Arthropod-Plant Interactions
https://doi.org/10.1007/s11829-020-09768-3

ORIGINAL PAPER

Biochemical components of wild relatives of chickpea confer resistance to pod borer, *Helicoverpa armigera*

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Received: 7 March 2020 / Accepted: 13 April 2020

Abstract

Efforts are being made to develop chickpea varieties with resistance to the pod borer, *Helicoverpa armigera* for reducing pesticide use and minimizing the extent of losses due to this pest. However, only low to moderate levels of resistance have been observed in the cultivated chickpea to this polyphagous pest. Hence, it is important to explore wild relatives as resistance sources to develop insect-resistant cultivars. Therefore, we studied different biochemical components that confer resistance to *H. armigera* in a diverse array of wild relatives of chickpea. Accessions belonging to wild relatives of chickpea exhibited high levels of resistance to *H. armigera* as compared to cultivated chickpea genotypes in terms of lower larval survival, pupation and adult emergence, decreased larval and pupal weights, prolonged larval and pupal developmental periods and reduced fecundity of the *H. armigera* when reared on artificial diet impregnated with lyophilized leaf powders. Amounts of proteins and phenols in different accessions of chickpea wild relatives were significantly and negatively correlated with larval weight, pupation and adult emergence. Phenols showed a negative correlation with pupal weight and fecundity, but positive correlation with pupal period. Total soluble sugars showed a negative correlation with larval period, but positive correlation with pupation and pupal weight, while tannins showed a positive correlation with larval weight, pupation and adult emergence. The flavonoid compounds such as chlorogenic acid, ferulic acid, naringin, 3,4-dihydroxy flavones, quercetin, naringenin, genistein, biochanin-A and formononetin that were identified through HPLC fingerprints, exhibited negative effects on survival and development of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powders. The wild relatives with diverse mechanisms of resistance conferred by different biochemical components can be used as sources of resistance in chickpea breeding programs to develop cultivars with durable resistance to *H. armigera* for sustainable crop production.

Keywords Chickpea · Wild relatives · *Helicoverpa armigera* · Antibiosis · Biochemical mechanism of resistance

Introduction

Chickpea (*Cicer arietinum* L.) ranks third among the pulse crops after dry beans and peas in terms of total production worldwide. Chickpea is grown in over 50 countries globally on an area of 12.65 million ha and production of 12.09 million tonnes, of which Asia alone accounts for 10.68 million ha area and 9.70 million tonnes production (FAO STAT 2016). Legume pod borer, *Helicoverpa armigera* (Hubner) is the most important constraint for chickpea production worldwide. The early instar *H. armigera* larvae feed on leaves of chickpea, while later instars shift to flowers and developing pods and cancause 80–90% reduction in yield despite several rounds of insecticide applications (Banu et al. 2005). It is very difficult to control this pest due to its high fecundity, multiple generations, polyphagous feeding habit, and long distance migration (Sarode 1999) and ability to develop resistance to insecticides (Sharma 2001; Kranthi et al. 2002). Therefore, there is need for deploying alternative methods of controlling this pest, of which host plant resistance can provide an effective means to minimize the extent of crop losses. However, only moderate levels of resistance have been identified in the available cultivated chickpea germplasm, while the wild relatives of chickpea have shown high levels of

Handling Editor: Heikki Hokkanen.

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Published online: 30 April 2020

resistance to *H. armigera* (Sharma et al. 2005a, b, 2006; Golla et al. 2018). Hence, there is a necessity to identify accessions with different components of resistance, to use in breeding programs to broaden the basis and increase the levels of resistance to this pest for sustainable crop production.

The wild relatives of chickpea that have shown high levels of resistance to *H. armigera* have not yet been characterized for biochemical mechanisms of resistance. Plant–herbivore interactions are the resultant of nutritional composition of the host plant, and the influence of morphological traits and secondary metabolites on the herbivores (Cates 1980). Primary and secondary metabolites of the host plant influence the insect behavior and their survival and development (Roeder and Behmer 2014). Secondary metabolites reduce the digestibility of plant tissues in the insect gut, and thereby affect the larval growth and development (Bennett and Wallsgrove 1994), whereas the effect of primary metabolites or nutritional factors depends on the relative amounts of different constituents (Behmer 2008). The suboptimal ratio between carbohydrates and proteins reduces the insect growth and development (Simpson and Raubenheimer 2009; Roeder and Behmer 2014).

Chickpeas are known for their inhibitory activity of gut proteinases (Saini et al. 1992; Patankar et al. 1999).Wild relatives of chickpea exhibit considerable diversity in protease inhibitor isoforms as compared to the cultivated chickpea (Patankar et al. 1999). Among the secondary metabolites, phenols play a significant role in conferring resistance to insect pests by adversely affecting larval growth and development by feeding inhibition and/or by reduced larval metabolism (Treutter 2006; Ballhorn et al. 2011). Development of plant with resistance to insect pests has often been attributed to high phenol content (Selvanarayanan and Narayanasamy 2006). Severity of the adverse effects of tannins in different insects ranges from no visual effect to reduction in growth and development, and finally mortality of the insect (Panzuto et al. 2002). However, the effects of tannins in insect gut depend on their concentration and chemical structure of the tannins, as well as pH and concentration of antioxidants in the insect gut (Galati et al. 2002; Hagerman et al. 2003). Flavonoid compounds are biosynthesized via the phenylpropanoid pathway (Dakora and Phillips 1996) in the host plant and affect insect feeding, survival, growth and fecundity (Musayimana et al. 2001; Napal et al. 2010). The flavonoid compounds such as quercetin, chlorogenic acid and rutin are widely distributed among crop plants and contribute to resistance against herbivores (Kennedy 2003; Simmonds 2003). Hence, a basic understanding of the interactions between the biochemical characters of wild relatives of chickpea and growth and development of *H. armigera* is highly important to identify biochemical constituents contributing to host plant resistance to this insect for use as selection criteria to develop chickpea cultivars with stable and high levels of resistance to this pest.

Materials and methods

Chickpea genotypes

Fifteen accessions of the wild relatives of chickpea belonging to seven species of *Cicer*, along with five accessions of cultivated chickpea (*Cicer arietinum*) were evaluated for resistance to pod borer, *H. armigera*. All the genotypes were grown under field conditions during post rainy seasons, 2014–2015 and 2015–2016 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India. Each accession was sown in a two row plot, and each row being 2 m long. There were two replications in a randomized complete block design. Seeds of the wild accessions were scarified at one end using scalpel to enhance water absorption, soaked in water for 24 h, and treated with thiram (3 g per kg of seed) before sowing. The seeds of cultivated chickpea were sown without scarification. The experimental trial was laid out with a spacing of 60 × 30 cm between the rows and plants in black soil (Vertisols), and the crop was raised under insecticide-free conditions.

In the glasshouse, all genotypes were planted in plastic pots (30 × 30 cm) filled with a potting mixture of black soil, sand and farmyard manure (2:1:1) at ICRISAT, Patancheru, Telangana, India. Five seedlings were raised in each pot and there were three pots for each accession in a completely randomized design. The plants in pots were watered as and when necessary. Desert coolers were used to maintain the temperature of 27 ± 5 °C, and relative humidity of > 65% in the glasshouse.

Helicoverpa armigera culture

The neonates of *H. armigera* used in bioassays were obtained from the laboratory reared culture atthe International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana State, India. Under laboratory conditions, the *H. armigera* larvae were reared individually on chickpea-based artificial diet (Babu et al. 2014) at 27 ± 2 °C temperature, 65 to 75% relative humidity, and 16: 8 h (L/D) photoperiod regime.

Survival and development of *H. armigera* on different wild relatives of chickpea

The antibiosis component of resistance to *H. armigera* in the wild relatives of chickpea was evaluated by rearing the neonate larvae on artificial diet impregnated with lyophilized

leaf powders of different chickpea genotypes (Narayanamma et al. 2008). The chickpea terminals or branches with tender green leaves were collected from the plants grown in the field and the glasshouse at the full vegetative growth stage, and placed in an icebox and eventually frozen at − 20 °C (REMI, Model-RQF 425, Japan). The leaves were freeze-driedto avoid changes in biochemical composition using lyophilizer (Modulyo D, Thermo Savant, Japan) at − 45 °C temperature and 436 mbar pressure for 3 to 4 days. The leaves were powdered and stored in a desiccator till used. Dried powder of chickpea leaves (20 g) was incorporated into the artificial diet as a replacement for part of the chickpea flour for rearing of *H. armigera*. The neonate larvae of *H. armigera* were released individually on the diet in a 25 cell-well plate with a fine camel hairbrush. Each treatment was replicated thrice (25 larvae in each replication) in completely randomized design. The cell-wells were maintained at 27 ± 2 °C temperature, 65 to 75% relative humidity and 12 h photoperiod after releasing the neonates onto the artificial diet. Data were recorded on larval survival, and the larval weights on 10th day after releasing the larvae into artificial diet. Pupal weights were recorded one day after pupation. Pupae from each replication were sterilized with 2% sodium hypochlorite solution and placed in a plastic jar containing moist vermiculite. Data were also recorded on larval and pupal periods. The adults were collected from the jars, and three pairs of adults that emerged on the same day on a particular genotype were placed inside a plastic cage, and the numbers of eggs laid were counted. Percentage of larval survival on 10th day, pupation and adult emergence were computed in relation to number of neonate larvae released in each replication.

