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Screening for host plant resistance to *Helicoverpa armigera* (Hubner) in chickpea using novel techniques

Jagdish Jaba[1,2]*, Meena Agnihotri[2] and Snehel Chakravarty[2,3]

College of Agriculture,
Govind Ballabh Pant University of Agriculture and Technology, Pantangar-263 145, Uttarakhand, India.
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ABSTRACT
Insights of antibiosis resistance in various chickpea cultivars against pod borer *Helicoverpa armigera* (Hubner) under laboratory condtions. The present study was carried out in order to investigate the level of resistance against *H. armigera* on different cultivars by adopting detach leaf and pod assay. In leaf detach assay, pooled mean of 2012 and 2013, significantly lower and maximum weight gain and percent gain recorded was 101.9 mg (88.5%); 382.3mg (317.4%), respectively for ICCV 097105 and ICCV 07306. During podding stage, lowest pod damage (%) was recorded 55 % (ICCV 92944) and highest pod damage recorded by ICC 3137, LL 550 and ICCV07306 (95 %).Highest pupal weight of one day old pupae was recorded on LL 550 (405.2 mg) and lower pupal weight in ICCV 097105 (202.4 mg). The present result gave clue for future in depth studies on weeds for developing either specific pheromone compound or repellant compound for the eco-friendly management of *H. armigera*.

Key words: Antibiosis, *Helicoverpa armigera*, Host plant resistance and detach assay.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops in India. Grain legumes are cultivated globally on an area of 70 million hectares, with annual production of over 78 million tons, and an average productivity of 846 kg ha^{-1} (FAO, 2012). In India, the total pulse production for the year 2013 14 was 18.43 million tons from an area of 26 million ha, (Anonymous, 2014). It is an important source of high value protein, and plays an important role in the nutrition of mainly vegetarian and poor people in Asia and Eastern Africa. In chickpea, gram pod borer, *Helicoverpa armigera* (Hubner) is one of the major constraints affecting its production. Pod borers cause an estimated annual loss of over $2 billion in the semi arid tropics, despite application of insecticides costing over $500 million annually (Sharma, 2005). Therefore, it is important to increase the levels of resistance to *H. armigera* in chickpea through host plant resistance, which requires an in-depth understanding of different mechanisms of resistance to the target insect, and precise evaluation of the available sources of resistance for different components of resistance. Assessment of antibiosis component of resistance on fresh plant parts under laboratory conditions is influenced by possible changes in the relative amounts of primary and secondary plant metabolites. Therefore, we evaluated a detach leaf/pod bioassay technique to assess for rapid screening of cultivars against *H. armigera* under lab conditions.

MATERIALS AND METHODS

The cultivars of chickpea were bio-assayed for resistance to *H. armigera* under no-choice conditions using the detached leaf and pod assay (Sharma *et al.,* 2003). The plants were grown in plastic pots (30 cm diameter, 30 cm deep) in the greenhouse. The pots were filled with a potting mixture and farmyard manure (2:1:1). The seeds were sown in pots, the plant were watered regularly. There were five plants for each cultivar. The pots were arranged in a completely randomized design.

The terminal leaf branches during vegetative and podding stages were evaluated for resistance to second and third instar larvae of *H. armigera* using the detached leaf and pod assay respectively. The chickpea branches were cut with scissors, and immediately placed in a slanting manner into 3% agar-agar medium in a 50-ml plastic petri plate . There were four replications for each cultivars in a completely randomized design. The larvae of *H. armigera* raised in the laboratory (Sharma *et al.,* 2001) were released on the chickpea leaves. The petri plates were kept in the laboratory at 27±2 *C, and 45–65% relative humidity. For detach leaf assay release ten larvae/replications and for pod assay release of single third- instars larvae to mature pods

*Corresponding author's e-mail: jaba.jagdish@gmail.com
[1,2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Telangna, India.
[2]Department of Entomology, College of Agriculture, G.B.Pant University ofAgriculture & Technology, Pantnagar, Uttarkhand, India
[3]Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India.

for each replications. Observations were recorded at five and three days after larval release respectively for leaf and podding stage assay, when the differences between the resistant and susceptible checks were maximum. First, the plants were rated for leaf feeding on a 1–9 scale (1 =< 10% leaf area damaged, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, and 9 => 80% leaf area damaged). The number of larvae surviving after the feeding period were recorded, and placed in 25 ml plastic cups. The weights of larvae were recorded pre and post feeding of food. The data were expressed as percentage larval survival and mean weight of the larvae. Data on leaf damage rating, larval survival, and larval weights were used to compute resistance (host suitability) index for each cultivars. For this purpose, larval weight (representing weight gain by the larvae) were expressed as a function of food consumed per larva [damage rating/number of larvae survived] .For pod assay data were recorded on initial weight of the larva, weight of the larva after the feeding period, and percentage pods damaged at 5 days after infestation. The weight gained (in percentage) by the larvae were computed as follows

Weight gain (%) =

$$\frac{\text{(Final weight of the larva – Initial weight of the larva)}}{\text{Final weight of larva}} \times 100$$

The values denoted poor host suitability or high resistance, while high values indicated better host suitability or susceptibility to the pest.

