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Effect of different sowing dates on Expression of Resistance to *Helicoverpa armigera* in different Chickpea Genotypes using Detached Leaf assay

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ABSTRACT
Global warming and climate change will have a major bearing on pest incidence and pest associated losses in field crops. Climatic factors will also alter the interactions between the insect pests and their host plants. Therefore, in this study, we used detached leaf assay for evaluating five chickpea genotypes for resistance to Helicoverpa armigera (L) across sowing dates. Leaf feeding and larval weights were greater on the November sown crop, but there were no significant differences in larval survival across sowings. Among the genotypes tested, there were no significant differences in the leaf damage and larval weight, but the larval survival was highest on ICC 3137, irrespective of sowings. Different genotypes behaved differently across sowing dates, suggesting differential effect of climatic factors on expression of resistance to H. armigera.
Keywords: chickpea, climate, *Helicoverpa armigera*, host plant resistance, detached leaf assay.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) also known as Bengal gram or gram, is the second most important food legume in Asia, North Africa, and Mexico. Recently, it has also become an important grain legume crop in North USA, Canada, and Australia. It is grown on 13.5 million hectares worldwide, with an average production of 8.8 million tonnes. India is the largest producer of chickpea in the world sharing 71.0 and 67.2% of the total area (9.6 m ha) and production (8.8 mt), respectively (FAOSTAT, 2013). Several biotic and abiotic constraints limit the production and productivity of chickpea, of insect pests are a major constraint to increase the production and productivity of chickpea (Sharma 2005 and Yadav *et al*., 2006; Sharma et al., 2012). Losses due to insect pest damage are likely to increase as a result of changes in cropping patterns, and global warming. The pod borer, *Helicoverpa armigera* (Hubner), is one of the most important constraints in chickpea production (Sharma, 2005). Its population peaks generally correspond to the full bloom and pod formation stage of the crop in the post rainy season. Climatic factors will also alter selection pressures within populations because most populations are adapted to their local environment. As a result, some of the cultivars that are resistant to insect pests, may exhibit susceptible reaction under climatic variability. Therefore, there is a need to generate information on the likely effects of climate variability on insect pests and genotype interactions to develop robust technologies that will be effective and economical (Sharma, 2010). Therefore, this study was undertaken to study the expression of resistance to *H. armigera* in different genotypes of chickpea across sowing dates.

MATERIAL AND METHODS

H. armigera culture

The larvae of *H. armigera* used in the bioassays were maintained in the laboratory at ICRISAT, Patancheru, Telangana State, India. The *H. armigera* larvae were reared on chickpea based artificial diet (Armes *et al*., 1992) at 27 ± 2°C (Table 1 and Table 2). The neonates were reared for 5 days in groups of

200 to 250 in 200 ml plastic cups having a 2 to 3 mm layer of artificial diet on the bottom and sides of the cup. Thereafter, the larvae were transferred individually to six cell-well plates (each cell-well measured 3.5 cm in diameter and 2 cm in depth) to avoid cannibalism. Each cell-well had a sufficient amount of the artificial diet (7 ml) to support larval development until pupation. The pupae were removed from cell-wells, sterilized with 2% sodium hypochlorite solution (with 4% available chlorine), and kept in groups of 50 in plastic jars containing moistened vermiculite. Upon emergence, 10 pairs of adults were released in an oviposition cage (30 x 30 x 30 cm). Adults were provided with 10% sucrose or honey solution (Girijan Co-operative Ltd., Visakhapatnam, India) on a cotton swab for feeding. Liners having a rough surface, were provided as a substrate for egg laying. The liners were removed daily, and the eggs were sterilized in 2% sodium hypochlorite solution. The liners were then dried and placed inside the plastic cups. After 4 days, the liners were removed. Freshly emerged neonate larvae were used for bioassays using detached leaf assay (Sharma *et al*., 2005).

Table 1. Artificial diet composition

Ingredients	**Quantity**
Chickpea flour	75.0 g
L-ascorbic acid	1.175 g
Sorbic acid	0.75 g
Methyl -p- hydroxy benzoate	1.25 g
Aureomycin	2.875 g
Yeast	12.0 g
Formaldehyde (40%)	1.0 ml
Vitamin stock solution	2.5 ml
Water	112.5 ml
Agar-agar solution	
Agar-agar	4.325 g
Water	200 ml

Table 2. Composition of vitamin stock solution (500 ml).