Biochemical characterization of different wild relatives of chickpea

Biochemical components in the lyophilized leaf powder of different genotypes of chickpea were estimated using standard protocols. Protein content was estimated as per Lowry et al. (1951), phenol content as per the method presented by Bray and Thorpe (1954), tannin content by vanillin hydrochloride method (Burns 1971), and total soluble sugar content as per Hedge and Hofreiter (1962).

Estimation of flavonoids in wild relatives of chickpea through HPLC fingerprints

Flavonoid compounds from different wild relatives of chickpea were extracted by the method described by Hahn et al. (1983) with slight modifications. Lyophilized leaf sample (100 mg) was homogenized in 5 ml of HPLC grade methanol with mortar and pestle and centrifuged at 8000 rpm for 20 min. The supernatant was collected and partitioned with three times the volume of hexane in a separation funnel, and the methanol phase was collected. This process was repeated three times. Collected methanol phase was concentrated to a volume of 2 ml in a vacuum rotavapor (R-215, Buchi, Switzerland). Concentrated samples were filtered through 0.22 µm millipore filter and injected into HPLC (High Performance Liquid Chromatography). The standards such as chlorogenic acid, ferulic acid, naringin, 3,4-dihydroxy flavones, quercetin, naringenin, genistein, formononetin and biochaninA were prepared at 1000 ppm concentrations, and filtered as described above.

Flavonoid compounds in different samples were chromatographed using HPLC equipped with Sunfire C_{18} column (4.6 × 250 mm) with 5 µm pore size, and Waters 2695 separations module system consisting of a PCM 11 reciprocating piston pump. The sample retention time was recorded with photodiode array detector (Waters 2996). Multistep gradient solvent system was used for separation of compounds which consisted of 2% acetic acid in Millipore water (Solvent A) and 2% acetic acid in acetonitrile (Solvent B). The separation was programmed isocratically: 5% of solvent B for 10 min, followed by a 7.5 min linear gradient to 15% of solvent B, which was run isocratically for 13.5 min, followed by a 10 min linear gradient to 50% of solvent B. This was run isocratically for 4 min, followed by a 5 min linear gradient to 15% of solvent B, and finally followed by a 5 min linear gradient to 5% of solvent B. Flow rate was 1 ml/min. Three replications were maintained for each genotype. The chromatographic data were recorded and processed by the Empower™. The flavonoid compounds were identified by retention times of the peaks calibrated with standards and quantification was done by comparing the peak area of the sample with peak area of the standard obtained with known concentrations at similar retention times.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using GENSTAT 14.0 version. The significance of differences between treatments was measured by *F*-test and the treatment means were compared by least significant difference (LSD) at $P = 0.05$. The data on survival and development of *H. armigera* on artificial diet with lyophilized leaf powders and biochemical characters of different genotypes were subjected to similarity matrix analysis with nearest neighbors to assess the diversity among wild relatives of chickpea with resistance to the pod borer, *H. armigera*.

Results

Survival and development of *H. armigera* on artificial diets with lyophilized leaf powder of wild relatives of chickpea grown under field conditions

Post-rainy season, 2014–2015

Significant differences were observed in survival and development of *H. armigera* when the neonates were reared on artificial diet impregnated with lyophilized leaf powder of different genotypes during the post-rainy season, 2014–2015 (Table 1). On 10th day after releasing the larvae on to the diet, lowest larval survival was recorded on the resistant check, ICC 506 EB (58.33%), which was not significantly different from IG 599076 (60.42%), ICCL 86111 (60.42%), PI 599066 (60.42%), IG 70012 (62.50%) and PI 599046 (62.50%). Highest larval survival (87.50%) was recorded on IG 72933, and the susceptible check, ICC 3137. Larval weight on 10th day was significantly lower on all the wild relatives as compared to that on JG 11 (19.94 mg) and the susceptible check, KAK 2 (17.46 mg). The lowest larval weights were recorded in insects reared on IG 69,979 (2.55 mg). Larval period of *H. armigera* was prolonged significantly in insects reared on the wild relatives of chickpea (> 25.0 days) as compared to the larvae fed on the susceptible check, ICC 3137 (23.52 days).

Pupation was significantly lower (27.08%) in *H. armigera* larvae reared on *C. bijugum* (IG 70012, IG 70018 and PI 599046), while highest pupation was observed in insects reared on JG 11 (54.17%). The weights of *H. armigera* pupae were significantly lower in insects reared on the wild relatives of chickpea as compared to the larvae reared on the susceptible check, ICC 3137 (417.27 mg), but not significantly different from the larvae reared on other cultivated chickpea. Longest pupal period was observed in insects reared on IG 70018 (15.82 days), followed by IG 70022 (15.41 days), PI 599066 (15.39 days) and PI 510663 (15.35 days), while the shortest pupal period was observed in insects reared on the susceptible checks, KAK 2 (12.17 days) and ICC 3137 (12.43 days). Adult emergence of *H. armigera* when reared on all the wild relatives was observed in a range of 16.67% (IG 70018 and PI 599046) and 33.33% (IG 72953, PI 510663, PI 568217 and PI 599077) and significantly lowest compared to susceptible checks, KAK 2 (47.92%) and ICC 3137 (45.83%). Fecundity of *H. armigera* females was lowest when the larvae were reared on PI 599066 (214.42 eggs/female), and highest in larvae reared on JG 11 (389.42 eggs/female), the later was not significantly different fromthose reared on the susceptible checks, ICC 3137 (349.25 eggs/female) and KAK 2 (340.17 eggs/female).

Post-rainy season, 2015–2016

Survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders was significantly different among the genotypes tested during post-rainy season, 2015–2016 (Table 2). Larval survival of *H. armigera* on 10th day was significantly lowest on IG 70006 (50.00%), followed by IG 70012 (58.33%) and IG 72933 (58.33%). Highest larval survival was observed on the susceptible check, KAK 2 (91.67%), which was not significantly different from JG 11 (89.58%), ICC 3137 (87.50%) and PI 599077 (87.50%). Significantly lower larval weights were recorded in larvae reared on artificial diets with leaf powder of the wild relatives of chickpea (3.61 mg on IG 70018 to 11.24 mg on IG 72953) as compared to the larvae reared on diets with leaf powder of JG 11 (17.12 mg) and the susceptible check, KAK 2 (16.19 mg). Larval period of *H. armigera* was prolonged by 2–3 days when the larvae were reared on wild relatives of chickpea as compared to that on the susceptible check, ICC 3137 (22.35 days). Longest larval period was observed in larvae reared on diets with leaf powder of *C. microphyllum* accession, ICCW 17148 (26.94 days), followed by *C. bijugum*, IG 70018 (26.77 days).

Significantly lower pupation was observed in larvae reared on diets with leaf powder of wild relatives of chickpea as compared to the larvae reared on diets with leaf powder of susceptible check, KAK 2 (72.92%), except on PI 599077 (64.58%) and PI 599109 (58.33%). Mean pupal weights were lower in insects reared on diets with leaf powder of wild relatives of chickpea (321.68 mg on IG 70012 to 410.63 mg on IG 72953) as compared to the larvae reared on diets with leaf powder of JG 11 (464.73 mg), which was not significantly different from susceptible checks, ICC 3137 (446.31 mg) and KAK 2 (427.42 mg). Pupal period of was significantly longer in larvae reared on diets with leaf powder of IG 70012 (15.81 days) and IG 70018 (15.73 days) as compared to the larvae reared on diets with leaf powder of JG 11 (11.63 days) and ICC 3137 (11.77 days). Adult emergence on the wild genotypes ranged 12.50% (IG 70006) to 39.58% (PI 599077 and ICCW 17148), which was significantly lower than on JG 11 (56.25%), ICC 3137 (54.17%) and KAK 2 (50.00%). Lowest fecundity was observed when the insects were reared on diets with leaf powder of IG 70018 (207.33 eggs/female), followed by IG 70012 (211.33 eggs/female), while highest fecundity was recorded in insects reared on JG 11 (382.33 eggs/female) and ICC 3137 (382.00 eggs/female).

