Pak. J. Bot., 43(2): 1265-1270, 2011.

IDENTIFICATION OF SUSCEPTIBLE AND TOLERANT GRAM (*CICER ARIETINUM* L.) GENOTYPES AGAINST GRAM POD BORER (*HELICOVERPA ARMIGERA*) (HUBNER)

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Abstract

The use of crop varieties resistant or tolerant to insect pests stress is an imperative approach in non-chemical crop protection. In the presented studies, 16 genotypes of gram (*Cicer arietinum* L. were utilized for field assessment against gram pod borer (*Helicoverpa armigera* Hubner) (Lepidoptera: Noctuidae) to evaluate their genotypic differences. Results on identification of susceptible and tolerant genotypes observed significant differences among the genotypes tested for same traits under consideration. Based on the data for larval population, percentage pod damage and yield components, genotypes CM 2100/96 and CM-4068/97 were relatively resistant, while, lines No. 96051 and PBC-2000 susceptible against preference of the *H. armigera* in contrast to other genotypes. Oviposition activities of moth were also monitored to trace the eggs of pest on gram, weeds and crops growing around the experimental field. It is suggested that gram genotypes located as sources of tolerance for *H. armigera* deserve due attention for resistance breeding strategies, if gram has to remain a viable crop.

Introduction

Pulses have a stretched and imperative history in world's agriculture because of their contributions to the diets of millions of people worldwide, and their inclusion as major feed ingredients in better animal production. Despite the ongoing efforts, the productivity in Pakistan for the major pulse crops is far below than the global averages, largely due to persisting problems of pest. The stumbling blocks in pulse crops appear to be the yield losses due to insect pests. Gram pod borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is a serious pest of many important crops and asserts a major share in crop losses every year, worldwide (Sharma *et al.,* 2005). It is a polyphagous pest of 181 plant species, including gram, pigeonpea, tomato, okra and cotton, and is expected to become an important pest on other crops such as sorghum, pearl millet, maize, tobacco, and groundnut (Manjunath *et al*., 1989). This pest starts infesting the shoot tips few weeks after crop emergence and feeds on buds, flowers and pods till harvesting, causing heavy yield losses. Larvae of *H. armigera* are voracious foliar feeders as early instars and later shift to the developing seeds and fruits leading to drastic reductions in yield (Reed & Pawar, 1982). The pod borer, *H. armigera*, is the most serious pest which causes high economic losses to the chickpea crop (Singh & Yadav, 2006; Sarwar *et al*., 2009).

This pest is perennial and persistently causes losses to the gram crop, and the commercial chickpea is an important source of *Helicoverpa* spp. populations (Sequeira, 2001). Farmers are unable to control this pest to desired level in spite of spending millions of dollars on pesticides. With the increasing pesticide utilizations, *H. armigera* is exhibiting resistance towards a wide range of insect killers (McCaffery *et al*., 1991). Therefore, the necessity to design future pest management strategies for controlling this pest becomes more apparent. Further, with increase in pest problems and resultant non judicious use of pesticides, there are concerns of environmental problems and ecological imbalance (Reddy & Zehr, 2004). The high input demands require that we should look at new technologies to be deployed to improve and sustain productivity. One of the most important crop improvement goals has been the enhancement of tolerance to biotic stresses and the development of resistant cultivars as means of pest control. In realizing this, the objectives of present work were screening and evaluating the response of 16 gram accessions for resistance on the basis of their inhibition potential toward gram pod borer. As diversified crop habitat can reduce the pest population on the main crop by sidetrack burden on other minor plants, study was also undertaken to recognize crop species that could serve as alternate hosts for oviposition of *Helicoverpa* adult moth.

