0.16	−0.16	−0.36
16.462	0.05	0.29	0.54*	0.44
17.555	0.92**	0.87**	0.65**	0.27
22.409	−0.28	−0.27	−0.24	−0.24
25.487	0.59*	0.54*	0.41	0.22
27.444	0.50	0.40	0.23	0.25
32.000	0.27	0.21	0.05	0.31

*, ** = Correlation coefficients significant at *P* 0.05 and 0.01, respectively

Table 4 Correlation of HPLC peaks in hexane extracts of pod surfaces of wild relatives of pigeonpea with resistance/susceptibility to pod borer, *H. armigera* (ICRISAT, Patancheru, India)

Retention time (Min.)	Eggs per 5 inflorescences	Larvae per 5 inflorescences	Pod damage in the field	Feeding on unwashed pods in no-choice tests
11.924	0.15	0.39	0.65**	0.53*
14.492	0.88**	0.88**	0.80**	0.44
15.496	0.93**	0.95**	0.85**	0.49
16.346	0.83**	0.87**	0.84**	0.48
17.741	0.74**	0.83**	0.91**	0.51*
20.408	−0.22	−0.25	−0.34	−0.15
22.290	−0.27	−0.27	−0.30	−0.42
23.389	−0.27	−0.30	−0.34	−0.37
26.797	−0.24	−0.26	−0.36	−0.63**
30.087	−0.22	−0.21	−0.26	−0.11
35.335	−0.28	−0.33	−0.40	−0.39
36.741	−0.25	−0.25	−0.18	−0.11

*, ** = Correlation coefficients significant at *P* 0.05 and 0.01, respectively

Diversity among wild relatives of pigeonpea based on HPLC fingerprints of methanol and hexane extracts and feeding preference of *H. armigera* larvae

Principal component analysis based on HPLC fingerprints of methanol extracts of wild relatives of pigeonpea placed the test material in five groups. ICPW 14 (*C. albicans*) and ICPW 160 (*C. sericeus*) were placed independently, while