Survival and development of *H. armigera* on artificial diets with lyophilized leaf powder of wild relatives of chickpea grown under glasshouse conditions

Survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders varied

Table 1 Expression of resistance to *H. armigera* in wild relatives of chickpea grown under field conditions (diet incorporation assay, post-rainy season, 2014–2015)

Species	Genotype	Larval survival 10 DAE (%)[#]	Mean larval weight 10 DAE (mg)	Larval period (days)	Pupation (%)[#]	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) [#]	Fecundity (eggs/female)[##]
C. chrossanicum	IG 599076	60.42 (51.02)^{ab}	4.34^{ab}	26.13^{cde}	31.25 (33.98)^{a}	354.11^{ab}	14.30^{bcde}	29.17 (32.63)^{abcde}	273.92 (16.56)^{bcdef}
C. cuneatum	IG 69979	83.33 (67.77)^{cd}	2.55^{a}	25.46^{abcde}	33.33 (35.22)^{ab}	356.76^{ab}	14.02^{abcde}	31.25 (33.98)^{abcdef}	233.25 (15.29)^{ab}
C. bijugum	IG 70006	77.08 (62.02)^{abcd}	4.40^{ab}	25.34^{abcde}	35.42 (36.51)^{abc}	355.05^{ab}	14.44^{cde}	18.75 (25.35)^{ab}	252.00 (15.89)^{abcd}
C. bijugum	IG 70012	62.50 (52.27)^{abc}	2.90^{a}	25.70^{cde}	27.08 (31.34)^{a}	336.74^{a}	14.49^{cde}	18.75 (25.63)^{abc}	226.42 (15.06)^{a}
C. bijugum	IG 70018	70.83 (57.54)^{abcd}	3.91^{ab}	26.66^{e}	27.08 (31.34)^{a}	328.60^{a}	15.82^{e}	16.67 (23.93)^{a}	222.42 (14.92)^{a}
C. bijugum	IG 70022	77.08 (61.42)^{abcd}	6.77^{abc}	26.69^{e}	31.25 (33.98)^{a}	351.03^{ab}	15.41^{de}	22.92 (28.58)^{abcd}	275.17 (16.60)^{bcdef}
C. reticulatum	IG 72933	87.50 (70.53)^{d}	10.31^{cd}	25.37^{abcde}	35.42 (36.51)^{abc}	372.91^{abc}	13.26^{abc}	25.00 (29.92)^{abcd}	326.25 (18.07)^{ghi}
C. reticulatum	IG 72953	77.08 (61.42)^{abcd}	7.60^{bc}	25.26^{abcde}	39.58 (38.94)^{abcd}	382.23^{abc}	13.50^{abcd}	33.33 (35.22)^{bcdef}	343.25 (18.54)^{ij}
C. pinnatifidum	PI 510663	72.92 (58.79)^{abcd}	3.58^{ab}	26.12^{cde}	41.67 (40.13)^{abcd}	354.28^{ab}	15.35^{de}	33.33 (35.26)^{bcdef}	285.75 (16.92)^{cdefg}
C. judaicum	PI 568217	85.42 (68.03)^{cd}	5.69^{ab}	26.13^{cde}	39.58 (38.98)^{abcd}	363.73^{abc}	13.35^{abc}	33.33 (35.22)^{bcdef}	215.50 (14.69)^{a}
C. bijugum	PI 599046	62.50 (52.35)^{abc}	3.36^{ab}	25.73^{cde}	27.08 (31.21)^{a}	341.76^{a}	14.22^{bcde}	16.67 (23.93)^{a}	216.00 (14.71)^{a}
C. bijugum	PI 599066	60.42 (51.02)^{ab}	3.19^{a}	25.67^{bcde}	31.25 (33.88)^{a}	344.54^{a}	15.39^{de}	22.92 (28.58)^{abcd}	214.42 (14.66)^{a}
C. judaicum	PI 599077	75.00 (60.00)^{abcd}	4.17^{ab}	26.37^{de}	43.75 (41.38)^{abcd}	352.38^{ab}	14.59^{cde}	33.33 (35.22)^{bcdef}	240.25 (15.51)^{abc}
C. pinnatifidum	PI 599109	75.00 (60.00)^{abcd}	4.43^{ab}	25.00^{abcde}	39.58 (38.94)^{abcd}	353.35^{ab}	14.05^{abcde}	27.08 (31.21)^{abcd}	256.92 (16.04)^{abcde}
C. microphyllum	ICCW 17148	83.33 (65.91)^{bcd}	4.57^{ab}	25.84^{cde}	31.25 (33.68)^{a}	326.81^{a}	14.71^{cde}	25.00 (29.23)^{abcd}	305.67 (17.50)^{fghi}
C. arietinum	JG 11 (C)	85.42 (67.60)^{cd}	19.94^{e}	23.65^{ab}	54.17 (47.42)^{d}	413.76^{c}	12.28^{a}	45.83 (42.58)^{ef}	389.42 (19.75)^{j}
C. arietinum	KAK 2 (S)	75.00 (60.00)^{abcd}	17.46^{e}	24.45^{abcd}	52.08 (46.22)^{cd}	403.73^{bc}	12.17^{a}	47.92 (43.80)^{f}	340.17 (18.45)^{hij}
C. arietinum	ICC 3137 (S)	87.50 (69.56)^{d}	16.03^{e}	23.52^{a}	50.00 (45.00)^{bcd}	417.27^{c}	12.43^{ab}	45.83 (42.58)^{ef}	349.25 (18.68)^{ij}
C. arietinum	ICCL 86111 (R)	60.42 (51.02)^{ab}	10.73^{cd}	24.16^{abc}	43.75 (41.38)^{abcd}	379.09^{abc}	13.06^{abc}	35.42 (36.51)^{cdef}	306.75 (17.50)^{efghi}
C. arietinum	ICC 506EB (R)	58.33 (49.96)^{a}	11.93^{d}	24.30^{abc}	41.67 (40.19)^{abcd}	381.13^{abc}	13.06^{abc}	37.50 (37.730)^{def}	291.00 (17.07)^{defgh}
	Fp	0.04	< .001	0.03	0.02	0.02	0.003	0.004	< .001
	Mean	59.91	7.39	25.38	37.81	363.46	13.99	32.86	16.62
	SE ±	4.60	1.26	0.59	3.01	16.26	0.56	3.21	0.44
	LSD ($P = 0.05$)	13.61	3.73	1.75	8.92	48.12	1.66	9.50	1.30

C commercial cultivar, *S* susceptible check, *R* resistant check

The values followed by same alphabet did not differ significantly at $P \leq 0.05$ (DMRT)

[#]Figures in the parentheses are Angular transformed values; *DAE* days after initiation of experiment

[##]Figures in the parentheses are square root ($\sqrt{x+0.5}$) transformed values

Table 2 Expression of resistance to *H. armigera* in wild relatives of chickpea grown under field condition (diet incorporation assay, post-rainy season, 2015–2016)