Ingredients	**Quantity**
Nicotinic acid	1.528 g
Calcium pantothenate	1.528 g
Riboflavin	0.764 g
Aneurine hydrochloride	0.382 g
Pyridoxine hydrochloride	0.382 g
Folic acid	0.382 g
D-Biotin	0.305 g
Cyano cobalamine	0.003 g
Water	500 ml

Detached leaf assay

Five chickpea genotypes (Two resistant ICCL 86111 and ICCV 10, Two commercial JG 11 and KAK 2 and one susceptible ICC 3137) grown in the field across four planting dates at monthly intervals from October

to January were bioassayed under controlled conditions in the laboratory [27 ± 2 ^{0}C; 65 - 75% RH, and photoperiod of 12:12 h. (L : D)]. The experiment was laid out in randomized block design (RBD) with three replications for each genotype, in a plot of four rows with a spacing of 60 cm between rows and 10 cm between plants with in a row. Terminal branches of chickpea (three to four fully expanded leaves/buds) were placed into solidified agar agar (3%) plastic cups (4.5cm x 11.5 cm diameter) (Sharma *et al*., 2005). Agar-agar (3%) was boiled, and 10 ml solution was poured into a 250 ml plastic cup kept in a slanting manner. The solidified agar-agar served as a substratum for holding the chickpea branches. The terminal branches were cut with scissors and immediately placed in a slanting manner into the agar-agar medium. Care was taken to see that the chickpea branches did not touch the inner walls of the cup. Ten neonates of *H. armigera* larvae were released on the chickpea leaves in each cup, and then covered with a lid to keep the chickpea terminals in a turgid condition.

The experiment was conducted in a completely randomized Design (CRD) with three replications for each genotype. The experiments were terminated when >80% of the leaf area was consumed in the susceptible genotype or when there were maximum differences between the resistant and susceptible genotypes (generally at 5 - 6 days after releasing the larvae on the leaves). The plants were scored for leaf feeding visually on a 1 - 9 scale (1 = <10%, and 9 = >80% leaf area consumed). Data were also recorded on larval survival, and weights of the larvae 4 h after terminating the experiment.

Results and Discussion.

***H. armigera* damage**

There were significant differences in the leaf damage by *H. armigera* across sowing dates. During, 2012 - 13, lowest (DR 3.3) damage was seen in the November sown crop. In 2013 - 2014, the lower damage was noticed in October, December and January sown crops, which were on par with each other as compared that on the November sown crop (DR 6.6). Similar trend was observed across seasons. There were no significant differences among the genotypes in 2012 - 13, and across seasons. In 2013 - 14, lowest damage was observed in KAK 2 (DR 3.6) and the highest in ICC 3137 (DR 5.0). The interaction effects were not significant (Table 3).

Larval survival (%)

The larval survival differed significantly across sowings, in both the seasons. In 2012 - 13, highest larval survival was observed in the crop sown in January (82.2%), which was on par with the December sowing (80.7%) and the lowest larval survival was observed in the October (63.6%) sown crop which was on par with the November sowing (64.7%). In 2013 - 14, the highest larval survival was recorded in the crop sown in November (72.0%). Across the seasons, there were no significant differences in larval survival across sowings.

Among the genotypes tested, there were significant differences in larval survival in both the seasons. In 2012 - 13, the highest larval survival was observed on ICC 3137 (77.5), which was on par with ICCV 10 (75.3%), ICCL 86111(73.1%) and KAK 2 (73.9%) and the lowest in JG 11 (64.2%). In 2013 - 14, the highest larval survival was recorded on ICC 3137 (75.8%) and the lowest on KAK 2 (49.2). Across sowings, significantly highest larval survival was observed on ICC 3137 (76.7%), while the other genotypes were on par with each other (Table 4).

Larval weight

There were no significant differences across the sowing dates in the mean larval weight in 2012 - 13. In 2013 - 14, significant differences were observed between the sowing dates, and the highest larval weight was recorded in the November (4.8 mg), and the lowest in the January (1.8 mg) sown crop. Across seasons, the highest larval weight was recorded in the November (3.9 mg), and the lowest in the January (2.3 mg) sown crop.