RESULTS AND DISCUSSION

Evaluation of cultivars of chickpea at the vegetative and podding stage for resistance to *H. armigera* by – detached leaf/pod assay under laboratory condition during 2012 and 2014: The results of pooled detach leaf assay for the year 2012 and 2013 are presented here. The 2nd instars of *H. armigera* larvae when fed on chickpea branches during vegetative stage using detached leaf assay, the damage rating DR ranged between 4.87- 8.75. The greater leaf feeding was observed on the check cultivar L550 (DR 8.75) followed by ICCCV 07306 (DR 8.25) and ICCV 07104 (DR 7.87). Significantly lower leaf feeding was observed on the ICCV097105 (DR 4.8) followed by ICCV 92944 (DR 5). The unit initial larva weight ranged between 120.9mg (ICCV 7112) - 186.7mg (L 550) (Table 1).The final weight gain by larvae ranged between 101.9mg to 382.3mg. The significantly lower weight gain and per cent gain was recorded in ICCV 097105 101.9 mg (88.5%) followed by 136.7 mg (105.7%) in ICCV 8108 and 145.4 mg (110.9%) in ICCV08107. The maximum weight gain and per cent gain was observed in ICCV 07306 with 382.3 mg (317.4%) followed by 329.3 mg (277.7%) in ICC3137, 313.4 mg (263.9%) in 5034 when compared to commercial check cultivars L550 and PG 186 with 278.5mg(193.6 %), 200.2mg(160.3%) respectively.

The pooled results of 2012 and 2013 for antibiosis studies of podding stage are presented meticulously below. During the podding stage, when a single third-instar larva

Table 1: Evaluation of cultivars of chickpea at the vegetative for resistance to *Helicoverpa armigera* – detached leaf assay (2012 and 2013)

Cultivars	Initial weight(mg)	Final weight(mg)	Weight gain(mg)	Damage rating	Weight gain(%)
ICCV 09103	121.3	294.2	173	6	157.1
ICCL86111	145.7	322.1	166.1	5.25	174.6
ICCV09115	142.7	389.4	280.9	7.125	227.2
ICCV08108	162.6	299.1	136.7	6	105.7
ICCV97105	126.7	221.2	101.9	4.85	88.5
ICCV07306	134.3	516.5	382.3	8.25	317.4
ICCV92944	158.8	350.3	192.7	5	134.4
JG 11	175	326.2	151.3	6.75	126.4
ICCV07112	120.9	390.1	269.3	7.25	272.6
ICCV08107	162.6	307.9	145.4	5.75	110.9
ICCL 86105	123.7	303.4	179.8	6.25	161
D 059	142.4	358.9	240.3	6.625	212.7
5034	155.2	468.5	313.4	7.25	263.9
ICC3137	126.7	455.9	329.3	8	277.7
ICC14872	146.2	387.3	283.1	6	302.1
ICC14364	182	481.7	299.8	7.125	236.4
ICCV07104	142.7	404.1	261.5	7.875	225.2
ICCV09118	160.3	384.9	215.8	7.75	162.6
ICCV10	130.7	322	192.9	6.25	173.1
ICCV95334	170.3	338	177.5	6.625	111
PG186	162	362.1	200.2	5.625	160.3
L550	186.7	465	278.5	8.75	193.6
SE±	63.14	75.52	97.78	0.9761	122.1
LSD (5%)	62.37	78.16	101.9	0.9643	127.3

was released on chickpea branches with young pods, the per cent pod damage ranged between 55 % to 95 % The lowest pod damage was recorded in ICCV92944 (55 %) and highest pod damage was recorded in ICC 3137, L550 and ICCV07306 with 95 %. The greater pod feeding was observed on the cultivars L 550, ICC 3137 and ICCV 07306 (DR 8.00). Significantly lower pod feeding was observed on the ICCL 86111, ICCV 092944, ICCV 097105 and ICC 14872 (DR 5) .The unit initial larva weight ranged between 114 mg (ICCV 08108) to 357 mg (L 550) (Table 2). Significantly lower weight gain was recorded in ICCV 08108 (114 mg). The maximum weight gain was observed in L 550 with 357 mg when compared to commercial check cultivars PG 186 with 195.3 mg gain. Highest pupal weight was recorded on L 550 (405.2 mg) while ICC 14872 and 5034 recorded the lowest pupal weight (204.1 and 240.1 mg) compared to susceptible check. L 550 (362.7 mg)