Species	Genotype	Larval survival 10 DAE (%)[#]	Mean larval weight 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) [#]	Fecundity (eggs/female)[##]
C. chrossanicum	IG 599076	75.00 (60.08)^{bcde}	4.26^{ab}	26.43^{ghij}	39.58^{ab}	346.02^{abc}	13.82^{cdefghi}	27.08 (31.21)^{de}	265.33 (16.29)^{bcdef}
C. cuneatum	IG 69979	79.17 (62.95)^{cdef}	4.43^{ab}	25.52^{efghi}	43.75^{abcd}	360.63^{abcde}	14.14^{defghij}	35.42 (36.45)^{fg}	230.00 (15.17)^{abcd}
C. bijugum	IG 70006	50.00 (45.00)^{a}	5.81^{abcde}	24.19^{bcde}	41.67^{abc}	353.91^{abcde}	14.15^{defghij}	12.50 (20.70)^{a}	255.00 (15.93)^{abcde}
C. bijugum	IG 70012	58.33 (49.87)^{ab}	8.15^{abcdefg}	25.04^{defg}	33.33^{a}	321.68^{a}	13.47^{abcdefg}	18.75 (25.63)^{b}	211.33 (14.52)^{a}
C. bijugum	IG 70018	62.50 (52.27)^{abc}	3.61^{a}	26.77^{ij}	35.42^{a}	328.21^{ab}	15.73^{ij}	20.83 (27.05)^{bc}	207.33 (14.40)^{a}
C. bijugum	IG 70022	62.50 (52.35)^{abc}	5.17^{abcd}	26.14^{fghij}	37.50^{ab}	358.28^{abcde}	15.81^{j}	25.00 (30.00)^{cd}	287.33 (16.95)^{ef}
C. reticulatum	IG 72933	58.33 (49.87)^{ab}	9.94^{efgh}	25.08^{defg}	41.67^{abc}	391.62^{cdefg}	12.95^{abcdef}	22.92 (28.58)^{bcd}	349.00 (18.68)^{ghi}
C. reticulatum	IG 72953	70.83 (57.31)^{abcd}	11.24^{fgh}	24.57^{cde}	52.08^{abcde}	410.63^{fgh}	13.67^{bcdefgh}	37.50 (37.73)^{fgh}	344.00 (18.54)^{ghi}
C. pinnatifidum	PI 510663	70.83 (57.37)^{abcd}	9.29^{cdefgh}	26.74^{hij}	52.08^{abcde}	351.44^{abcd}	15.62^{hij}	33.33 (35.22)^{fg}	277.00 (16.66)^{def}
C. judaicum	PI 568217	62.50 (52.27)^{abc}	9.58^{defgh}	26.27^{fghij}	43.75^{abcd}	369.78^{bcdef}	12.19^{abcd}	31.25 (33.98)^{ef}	223.00 (14.93)^{ab}
C. bijugum	PI 599046	64.58 (53.92)^{abc}	4.74^{abc}	25.36^{efgh}	43.75^{abcd}	341.07^{ab}	14.23^{efghij}	22.92 (28.58)^{bcd}	227.00 (15.06)^{abc}
C. bijugum	PI 599066	62.50 (52.27)^{abc}	3.82^{ab}	25.50^{efghi}	31.25^{a}	339.16^{ab}	14.64^{fghij}	22.92 (28.58)^{bcd}	216.33 (14.72)^{a}
C. judaicum	PI 599077	87.50 (69.30)^{def}	8.49^{bcdefg}	24.88^{cdef}	64.58^{de}	345.14^{ab}	15.32^{ghij}	39.58 (38.98)^{gh}	228.00 (15.11)^{abc}
C. pinnatifidum	PI 599109	75.00 (60.08)^{bcde}	7.24^{abcdef}	23.63^{abc}	58.33^{bcde}	347.50^{abc}	13.49^{abcdefg}	35.42 (36.45)^{fg}	272.33 (16.47)^{cdef}
C. microphyllum	ICCW 17148	79.17 (62.95)^{cdef}	4.32^{ab}	26.94^{j}	52.08^{abcde}	340.08^{ab}	13.56^{abcdefg}	39.58 (38.98)^{gh}	295.33 (17.19)^{efg}
C. arietinum	JG 11 (C)	89.58 (71.26)^{ef}	17.12^{j}	22.93^{ab}	72.92^{e}	464.73^{i}	11.63^{a}	56.25 (48.59)^{i}	382.33 (19.50)^{i}
C. arietinum	KAK 2 (S)	91.67 (73.22)^{f}	16.19^{ij}	22.93^{ab}	72.92^{e}	427.42^{ghi}	12.11^{abc}	50.00 (45.00)^{i}	351.33 (18.72)^{hi}
C. arietinum	ICC 3137 (S)	87.50 (69.30)^{def}	13.28^{hij}	22.35^{a}	70.83^{e}	446.31^{hi}	11.77^{ab}	54.17 (47.40)^{i}	382.00 (19.51)^{i}
C. arietinum	ICCL 86111 (R)	75.00 (60.32)^{bcde}	12.34^{ghi}	23.88^{bcd}	62.50^{cde}	395.94^{defg}	12.35^{abcde}	39.58 (38.94)^{gh}	341.00 (18.48)^{ghi}
C. arietinum	ICC 506EB (R)	81.25 (64.37)^{cdef}	13.38^{hij}	23.81^{bcd}	62.50^{cde}	398.65^{efg}	12.46^{abcde}	43.75 (41.41)^{h}	307.00 (17.52)^{fgh}
	Fp	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< .001	< .001
	Mean	58.82	8.62	24.95	50.62	371.91	13.65	34.97	16.72
	SE ±	3.69	1.39	0.42	6.27	13.59	0.58	1.20	0.47
	LSD ($P = 0.05$)	10.92	4.10	1.23	18.54	40.22	1.71	3.54	1.35

C commercial cultivar, *S* susceptible check, *R* resistant check

The values followed by same alphabet did not differ significantly at $P \leq 0.05$ (DMRT)

[#]Figures in the parentheses are Angular transformed values; *DAE* days after initiation of experiment

[##] Figures in the parentheses are square root ($\sqrt{x+0.5}$) transformed values

significantly among the genotypes grown under glasshouse condition (Table 3). Lowest larval survival on 10th day was observed in larvae reared on artificial diets with the leaf powder of IG 70018 (47.92%), followed by those reared on diets with ICCW 17148 (52.08%) and IG 70012 (52.08%). The highest larval survival was observed on the susceptible check, ICC 3137 (81.25%). Larval weights were significantly lower when the insects reared on diets with leaf powder of the wild genotypes as compared to that on the susceptible check, KAK 2 (14.63 mg), except on diets with leaf powder of IG 72933 (10.12 mg) and IG 599076 (9.15 mg). Lowest larval weight was recorded on ICCW 17148 (1.69 mg), followed by PI 599046 (2.02 mg). Larval period was longest in insects reared on diets with leaf powder of IG 70018 (27.18 days), followed by ICCW 17148 (26.80 days), while the shorter larval period was observed on JG 11 (24.38 days) followed by ICC 3137 (24.70 days) and KAK 2 (24.80 days).

Pupation was significantly lower in insects reared on diets with leaf powder of ICCW 17148, PI 599046, IG 70018, IG 70012 and IG 70022 (20.83 to 35.42%) than those reared on diets with leaf powder of the susceptible check, KAK 2 (50.00%). Lower pupal weights were observed in insects reared on diets with leaf powder of wild relatives of chickpea (313.54 to 362.20 mg) as compared to those reared on diets with leaf powder of the susceptible check, ICC 3137 (388.23 mg), which was not significantly different from KAK 2 (380.03 mg), ICC 506EB (363.61 mg), JG 11 (362.79 mg) and ICCL 86,111 (362.23 mg).

Pupal period was delayed when the *H.armigera* larvae were reared on diets with leaf powder of wild relatives of chickpea (ranging from 13.33 days in IG 72953 to 16.14 days in PI 599066) as compared to the larvae reared on diets with leaf powder of the susceptible check, KAK 2 (12.22 days). Lowest adult emergence was observed in insets reared on diets with leaf powder of ICCW 17148 (10.42%), followed by IG 70018 (14.58%) and PI 599046 (14.58%). highest adult emergence was observed in insects reared on KAK 2 (45.83%). Lowest fecundity of *H. armigera* was observed when the insects were reared on PI 599046 (205.00 eggs/female), and highest on JG 11 (396.50 eggs/female), which was not significantly different from IG 72953 (342.50 eggs/female), KAK 2 (329 eggs/female), ICC 3137 (316.50 eggs/female), ICCW 17148 (316.00 eggs/female) and IG 72933 (303.50 eggs/female).

Biochemical characterization of wild relatives of chickpea

Protein content

There were significant differences in protein content among the wild relatives and the cultivated genotypes of chickpea (Table 4). During the post-rainy season 2014–2015, greater amounts of protein content were recorded in KAK 2 (16.41%) and PI 599066 (15.89%), while lowest was observed in ICC 3137 (11.42%) which was not significantly different from PI 568217 (11.99%) and ICCW 17,148 (12.39%). During the post-rainy season 2015–2016, the *C. bijugum* genotypes IG 70018, PI 599046, IG 70012, PI 599066, IG 70022 and IG 70006 recorded higher protein content (15.40 to 12.38%) than ICC 506EB (8.27%). Under glasshouse conditions, lowest protein content was recorded in PI 599077 (7.73%), which was not significantly different from IG 69979 (7.87%), PI 568217 (8.16%), PI 599109 (8.18%) and ICC 506EB (8.65%), while highest protein content was recorded in IG 70012 (12.20%).