Materials and Methods

These varietals ranking of gram genotypes against gram pod borer *H. armigera* were performed at the experimental farm, Nuclear Institute of Agriculture, Tandojam. Screening was conducted for two growing seasons (2002-2003 and 2003-2004) to assess the genotypic differences for pest damage and yield. Sixteen genotypes of *Cicer arietinum* used for field evaluation were acquired from the germplasm collections held at the Nuclear Institute of Agriculture and Biology, Faisalabad, and seed samples were taken from varietals trial plots. The genotypes evaluated were: CM-2100/96, CM-4068/97, CM-3021/97, CM-3000/97, CM-3837/97, CM-4212/97, CM-1441/98, CM-1223/98, CM-1991/94, CM-1463-2/94, CM-2000, BITTLE-98, No. 96052, No. 96051, PBC-2000 and CM-98. The experiment was performed after application of a pre-sowing irrigation with water drawn from a tube-well; the land was ploughed, leveled and bunded to make plots each measuring 3 m^2, separated from each other by a 1-m wide path. During November of each year of the experiment, genotypes were sown by hand drill in lines within 3 m x 1 m plot spaced with 30 cm between rows and 10 cm between plants within rows. Seeds of each genotype were sown in randomized block design, with three replicates of each treatment. In each plot, genotypes were randomized to minimize any effects of variations within the field. No plant protection measures were taken at all to prevent insect attack on plants. After crop emergence, no irrigation water was applied in the field. Customary agronomic practices were followed for raising the crop.

Differences in genotypes tolerance due to differential pod borer's losses were recorded twice a month. For assessing of data on insect population from three rows, 5 plants were randomly chosen in each treatment from the outer or inner rows, at the seedling stage after 30 days of crop emergence. The observations recorded were number of larval population per plant from leaves and inflorescences. At crop maturity, data were also recorded on % pod damage due to pod borer by counting the number of healthy and damaged pods in samples taken at random. As a part of the trial, samplings for oviposition of *Helicoverpa* were made at vegetative, flowering and grain filling stages of the gram. Oviposition activities of moth were further monitored to trace the eggs of pest on weeds and other crops growing around the experimental field. After the crop full-fledged, plants were harvested, threshed and finely weighed. All the rows within a replicate were gathered for quantifying grain yield per plot. Statistical analysis of results of the field experiments was scrutinized by ANOVA using a randomized block design package.

Results and Discussion

Results on varietals ranking of tested genotypes depicted that all the genotypes showed variable responses to the traits under observations (Tables 1 & 2). Among all the included 16 selections, CM-2100/96 was observed to be the most stable for the combinations of selected criterion; on the other hand, No. 96051 appeared cumbersome as far as possible, because of being highly affected in its survival for insect pest. In first growing season 2002-2003, pod borer larval numbers were 2.25-5.33 per plant in the investigated genotypes. On the observation of % pod borer damage, it was found 10.53-39.14% in samples collected at random. Grain yield per 3 m^2 plot was 423.3-38.33 gm in all genotypes. In the second year observations for season 2003-2004, the differences in the susceptibility of the test genotypes were not greater, but the trends were 0.85-3.04 larval numbers per plant, 15.96-36.87% pod damage in samples taken at random, and 165.0-310.0 gm grain yield/ 3 m^2

Based on the both years differences in resistant and susceptible scores among genotypes, results were highly significant (Table 2). Genotypes CM-2100/96, CM-4068/97 and CM-1223/98 had numerically less numbers of larvae (1.55, 1.81 and 1.96) per plant than the other lines. The differences in larval number were large (4.18, 3.80 and 3.41 per plant) in No. 96051, PBC-2000 and CM-1463-2/94. Pod borer damaged pods were lower (13.24, 14.41 and 16.74%) in CM-2100/96, CM-4068/97 and CM-1223/98, while the numbers were greater (38.00, 36.00 and 32.87%) in No. 96051, PBC-2000 and CM-1463-2/94 respectively, as compared with left behind selections. Amongst these genotypes, CM-2100/96 and CM-4068/97 yielded 366.7 gm and 330.0 gm seeds per plot (1222.3 and 1100.0 kg/he) as compared with 101.7 gm and 116.7 gm (339.0 and 389.0 kg/he) in No. 96051 and PBC-2000 respectively. The other lines were intermediate with less or more significantly different pests incidence within both susceptible and resistant genotypes, and the results were consistent in both years with slight changes. For simplicity, distribution of lines from resistant to susceptible scores, could be determined as: CM-2100/96, CM-4068/97, CM-1223/98, CM-98, CM-3837/97, CM-3021/97, CM-3000/97, CM-4212/97, CM-2000, No. 96052, CM-1441/98, CM-1991/94, BITTLE-98, CM-1463-2/94, PBC-2000 and No. 96051, although some of the differences were not significant statistically. In general, all the lines took a time of 120 days to mature under non-sprayed conditions.