Among the genotypes tested, the highest larval weight was recorded in ICCV 10 (3.9 mg) and the lowest was recorded in KAK 2 (2.5 mg) in 2012 - 13. In 2013 - 14, and across seasons, there were no significant differences in larval weight among the genotypes tested. Interaction effects were not significant (Table 5).

Leaf feeding and larval weights were greater in the November sown crop, but there were no significant differences in larval survival across sowings. Among the genotypes tested, there were no significant differences in the leaf damage and larval weight, but the larval survival highest on ICC 3137 irrespective of sowings. Shankar *et al*. (2014) observed highest leaf damage and larval weight of *S. exigua on* ICC 3137, as compared to that on ICCL 86111. Earlier reports (Cowgill and Lateef, 1996 and Sharma *et al*., 2005) also revealed the existence of significant influence of chickpea genotypes on larval weight. Leaf feeding by the larvae was significantly lower on ICC 506 than on ICCC 37 when the seedlings were infested with 20 neonates per 5 plants at 15 day after seedling emergence or 10 neonates per three plants at the flowering stage. Larval weights were significantly lower on ICC 506 than on ICCC 37 across growth stages, and infestation levels (Shankar *et al*., 2014). Narayanamma *et al*. (2008) reported that larval weights were significantly lower in larvae reared on leaves/pods of ICC 12475, ICC 12476, ICC 12477 and

ICCV2 as compared to those reared on the susceptible check, ICCC 37. Future studies should focus on simultaneously testing the effects of multiple environmental factors on insect-plant interactions, to gain a realistic perspective of how global climatic changes may impact the production of secondary chemicals and its potential implications for co evolutionary associations between the interacting plant and insect species.

Table 3. Variation in the damage rating of *H. armigera* in different chickpea genotypes across various sowing dates. (2012/13 and 2013/14 post rainy season, ICRISAT, Patancheru, Telangana, India)

Genotype	HDR[1]														
	2012 - 2013					2013 - 2014					Pooled				
	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean
ICC 3137	4.4	3.2	2.7	4.7	3.8[a]	6.3	7.2	4.0	2.7	5.0[b]	5.4	5.2	3.3	3.7	4.4[a]
ICCL 86111	3.6	2.3	5.8	4.3	4.0[a]	3.0	6.7	4.0	3.0	4.2[a b]	3.3	4.5	4.9	3.6	4.1[a]
ICCV 10	4.1	3.8	2.8	3.4	3.5[a]	3.7	5.7	4.3	3.7	4.3[a b]	3.9	4.7	3.6	3.5	3.9[a]
JG 11	2.6	4.2	3.3	3.4	3.4[a]	2.7	7.2	4.7	3.8	4.6[a b]	2.6	5.7	4.0	3.6	4.0[a]
KAK 2	3.6	2.8	5.0	4.0	3.8[a]	3.3	6.3	1.3	3.5	3.6[a]	3.5	4.6	3.2	3.8	3.7[a]
Mean	3.7[ab]	3.3[a]	3.9[b]	3.9[b]		3.8[a]	6.6[b]	3.7[a]	3.3[a]		3.7[a]	4.9[b]	3.8[a]	3.6[a]	
	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)
Genotype (G)	0.334	1.2	0.2	NS	21.5	0.055	2.6	0.3	0.9	26.0	0.634	0.6	0.3	NS	36.9
Sowing (S)	0.083	2.4	0.2	0.6		<0.001	26.7	0.3	0.8		0.003	5.1	0.3	0.8	
G X S	<0.001	5.0	0.5	1.3		0.010	2.7	0.7	1.9		0.159	1.4	0.6	NS	

Figures followed by the same letter within a column and row are not significantly different at P ≤ 0.05.

HDR[1] - Leaf damage rating (1 = <10%, and 9 = >90%).