Phenol content

Significant differences were observed in phenol content among the genotypes tested (Table 4). During the post-rainy season 2014–2015, accessions belonging to wild relatives of chickpea exhibited significantly higher amounts of phenols (6.55 mg/g in PI 599077 to 7.97 mg/g in PI 599046) as compared to the cultivated chickpea (5.93 mg/g in ICCL 86111 to 6.15 mg/g in ICC 506 EB), except in *C. reticulatum*, IG 72953 (4.10 mg/g) and IG 72933 (4.52 mg/g) and *C. chrossanicum*, IG 599076 (6.15 mg/g). During the post-rainy season 2015–2016, highest phenol content was observed in PI 599046 (6.50 mg/g),which was not significantly different from IG 70006 (6.41 mg/g), while lowest protein content was recorded in IG 599076 (4.07 mg/g) which was not significantly different from the susceptible check, ICC 3137 (4.16 mg/g). Under glasshouse conditions, phenol content varied significantly among genotypes tested, and ranged from 3.06 mg/g (ICC 3137) to 5.90 mg/g (IG 70006).

Total soluble sugars

Significant differences were observed in total soluble sugars among different genotypes of chickpea (Table 4). During the post-rainy season 2014–2015, the genotypes ICCW 17148, PI 568217, IG 599076, PI 599109, IG 72933, PI 510663, IG 72953, IG 69,979 and PI 599046 had significantly lower amounts of total soluble sugars (8.04 to 9.61%) than the susceptible check, KAK 2 (13.60%). During the post-rainy season 2015–2016, lower amounts of total soluble sugars were recorded in IG 599076 (10.35%), followed by IG 69979 (11.05%), while highest amounts of soluble sugars were recorded in KAK 2 (17.18%), which was not significantly different from ICC 506EB (16.54%), ICC 3137 (16.05%) and JG 11 (15.86%). Under glasshouse conditions, amounts of total soluble sugars were significantly lower in IG 72933 (7.38%), PI 599077 (7.87%) and IG 599076 (7.91%) than in IG 70022 (16.35%) and IG 70018 (14.96%).

Table 3 Expression of resistance to *H. armigera* in wild relatives of chickpea grown under glasshouse conditions (diet incorporation assay)

Species	Genotype	Larval survival 10 DAE (%)	Mean larval weight 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) #	Fecundity (eggs/female)##
C. chrossanicum	IG 599076	60.42^{abcd}	9.15^{bcdef}	25.83^{abcdef}	41.67^{cd}	362.20^{cde}	14.78^{bcdef}	35.42 (36.51)^{fghi}	282.50 (16.82)^{abc}
C. cuneatum	IG 69979	60.42^{abcd}	8.65^{abcdef}	25.41^{abcde}	37.50^{bcd}	352.88^{bcd}	13.89^{abcdef}	29.17 (32.46)^{cdef}	236.50 (15.34) ^{ab}
C. bijugum	IG 70006	58.33^{abc}	4.97^{abc}	26.50^{def}	43.75^{cd}	356.19^{bcde}	14.73^{abcdef}	25.00 (29.92)^{bcd}	249.00 (15.79) ^{ab}
C. bijugum	IG 70012	52.08^{ab}	6.52^{abcd}	26.37^{cdef}	35.42^{abcd}	351.79^{bcd}	15.50^{def}	22.92 (28.58)^{bc}	241.50 (15.51) ^{ab}
C. bijugum	IG 70018	47.92^{a}	3.42^{ab}	27.18^{f}	33.33^{abc}	328.98^{ab}	15.90^{ef}	14.58 (22.40)^{a}	237.50 (15.38) ^{ab}
C. bijugum	IG 70022	58.33^{abc}	3.94^{ab}	26.61^{def}	35.42^{abcd}	343.77^{bc}	15.00^{bcdef}	22.92 (28.39)^{bc}	263.00 (16.16) ^{ab}
C. reticulatum	IG 72933	70.83^{bcde}	10.12^{bcdef}	25.67^{abcdef}	41.67^{cd}	354.20^{bcd}	13.57^{abcde}	31.25 (33.98)^{defg}	303.50 (17.38) ^{abc}
C. reticulatum	IG 72953	68.75^{bcde}	7.61^{abcde}	25.96^{abcdef}	41.67^{cd}	353.83^{bcd}	13.33^{abcd}	29.17 (32.63)^{cdef}	342.50 (18.51) ^{bc}
C. pinnatifidum	PI 510663	64.58^{abcde}	6.91^{abcd}	25.50^{abcde}	41.67^{cd}	357.12^{bcde}	15.07^{cdef}	33.33 (35.26)^{efghi}	294.50 (17.15) ^{abc}
C. judaicum	PI 568217	66.67^{abcde}	8.23^{abcdef}	26.00^{abcdef}	47.92^{cd}	357.68^{bcde}	14.50^{abcdef}	37.50 (37.73)^{ghi}	208.00 (14.43)^{a}
C. bijugum	PI 599046	58.33^{abc}	2.02^{a}	26.10^{bcdef}	22.92^{ab}	342.45^{abc}	14.21^{abcdef}	14.58 (22.40)^{a}	205.00 (14.32)^{a}
C. bijugum	PI 599066	60.42^{abcd}	6.07^{abcd}	25.84^{abcdef}	43.75^{cd}	349.91^{bcd}	16.14^{f}	27.08 (31.21)^{bcde}	212.50 (14.59)^{a}
C. judaicum	PI 599077	60.42^{abcd}	7.07^{abcd}	25.80^{abcdef}	45.83^{cd}	359.62^{bcde}	13.86^{abcdef}	31.25 (33.98)^{defgh}	252.50 (15.90) ^{ab}
C. pinnatifidum	PI 599109	62.50^{abcde}	5.74^{abcd}	26.37^{cdef}	39.58^{cd}	359.20^{bcde}	14.60^{abcdef}	20.83 (27.05)^{b}	241.50 (15.50) ^{ab}
C. microphyllum	ICCW 17148	52.08^{ab}	1.69^{a}	26.80^{ef}	20.83^{a}	313.54^{a}	15.85^{def}	10.42 (18.74)^{a}	316.00 (17.78) ^{bc}
C. arietinum	JG 11 (C)	79.17^{de}	11.16^{cdef}	24.38^{a}	47.92^{cd}	362.79^{cde}	12.92^{abc}	39.58 (38.94)^{gij}	396.50 (19.91)^{c}
C. arietinum	KAK 2 (S)	75.00^{cde}	14.63^{f}	24.80^{abc}	50.00^{d}	380.03^{de}	12.22^{a}	45.83 (42.60)^{j}	329.00 (18.14)^{ab}
C. arietinum	ICC 3137 (S)	81.25^{e}	14.34^{ef}	24.70^{ab}	47.92^{cd}	388.23^{e}	12.50^{ab}	37.50 (37.65)^{ghi}	316.50 (17.80)^{bc}
C. arietinum	ICCL 86111 (R)	70.83^{bcde}	11.29^{cdef}	25.39^{abcde}	45.83^{cd}	362.23^{cde}	14.35^{abcdef}	33.33 (35.26)^{efghi}	272.50 (16.50)^{ab}
C. arietinum	ICC 506EB (R)	68.75^{bcde}	12.35^{def}	25.02^{abcd}	45.83^{cd}	363.61^{cde}	13.66^{abcdef}	33.33 (35.22)^{efghi}	275.00 (16.56)^{ab}
	Fp	0.039	< 0.001	0.035	0.01	0.015	0.04	< .001	0.02
	Mean	63.85	7.79	25.81	40.52	355.01	14.33	32.05	16.47
	SE ±	5.83	2.09	0.48	4.66	9.43	0.73	1.47	0.92
	LSD ($P = 0.05$)	17.27	5.86	1.41	13.79	27.92	2.16	4.34	2.73

C commercial cultivar, *S* susceptible check, *R* resistant check

The values followed by same alphabet did not differ significantly at $P \leq 0.05$ (DMRT)

#Figures in the parentheses are Angular transformed values; *DAE* days after initiation of experiment

##Figures in the parentheses are square root ($\sqrt{x+0.5}$) transformed values

Table 4 Biochemical characterization of wild relatives of chickpea exhibiting different levels of resistance to *H. armigera*