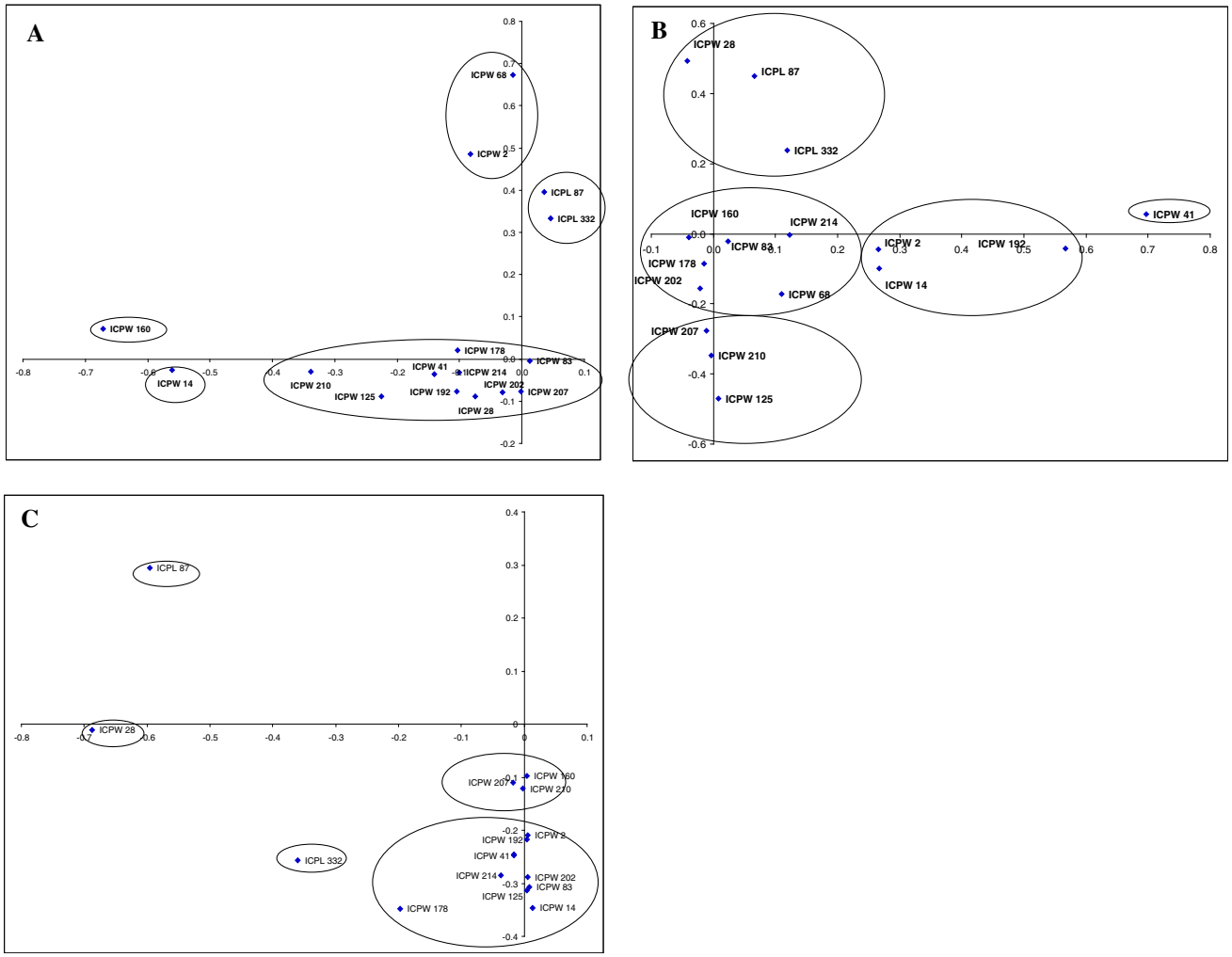

Fig. 1 Diversity among wild relatives of pigeonpea based on principal component analysis. **a** HPLC fingerprints of methanol extract, **b** HPLC fingerprints of hexane extract, and **c** biological interactions of *H. armigera* with wild relatives of pigeonpea

the moderately resistant (ICPL 332) and the susceptible (ICPL 87) checks were placed in the same group (Fig. 1). ICPW 2 (*C. acutifolius*) and ICPW 68 (*C. platycarpus*) were placed in one group, and the remaining 10 accessions were placed in another group. Principal component analysis based on fingerprints of pod surface extracts in hexane placed the test material in four groups. ICPW 41 (*C. lineatus*) was placed independently, while the susceptible (ICPL 87) and the moderately resistant (ICPL 332) checks were grouped along with ICPW 28 (*C. cajanifolius*)—the closely related wild relative of pigeonpea. Another group comprised of ICPW 2 (*C. acutifolius*), ICPW 14 (*C. albicans*), and ICPW 192 (*F. bracteata*), while the remaining nine genotypes were placed in one group. Principal component analysis based on biological interactions of *H. armigera* with wild relatives of pigeonpea placed the test material in five groups. The moderately resistant (ICPL 332) and the susceptible (ICPL 87) checks were placed independently in separate groups, as was the closely related wild relative of pigeonpea, *C. cajanifolius* (ICPW 28). ICPW 160 (*C. sericeus*), ICPW 207 (*P. scariosa*), and ICPW 210 (*R. aurea*) were placed in one group, while the remaining nine accessions were placed in another group. All the *C. scarabaeoides* accessions were always placed in one group based on methanol or hexane extract fingerprints, or on biological interactions of *H. armigera* with wild relatives of pigeonpea.

Discussion

The *H. armigera* larval feeding, in general, was greater on the pods of cultivated pigeonpea as compared to those of the wild relatives, and these differences may be due to physico-chemical characteristics of different species/accessions (Sharma et al. 2009). The *H. armigera* larvae

spend more time while feeding on the pods of cultivated pigeonpea than on the pods of the wild relative, *C. scarabaeoides* (Shanower et al. 1997; Romeis et al. 1999). Several chemical compounds are present on the pod surface of cultivated pigeonpea, and some of these compounds are absent in the wild relatives (Green et al. 2002, 2003), which may be responsible for differences in feeding behavior of *H. armigera* larvae on cultivated and wild pigeonpeas. Presence of dense non-glandular trichomes on the pods of some of the wild relatives of pigeonpea is another reason for low pod damage on these species (Peter et al. 1995; Shanower et al. 1997; Sharma et al. 2009). First- and second-instars *H. armigera* prefer to feed on the pods of ICPL 87 (with glandular trichomes) as compared to those of *C. scarabaeoides* accession, ICPW 83 (with non-glandular trichomes), and on ICPW 83 pods from which the trichomes have been removed (Sharma et al. 2001).