The results of both experiments are in confirmation with Sharma *et al.,* 1997 who revealed that it is difficult to identify stable sources of resistance under natural infestation. The results are in partial accordance with Narayanamma *et al.,* 2013 who reported that, lower leaf feeding was observed on the resistant check, ICC12475. Survival rate and larval weights were lowest on the resistant check, ICC 12475 suggesting that antibiosis is one of the components of resistance to *H. armigera* in chickpea. Brar and Singh. 2015 confounded that, the chickpea genotype GL 25016 recorded lowest leaf damage score of 2.0, lowest larval survival was found on RSG 963 (53.33%). The minimum larval and pupal weight of 1.01 and 0.156 g was also observed in genotype 5282.

Sharma *et al.* (2005) reported the antibiosis resistance mechanism to pod borer, *H. armigera* in wild cultigens of chickpea. The accessions ICC 17257, 1G 70002, IG 70003, IG 70012 *(Cicer bijugum*), IG 69948 (*C. pinnatifidum*), IG 69979 (*C. cuneatum*), IG 70032, IG 70033, IG 70038 and IG 72931 (*C. judaicum*) showed lower leaf feeding, drastic reduction in larval weight and pupal weight which showed poor host suitability index at the vegetative / podding stages of crop growth as compared to the cultivated chickpeas. The percentage pods damage recorded < 52 % pods damaged compared to 90 % pods damaged in Annigeri . The detached leaf assay not only gives an idea of the relative feeding by the larvae on different cultivars but also provides useful information on antibiosis component of resistance in terms of larval weight (Sharma *et al.,* 2005).Therefore, efforts should be made to establish a clear relationship in terms of survival and development of *H. armigera* on the fresh plant parts, and overall expression of same cultivars for resistance to *H. armigera* under field conditions.

Table 2: Pooled relative pod damage and weight gain by the third-instar larvae of *Helicoverpa armigera* on chickpea cultivars (2012 and 2013)

Cultivars	Initial weight (mg)	Final weight (mg)	Weight gain (mg)	Pupal weight (mg)	Number of Pod damage	Pod damage (%)	Damage rating
ICCV 09103	146.2	380.1	233.9	354.3	4.1	82.5	7
ICCL86111	180.4	372.5	180.8	261.5	3.4	67.5	5
ICCV09115	138.0	264.7	126.7	283.8	4.3	85	7
ICCV08108	135.3	249.3	114.0	305.3	3.5	70	6
ICCV97105	128.3	248.5	120.6	202.4	3.5	70	5
ICCV07306	162.1	340.1	210.3	332.3	4.8	95	8
ICCV92944	139.0	304.4	165.8	259.4	2.8	55	5
JG 11	128.2	296.5	167.8	263.2	4.5	90	7
ICCV07112	133.8	359.6	225.8	295.0	3.8	75	7
ICCV08107	127.6	389.4	261.8	275.2	4.0	80	6
ICCL 86105	156.4	293.1	166.5	255.1	4.5	90	7
D 059	135.7	319.4	183.8	289.1	4.3	85	6
5034	165.7	397.8	232.1	240.7	4.5	90	7
ICC3137	129.8	470.8	341.0	290.5	4.8	95	8
ICC14872	135.0	373.3	240.9	204.1	2.8	55	5
ICC14364	125.4	348.7	223.2	362.7	4.3	85	7
ICCV07104	134.7	420	285.3	334.1	4.5	90	7
ICCV09118	129.9	376.8	246.8	309.4	4.5	90	7
ICCV10	147.9	353.6	205.7	242.8	4.3	85	6
ICCV95334	172.1	334.7	162.5	276.1	4.0	80	6
PG186	135.7	328.1	195.3	279.4	3.4	62.5	6
L550	139.6	496.6	357.0	405.2	4.8	95	8
SE±	34.51	36.93	42.27	47.53	0.97		0.97
LSD (5%)	34.09	37.82	43.56	49.43	0.96		0.96

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