Species	Genotype	Post-rainy season, 2014–2015				Post-rainy season, 2015–2016				Glasshouse condition			
		Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)
C. chrossanicum	IG 599076	13.88	6.15	9.01	7.58	11.72	4.07	10.35	8.55	8.98	2.76	7.91	9.03
C. cuneatum	IG 69979	13.78	7.02	9.58	8.61	10.34	5.04	11.05	9.55	7.87	3.42	9.51	8.39
C. bijugum	IG 70006	15.67	7.46	10.70	9.48	12.38	6.41	12.44	9.15	9.88	5.90	12.57	10.15
C. bijugum	IG 70012	15.49	7.78	11.46	7.97	14.00	6.03	13.65	9.15	12.20	5.65	11.98	9.97
C. bijugum	IG 70018	14.96	7.94	10.23	8.88	15.40	5.40	13.41	8.30	11.99	5.42	14.96	8.97
C. bijugum	IG 70022	14.65	7.35	11.49	8.64	12.44	6.22	14.02	8.73	11.65	5.18	16.35	9.24
C. reticulatum	IG 72933	13.33	4.52	9.17	9.24	10.96	5.06	11.19	10.09	9.17	3.16	7.38	9.09
C. reticulatum	IG 72953	13.42	4.10	9.46	8.48	11.45	4.38	11.23	10.61	9.65	3.57	9.05	10.24
C. pinnatifidum	PI 510663	15.60	7.17	9.45	7.36	11.58	5.15	12.35	5.39	8.60	4.42	8.27	11.21
C. judaicum	PI 568217	11.99	6.84	8.19	9.27	10.03	4.47	11.34	9.24	8.16	3.42	10.50	11.00
C. bijugum	PI 599046	15.73	7.97	9.61	8.79	14.42	6.50	14.12	7.88	11.52	5.49	13.57	9.24
C. bijugum	PI 599066	15.89	7.76	10.58	8.82	13.44	5.42	14.70	7.09	11.93	4.87	9.67	13.64
C. judaicum	PI 599077	13.63	6.55	11.08	10.27	10.61	4.28	13.97	11.21	7.73	3.31	7.87	11.73
C. pinnatifidum	PI 599109	15.59	7.41	9.08	7.58	11.87	4.82	11.55	5.97	8.18	5.18	10.89	11.48
C. microphyllum	ICCW 17148	12.39	7.55	8.04	8.24	11.72	4.30	11.91	10.30	11.79	4.55	13.54	9.00
C. arietinum	JG 11	14.51	7.58	12.19	9.73	11.96	4.66	15.86	11.42	10.91	3.31	8.28	13.12
C. arietinum	KAK 2	16.41	5.97	13.60	8.97	13.01	4.47	17.18	10.64	10.75	4.18	9.34	16.30
C. arietinum	ICC 3137	11.42	5.99	10.59	5.97	10.92	4.16	16.05	10.79	9.44	3.06	8.90	12.55
C. arietinum	ICCL 86111	13.13	5.93	10.51	8.30	11.33	4.98	13.60	9.18	9.32	4.93	11.00	15.82
C. arietinum	ICC 506EB	13.52	6.15	11.62	7.97	8.27	4.57	16.54	7.94	8.65	3.55	10.46	12.39
	Fp	0.002	< 0.001	< 0.001	NS	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Mean	14.25	6.76	10.28	8.5	11.89	5.02	13.33	9.06	9.92	4.27	10.60	11.13
	SE ±	0.70	0.13	0.59	0.83	0.44	0.06	0.56	0.10	0.37	0.16	0.55	0.31

Table 4 (continued)

Species	Genotype	Post-rainy season, 2014–2015				Post-rainy season, 2015–2016				Glasshouse condition			
		Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)
	LSD ($P=0.05$)	2.07	0.38	1.75	-	1.31	0.18	1.67	0.30	1.09	0.46	1.64	0.93

Tannin content

During the post-rainy season 2014–2015, there were no significant differences in tannin content among the genotypes tested (Table 4). During the post-rainy season 2015–2016, tannin content was lower in PI 510663 (5.39 mg/g) and PI 599109 (5.97 mg/g) as compared to JG 11 (11.42 mg/g) and PI 599077 (11.21 mg/g). Significant differences were observed in tannin content among different chickpea genotypes under glasshouse conditions. KAK2 recorded higher tannin content (16.30 mg/g) than in IG 69979 (8.39 mg/g).

Flavonoids content in wild relatives of chickpea estimated through HPLC fingerprints

Significant differences were observed in flavonoid compounds identified through HPLC fingerprints at different retention times (RT from 8.11 to 25.70 min) among the genotypes tested (Table 5). Among the flavonoid compounds, chlorogenic acid (RT 8.11 min) was present only in four genotypes, IG 70012 (2.90 mg/g), PI 599066 (2.43 mg/g), KAK 2 (1.42 mg/g) and ICC 3137 (0.36 mg/g), while ferulic acid (RT 12.68 min), naringin (RT 13.01 min), 3,4-dihydroxy flavones (RT 17.43 min) and naringenin (RT 19.78 min) were present in a few genotypes of the wild relatives of chickpea, but were absent in the cultivated chickpea. Quercetin (RT 17.79 min) was present in all the genotypes, except in ICC 506EB, and the highest concentration was recorded in PI 599046 (12.31 mg/g), which was not significantly different from IG 70022 (12.16 mg/g). Lowest was amount of quercetin were recorded in ICC 3137 (0.55 mg/g), which was on par with ICCL 86111 (0.57 mg/g), JG 11 (0.67 mg/g) and IG 69979 (0.74 mg/g). Genistein (RT 20.39 min) was present in all the genotypes, and its highest concentration was recorded in IG 70022 (9.57 mg/g), followed by PI 599066 (9.07 mg/g), while lowest amounts were recorded in ICC 506EB (0.42 mg/g). Formononetin (RT 22.76 min) was present in all the genotypes, except in IG 69979, while highest concentration was present in IG 70018 (4.39 mg/g) and PI 599046 (4.36 mg/g) than in (ICCW 17148 and ICC 3137 0.60 mg/g). Biochanin-A (RT 25.70 min) was present in all the genotypes, except in IG 69979 and IG 70,018. Greater amounts of Biochanin-A were recorded in IG 599076 (8.68 mg/g) and PI 568217 (6.23 mg/g) than in PI 599109 (0.89 mg/g) and PI 599066 (0.93 mg/g).

Association of biochemical components in wild relatives of chickpea with survival and development of *H. armigera*

Biochemical components of wild relatives showed significant influence on survival and development of *H. armigera* (Table 6). Proteins content was significantly and negatively

Table 5 Flavonoid compounds (mg g^{-1} of sample) in wild relatives of chickpea estimated through HPLC fingerprinting

	Compounds	Chlorogenic acid	Ferulic acid	Naringin	3,4-Dihydroxy flavones	Quercetin	Naringenin	Genistein	Formononetin	Biochanin-A
Species	RT (min)→ Genotype↓	8.11	12.68	13.01	17.43	17.79	19.78	20.39	22.76	25.70
C. chrossanicum	IG 599076	–	–	3.16	–	3.51	–	0.68	2.60	8.68
C. cuneatum	IG 69979	–	–	4.81	0.74	0.74	0.86	0.66	–	–
C. bijugum	IG 70006	–	–	3.96	1.60	10.75	0.16	6.74	2.90	1.13
C. bijugum	IG 70012	2.90	–	4.82	1.78	11.55	0.18	8.26	2.46	1.13
C. bijugum	IG 70018	–	0.76	–	1.63	8.57	–	7.01	4.39	–
C. bijugum	IG 70022	–	0.92	4.15	1.66	12.16	0.35	9.57	3.12	1.48
C. reticulatum	IG 72933	–	–	–	–	1.81	–	2.85	0.86	1.53
C. reticulatum	IG 72953	–	–	–	–	2.00	–	1.69	0.85	1.32
C. pinnatifidum	PI 510663	–	0.19	–	1.55	4.62	–	3.08	1.59	5.03
C. judaicum	PI 568217	–	–	1.97	0.14	1.84	–	0.94	0.82	6.23
C. bijugum	PI 599046	–	1.02	4.61	1.59	12.31	–	8.50	4.36	1.98
C. bijugum	PI 599066	2.43	–	4.27	1.79	11.54	0.74	9.07	2.05	0.93
C. judaicum	PI 599077	–	–	4.35	0.51	2.32	–	1.15	0.90	3.21
C. pinnatifidum	PI 599109	–	0.76	1.69	1.12	3.27	–	3.30	1.05	0.89
C. microphyllum	ICCW 17148	–	–	–	0.13	2.27	–	0.88	0.60	5.16
C. arietinum	JG 11	–	–	–	–	0.67	–	1.58	0.72	3.31
C. arietinum	KAK 2	1.42	–	–	–	1.23	–	2.26	1.49	1.42
C. arietinum	ICC 3137	0.36	–	–	–	0.55	–	1.76	0.60	1.04
C. arietinum	ICCL 86111	–	–	–	–	0.57	–	0.84	0.69	6.00
C. arietinum	ICC 506EB	–	–	–	–	–	–	0.42	0.73	2.71
	Fp	0.02	0.01	< 0.001	0.001	< 0.001	0.02	< .001	< .001	< .001
	Mean	1.78	0.73	4.15	1.20	4.86	0.46	3.56	1.72	2.78
	SE±	0.27	0.07	0.04	0.20	0.13	0.09	0.07	0.11	0.81
	LSD ($P=0.05$)	1.21	0.27	0.12	0.65	0.40	0.37	0.19	0.32	2.40

correlated with larval weight, pupation and adult emergence ($r = -0.26, -0.31$ and -0.26, respectively). Phenols content was significantly and negatively correlated with larval weight, pupation, pupal weight, adult emergence and fecundity ($r = -0.35, -0.41, -0.25, -0.37$ and -0.30, respectively), while pupal period was correlated ($r = 0.27$) positively. Total soluble sugars were positively correlated with pupation ($r = 0.35$) and pupal weight ($r = 0.25$), but negatively correlated with larval period ($r = -0.21$). Tannins showed a significant and positive association with larval weight, pupation and adult emergence ($r = 0.28$, 0.25 and 0.25, respectively).