Feeding by the third-instar larvae of *H. armigera* larvae was significantly lower on the pods of *C. sericeus* (except ICPW 159 pods washed with hexane), *C. scarabaeoides* (except the unwashed pods of ICPW 94; water-washed pods of ICPW 116, ICPW 141, and ICPW 152; and hexane-washed pods of ICPW 281), *F. bracteata*, *F. stricta*, and *R. aurea* than the unwashed and water-, methanol-, and hexane-washed pods of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *D. ferruginea*, *P. scariosa*, *R. bracteata*, and the cultivated pigeonpea, *C. cajan* genotypes, ICPL 87 and ICPL 332 (although there were a few exceptions), suggesting that these wild relatives were resistant to *H. armigera* and that presence of feeding stimulants and/or absence of antifeedants contributed to the resistance of these species to *H. armigera*. Similar observations on the relative resistance of these species/accessions have earlier been recorded under field conditions (Sharma et al. 2009). When the larvae were provided with a choice between the unwashed and hexane-washed pods, the larvae preferred to feed on the hexane-washed pods, indicating that hexane removed some of the antifeedant compounds from the pod surface of pigeonpea and its wild relatives. Similar observations have earlier been reported by Green et al. (2002). However, when the larvae were provided with a choice between the methanol-washed and unwashed pods, the larvae preferred to feed on the unwashed pods of the same accession, indicating that methanol removed the phagostimulant compounds from the pod surface. These results were further confirmed by impregnating the methanol extracts in glass fiber disks. Methanol extract of ICPL 87 also stimulated feeding by the third-, fourth-, and fifth-instar larvae of *H. armigera*, but the differences were non-significant.

There were both qualitative and quantitative differences in the HPLC profiles of methanol and hexane extracts of different accessions of wild relatives of pigeonpea, and some of these peaks were associated with resistance/susceptibility to *H. armigera*. Pod surface chemicals influenced the host selection behavior of *H. armigera* larvae, and it is important to identify these compounds for use as selection markers to develop cultivars with resistance to *H. armigera*. A complete understanding of the compounds on pod surface contributing to resistance/susceptibility to *H. armigera* would facilitate the selection of accessions with different mechanisms of resistance to *H. armigera*. Accessions with low amounts of phagostimulants and high amounts of antifeedants will be useful for wide hybridization to develop varieties with broad-based resistance to this pest.

Principal component analysis based on chemical fingerprints of methanol and hexane extracts, and biological interactions of *H. armigera* with wild relatives of pigeonpea placed the test material in different groups. Based on HPLC fingerprints of methanol extract, the resistant and the susceptible checks belonging to cultivated pigeonpea were placed in the same group, while the hexane extract HPLC fingerprints placed the resistant and the susceptible checks along with ICPW 28 (*C. cajanifolius*)—the closely related wild relative of pigeonpea in the same group. Biological data placed the moderately resistant (ICPL 332) and the susceptible (ICPL 87) checks independently in separate groups, as was the closely related wild relative of pigeonpea, *C. cajanifolius* (ICPW 28). Based on methanol or hexane extract HPLC fingerprints or on biological interactions, the accessions belonging to *C. scarabaeoides* were placed in same group, suggesting that both chemical and biological data confirmed the similarity/diversity in the wild relatives of pigeonpea, and this information can be used to identify accessions with different combinations of characteristics associated with resistance to *H. armigera* for use in crop improvement programs.

Feeding by the third-instar *H. armigera* larvae was significantly lower on the pods of *C. sericeus* (except ICPW 159 pods washed with hexane), *C. scarabaeoides* (except the unwashed pods of ICPW 94; water-washed pods of ICPW 116, ICPW 141, and ICPW 152; and hexane-washed pods of ICPW 281), *F. bracteata*, *F. stricta*, and *R. aurea* than the unwashed, and water-, methanol-, or hexane-washed pods of *C. cajanifolius* and the cultivated pigeonpea, suggesting that the accessions with lower amounts of phagostimulants and greater amounts of antifeedants than those in the cultivated pigeonpea may be used to increase the levels and diversify the basis of resistance to pod borer, *H. armigera.*

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