Table 4. Variation in the larval survival(%) of *H. armigera* in different chickpea genotypes across various sowing dates. (2012/13 and 2013/14 post rainy season, ICRISAT, Patancheru, Telangana, India)

Genotype	Larval survival (%)														
	2012 - 2013					2013 - 2014					Pooled				
	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean
ICC 3137	80.0	64.4	76.7	88.9	77.5[b]	90.0	83.3	63.3	66.7	75.8[c]	85.0	73.9	70.0	77.8	76.7[b]
ICCL 86111	65.6	63.3	80.0	83.3	73.1[b]	56.7	66.7	46.7	46.7	54.2[ab]	61.1	65.0	63.3	65.0	63.6[a]
ICCV 10	61.1	71.1	83.3	85.6	75.3[b]	53.3	73.3	56.7	60.0	60.8[b]	57.2	72.2	70.0	72.8	68.1[ab]
JG 11	46.7	62.2	76.7	71.1	64.2[a]	33.3	73.3	63.3	63.3	58.3[ab]	40.0	67.8	70.0	67.2	61.3[a]
KAK 2	64.4	62.2	86.7	82.2	73.9[b]	60.0	63.3	40.0	33.3	49.2[a]	62.2	62.8	63.3	57.8	61.5[a]
Mean	63.6[a]	64.7[a]	80.7[b]	82.2[b]		58.7[a]	72[b]	54.0[a]	54.0[a]		61.1[a]	68.3[a]	67.3[a]	68.1[a]	
	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)
Genotype (G)	0.034	2.9	3.0	8.6	14.2	<0.001	9.5	3.3	9.3	18.9	0.005	4.0	3.2	9.1	23.9
Sowing (S)	<0.001	14.1	2.7	7.7		<0.001	8.5	2.9	8.4		0.242	1.4	2.9	NS	
G X S	0.408	1.1	6.0	NS		0.007	2.9	6.5	18.7		0.142	1.5	6.5	NS	

Figures followed by the same letter within a column and row are not significantly different at P ≤ 0.05.

Table 5. Variation in the larval weights of *H. armigera* in different chickpea genotypes across various sowing dates. (2012/13 and 2013/14 post rainy season, ICRISAT, Patancheru, Telangana, India)

Genotype	Mean larval weight (mg)														
	2012 - 2013					2013 - 2014					Pooled				
	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean	30th October	30th November	30th December	30th January	Mean
ICC 3137	4.0	3.8	3.5	2.4	**3.4 ab**	4.7	4.3	1.8	1.7	**3.1 a**	4.3	4.1	2.7	2.1	**3.3 a**
ICCL 86111	3.0	2.5	3.5	2.4	**2.8 ab**	2.3	5.4	2.8	1.2	**2.9 a**	2.7	3.9	3.1	1.8	**2.9 a**
ICCV 10	4.6	3.3	2.7	5.1	**3.9 b**	3.1	3.9	3.0	3.2	**3.3 a**	3.8	3.6	2.9	4.2	**3.6 a**
JG 11	4.6	3.0	3.6	2.4	**3.4 ab**	3.0	4.6	3.0	1.2	**2.9 a**	3.8	3.8	3.3	1.8	**3.2 a**
KAK 2	3.5	2.2	2.4	2.0	**2.5 a**	3.7	5.9	3.4	1.5	**3.6 a**	3.6	4.0	2.9	1.8	**3.1 a**
Mean	**3.9 a**	**2.9 a**	**3.1 a**	**2.9 a**		**3.4 b**	**4.8 c**	**2.8 b**	**1.8 a**		**3.6 bc**	**3.9 c**	**3.0 ab**	**2.3 a**	
	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)	Fp	Vr	SE ±	LSD (P 0.05)	CV (%)
Genotype (G)	**0.132**	**1.9**	**0.4**	**1.2**	**43.4**	**0.6**	**0.7**	**0.4**	**NS**	**39.4**	**0.447**	**0.9**	**0.3**	**NS**	**44.5**
Sowing (S)	**0.145**	**1.9**	**0.4**	**1.0**		**<0.001**	**15.4**	**0.3**	**0.9**		**<0.001**	**7.4**	**0.3**	**0.7**	
G X S	**0.637**	**0.8**	**0.8**	**NS**		**0.2**	**1.4**	**0.7**	**NS**		**0.276**	**1.2**	**0.6**	**NS**	

Figures followed by the same letter within a column and row are not significantly different at P ≤ 0.05.

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