Association of flavonoids with survival and development of *H. armigera*

Significant correlations were observed between flavonoid content of wild relatives of chickpea and the biological parameters of *H. armigera* (Table 7). Chlorogenic acid showed a significant and negative correlation with larval survival, larval weight, pupation, pupal weight, adult emergence and fecundity ($r = -0.90, -0.80, -0.84, -0.90, -0.84$ and -0.82, respectively), while larval period ($r = 0.93$) and pupal period ($r = 0.69$) were correlated positively. Amounts of ferulic acid were significantly and negatively correlated with pupation, pupal period, adult emergence and fecundity ($r = -0.64, -0.46, -0.70$ and -0.51, respectively). Naringin showed a significant and negative correlation with larval weight ($r = -0.50$), pupal weight ($r = -0.44$) and fecundity ($r = -0.71$). 3,4-Dihydroxy flavones had significant negative correlation with larval survival ($r = -0.58$), pupation ($r = -0.45$) and adult emergence ($r = -0.53$). Quercetin was significantly and negatively correlated with all the biological parameters, except larval period ($r = 0.50$) and pupal period ($r = 0.67$). Naringenin was significantly and negatively correlated with larval weight ($r = -0.62$) and fecundity ($r = -0.62$), but positively correlated with adult emergence ($r = 0.80$). Genistein showed a significant and positive correlation with pupal period ($r = 0.58$) and negative correlation with pupation, pupal weight, adult emergence and fecundity ($r = -0.58, -0.47, -0.69$ and -0.48, respectively). Formononetin showed a significant and positive correlation with larval and pupal periods ($r = 0.53$ and 0.63, respectively), but a negative correlation was observed with the other biological parameters. Among the flavonoid

Table 6 Association of biochemical components in wild relatives of chickpea with survival and development of *H. armigera*

	Larval survival (%)	Larval weight (mg)	Larval period (days)	Pupation (%)	Pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity
Protein (%)	0.05	−0.26*	0.01	−0.31*	−0.14	0.16	−0.26*	−0.11
Phenols (mg/g)	−0.03	−0.35**	0.13	−0.41**	−0.25*	0.27*	−0.37**	−0.30*
Total soluble sugars (%)	−0.03	0.15	−0.21*	0.35**	0.25*	−0.08	0.11	0.06
Tannins (mg/g)	0.07	0.28*	−0.06	0.25*	0.19	−0.15	0.25*	0.17

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

Table 7 Association of flavonoids in wild relatives of chickpea with survival and development of *H. armigera*

	Larval survival (%)	Larval weight (mg)	Larval period (days)	Pupation (%)	Pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity
Chlorogenic acid	−0.90**	−0.80**	0.93**	−0.84**	−0.90**	0.69**	−0.84**	−0.82**
Ferulic acid	−0.29	0.36	−0.13	−0.64**	−0.17	−0.46*	−0.70**	−0.51*
Naringin	0.25	−0.50*	−0.31	−0.13	−0.44*	−0.13	−0.16	−0.71**
3,4-Dihydroxy flavone	−0.58**	0.11	−0.12	−0.45*	−0.19	0.24	−0.53*	0.06
Quercetin	−0.52*	−0.51*	0.50*	−0.68**	−0.60**	0.67**	−0.77**	−0.59**
Naringenin	0.05	−0.62**	−0.39	0.19	0.24	−0.18	0.80**	−0.62**
Genistein	−0.30	−0.37	0.40	−0.58**	−0.47*	0.58**	−0.69**	−0.48*
Formononetin	−0.41	−0.49*	0.53*	−0.71**	−0.57**	0.60**	−0.73**	−0.59**
Biochanin A	−0.23	−0.17	0.20	−0.13	−0.15	0.05	0.05	−0.06

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

compounds, Biochanin-A did not show any significant correlations with the biological parameters of *H. armigera*.

Similarity matrix analysis

Similarity matrix analysis based on biochemical characters of wild relatives of chickpea and survival and development of *H. armigera* on artificial diet with lyophilized leaf powders of different genotypes of chickpea and its wild relatives separated the test genotypes into seven groups (coefficient 0.95) (Fig. 1). The genotypes belonging to *C. chrossanicum* (IG 599076), *C. cuneatum* (IG 69979), *C. judaicum* (PI 568217 and PI 599077) and *C. pinnatifidum* (PI 599109 and PI 510663) were grouped together. The genotypes belonging to *C. reticulatum*, which is the progenitor of cultivated chickpea (IG 72933 and IG 72953) were placed in one group, and were closer to the other group consisting of cultivated resistant checks, ICCL 86111 and ICC 506EB. All the genotypes belonging to *C. bijugum* (IG 70006, IG 70012, PI 599066, PI 599046, IG 70022 and IG 70018) were placed in one group. The genotype, ICCW 17148 which belongs to the tertiary gene pool (*C. microphyllum*) was placed independently, while the susceptible check, KAK 2 was grouped along with the commercial check, JG 11, which was nearer to another susceptible check, ICC 3137.

Discussion

Significant differences were observed in survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders of different genotypes of wild relatives of chickpea across the seasons. The wild relatives exhibited high levels of antibiosis to *H. armigera* as compared to cultivated chickpea in terms of lower larval survival, pupation and adult emergence, decreased larval and pupal weights, prolonged larval and pupal developmental periods and reduced fecundity. The genotypes, IG 70018, IG 70012, PI 599066, PI 599046, IG 70006 and IG 70022 (*C. bijugum*) exhibited high levels of resistance, while IG 69979 (*C. cuneatum*), ICCW 17148 (*C. microphyllum*), PI 599077, PI 568217 (*C. judaicum*) and IG 599076 (*C. chrossanicum*) exhibited moderate levels of resistance in terms of reduced survival, lower weights, delayed developmental periods

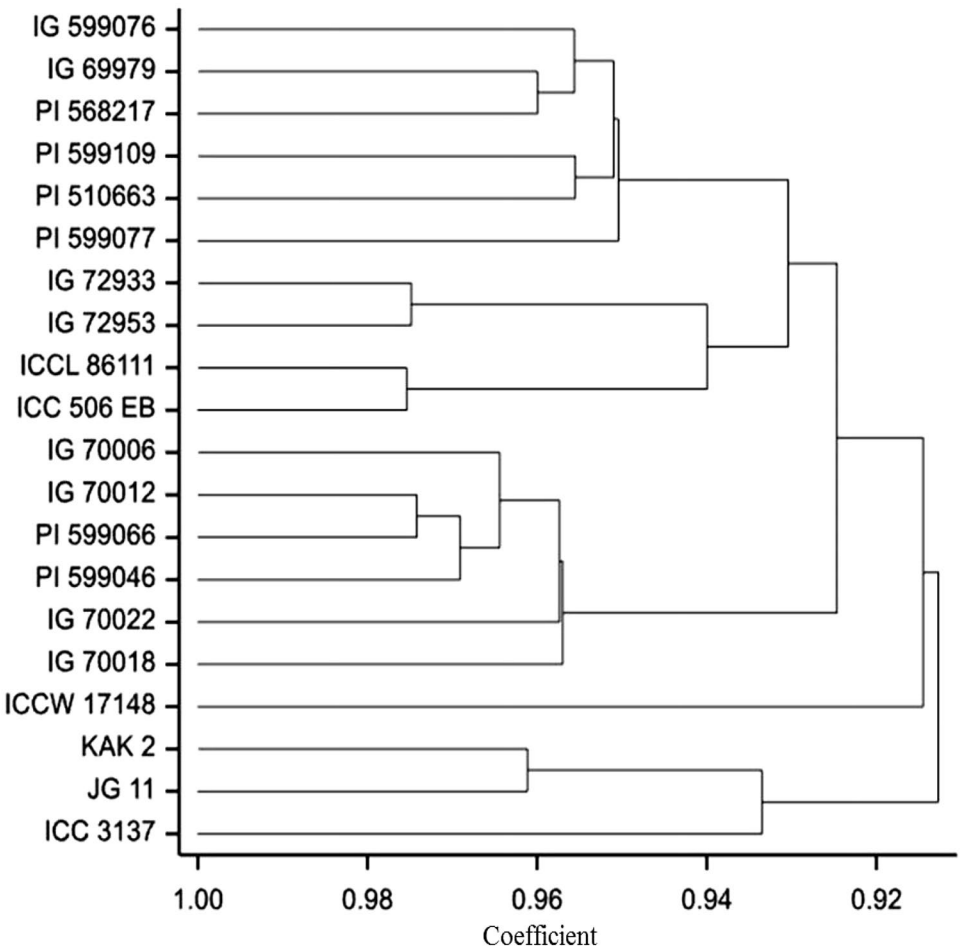

Fig. 1 Dendrogram representing similarities between different accessions of wild relatives of chickpea based on biochemical characters and expression of resistance to *H. armigera*

and reduced fecundity of *H. armigera* as compared to the susceptible checks, KAK 2 and ICC 3137. Higher levels of antibiosis against *H. armigera* in wild relatives as compared to cultigen in terms of reduced survival and delayed developmental periods had also been reported in earlier studies in chickpea (Sharma et al. 2005a, 2006) and pigeonpea (Sujana et al. 2008; Shanower et al. 1997). Narayanamma et al. (2008) also reported that, F_1 hybrids based on resistant genotypes of chickpea recorded lower larval survival, pupation, and pupal weights as compared to the susceptible check, suggesting the transfer of antibiosis mechanism of resistance to the progeny from resistant parents. The studies indicated that antibiosis seems to be the major component of resistance to *H. armigera* in the wild relatives of chickpea, which might be due to higher amounts of plant secondary metabolites or poor nutritional quality.

Significant differences were observed in biochemical composition among different accessions of wild relatives of chickpea. Proteins and phenols showed negative correlation with larval weight, pupation and adult emergence. Phenols also showed a negative correlation with pupal weight, fecundity, but positive correlation with pupal period. However, Kanchana et al. (2005) reported that protein content had positive correlation with pod damage in cultivated chickpea. These differences could be due to presence of higher amounts of protease inhibitors in wild relatives as compared to the cultivated genotypes (Patankar et al. 1999; Parde et al. 2012; Udamale et al. 2013). Protease inhibitors are reserve proteins present in plants that inhibit insect feeding and digestion of the ingested food by the insects (Blanco-Labra et al. 1995). Chickpea protease inhibitors exhibit differential inhibitory activity against *H. armigera* gut proteinases (Giri et al. 1998). The antibiosis effects of protease inhibitors such as extended larval period, reduction in larval weight, survival and adult emergence was observed in *H. armigera* fed on diet with chickpea trypsin inhibitor (Kansal et al. 2008). Higher phenolic content in resistant genotypes as compared to the susceptible ones might contribute to resistance against *H. armigera* (Rupalighodeswar et al. 2003; Kaur et al. 2014). The phenols accumulated in host plant lead to toxicity in insects (Walling 2000; Bhonwong et al. 2009) by increasing the defensive enzyme activity and mediating the transduction pathways, which results in oxidation of toxic substances such as quinines (Maffei et al. 2007; Bhonwong et al. 2009). Several other authors have also reported that total phenols exhibited significant negative correlation with percent pod damage by *H. armigera* (Girija et al. 2008; Sunitha et al. 2008; Anantharaju and Muthiah 2008; Sharma et al. 2009).

Total soluble sugars showed a significant negative correlation with larval period, and positive correlation with pupation and pupal weight, while tannins showed a positive correlation with larval weight, pupation and adult emergence. From these observations, it was evident that, higher concentrations of these components favored better survival and development of *H. armigera*, leading to increased susceptibility of host plant to this pest. Low sugar and high phenol content has also been recorded in the resistant cultivars of pigeonpea against *H. armigera* (Sahoo and Patnaik 2003; Sharma et al. 2009). On the contrary, it is well established that tannins act as feeding deterrents and reduce the survival and development of insects (Bernards and Bastrup-Spohr 2008; Sharma et al. 2009) by precipitating proteins non-specifically, thereby, inhibiting the digestion process (Bernards and Bastrup-Spohr 2008). However, protein binding activities of tannins depend on their chemical structure and other factors such as pH of the gut and concentration of antioxidants (Galati et al. 2002; Hagerman et al. 2003). Tannins not only act as feeding deterrents on the non-adapted insects, but also act as feeding stimulants on the adapted insects (Schultz 1989). Insects exhibit differential response to tannins of the host plant, based on their adoption capabilities to polyphenols (Barbenhenn et al. 2003). Protein and carbohydrate ratio also influences the toxicity of secondary metabolites (Raubenheimer 1992; Simpson and Raubenheimer 2001). While feeding on the plants, the herbivores ingest not only tannins, but also all other biomolecules in the host plant that effect their survival and development. The biological effects of tannins depend on their concentration and structure (Salminen and Karonen 2011).

There were significant differences among the genotypes tested with respect to flavonoid composition. The flavonoid compounds such as ferulic acid, naringin, 3,4-dihydroxy flavones and naringen were completely absent in cultivated chickpea, but were present only in a few wild relatives of chickpea. Wild relatives of chickpea had higher concentration of flavonoids as compared to the cultivated chickpea. Among the wild relatives, *C. bijugum* genotypes had higher concentration than in other species. More numbers of flavonoid compounds was present in IG 70012 and PI 599066 (*C. bijugum*) than in other genotypes. The flavonoid compounds exhibited negative correlations with larval survival, pupation, adult emergence and fecundity, whereas positive correlations were observed with the larval and pupal periods, which resulted in negative effects on survival and development of *H. armigera*. The flavonoid components present in wild relatives of chickpea such as judaicin 7-o-glucoside, 2-methoxy judaicin, judaicin and maakiain showed antifeedant activity, resulting in reduced larval weights of *H. armigera* (Simmonds and Stevenson 2001). Induction of flavonoids such as chlorogenic acid, syringic acid, quercetin, caffeic acid, vanillic acid and ferulic acid was greater in the resistant genotypes of groundnut in response to damage by *H. armigera* (War et al. 2016).The negative effects of flavonoids on insect performance in terms of prolonged developmental period, increased mortality, decreased survival, weight and

fecundity had also been observed in many insect species, including *Acyrthosiphon pisum* Harris (Goławska et al. 2014), *Epirrita autumnata* Borkhausen (Lahtinen et al. 2004; Valkama et al. 2005), *Mamestra configurata* Walker (Onyilagha et al. 2004), *Trichoplusia ni* Hubner (Beninger et al. 2004), *Nipaecoccus viridis* Newstead (Lahtinen et al. 2006) and *Eriosoma lanigerum* Hausmann (Atteyat et al. 2012). The defense compounds of the host plant could act either by decreasing consumption and digestion or by acting as toxins (Scriber and Slansky 1981). Chlorogenic acid binds to the free amino acids and proteins leading to reduction in digestibility of plant tissue (Felton et al. 1992). Caffeic acid andchlorogenic acid caused protein oxidation leading to increased gut toxicity in herbivores (Summers and Felton 1994). The digestive enzyme activities were low in the midgut of *H. armigera* larvae when reared on diet treated with flavonoids such as chlorogenic acid, caffeic acid and protocatechuic acid (War et al. 2013). Though there are several reports on negative effects of flavonoids on the host plant, the exact mechanism by which flavonoids modulate the behavior of insects remains unknown (Simmonds 2003).

Reduced larval survival and weights leads to delay in developmental period due to secondary metabolites or poor nutritional quality. The changes in insect feeding behavior and poor food digestion lead to smaller pupae and reduced fecundity. The slower growth and prolonged development of larvae may also increase the probability of the larvae being subjected to predation and/or parasitism. Hence, the secondary metabolites of the host plant also play an important role in tritrophic interactions (Van Emden 1987). The wild relatives of chickpea with higher levels of antibiosis could be used as diverse sources of resistance to develop cultivars with stable resistance to *H. armigera* for sustainable crop production.

Acknowledgements We thank the entomology staff at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India for their support in carrying out the experiments. The financial support provided by Department of Science and Technology (DST), New Delhi, India, under the INSPIRE Fellowship scheme to Dr Siva Kumar is gratefully acknowledged.

Funding This study was not funded by any organization.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent This is not applicable as this article does not contain any studies with human participants performed by any of the authors.

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