The larval counts and percentage of pod damage in the tolerant selections like CM-2100/96 in comparison with susceptible No. 96052 indicated that resistant lines recovered quickly from initial floral damage to produce a second flush of flowers. Further, it seems that after this initial damage in the resistant lines, the pest larvae were unable to cause significant damage to the new flowers and young pods. Conversely, in the susceptible lines, pest continued to cause higher flower and pod injury that not only resulted in crop losses but also reduced yield.

Several factors probably contributed to the observed lack or increase in resistance of gram genotypes to pest. Thus, our results and those of Sharma *et al*., (1999) suggest that the legume pod borer resistant reaction in legume is conditioned by a combination of factors such as oviposition, antibiosis and tolerance. They also reported that larvae reared on the resistant line had significantly lowered larval and pupal mass than those reared on the susceptible. The fecundity of the females emerging from the larvae reared on the pods of resistant genotypes was also low (43.4 eggs) compared with that of the susceptible control (100.6 eggs). Tolerance is clearly indicated in this study by the high level of recovery from pod borer damage in the resistant selections because of a yield compensation mechanism conditioned by a second flush of flowers and the resistant lines showed clear non-preference for oviposition. For a more complete understanding of resistance, additional studies are needed to relate oviposition, larval density, pod damage, recovery and seed yield.

Table 1. Screening of different gram genotypes against pod borers during 2002-2003 & 2003-2004.

Genotype	Larval population/ plant	Pods infestation (%)	Yield/ Plot (3 m^2) (gm)	Larval population/ plant	Pods infestation (%)	Yield/plot (3 m^2) (gm)
	2002-2003			2003-2004		
CM-2100/96	2.25 i	10.53 k	423.3 a	0.8533 l	15.96 i	310.0 a
CM-4068/97	2.58 hi	11.04 k	365.0 b	1.047 kl	17.78 i	295.0 ab
CM-3021/97	3.50 def	16.68 ghi	261.7 e	1.757 fghi	26.02 ef	240.0 def
CM-3000/97	3.83 cde	17.10 gh	236.7 f	1.663 ghi	24.82 fg	250.0 cde
CM-3837/97	3.25 efg	15.61 hi	278.3 de	1.520 hij	24.17 fg	250.0 cde
CM-4212/97	4.16 cd	24.33 e	146.7 h	1.473 ij	23.01 g	260.0 cd
CM-1441/98	4.00 cd	17.84 g	188.3 g	1.900 efgh	27.00 e	240.0 def
CM-1223/98	2.75 ghi	13.48 j	315.0 c	1.187 jkl	20.01 h	280.0 abc
CM-1991/94	4.25 c	22.10 f	146.7 h	2.090 cdef	30.68 d	215.0 fg
CM-1463/94	4.50 bc	30.56 c	85.00 i	2.377 bc	35.18 ab	190.0 ghi
CM-2000	3.91 cd	18.17 g	176.7 g	1.950 defg	30.15 d	220.0 efg
BITTLE-98	4.41 c	28.03 d	100.0 i	2.233 bcde	32.95 c	200.0 gh
No. 96052	4.16 cd	27.76 d	101.7 i	2.327 bcd	34.24 bc	200.0 gh
No. 96051	5.33 a	39.14 a	38.33 k	3.043 a	36.87 a	165.0 i
PBC-2000	5.08 ab	36.03 b	63.33 j	2.520 b	35.97 ab	170.0 hi
CM-98	3.08 fgh	15.13 ij	291.7 d	1.373 ijk	20.83 h	270.0 bcd
LSD value	0.59	1.73	18.58	0.380	2.017	28.70

Table 2. Pooled data indicating screening of different chickpea genotypes against pod borers (winter 2003 & 2004).

S. No.	Genotype	Larval population/ plant	Pods infestation (%)	Yield/plot (3 m^2) (gm)	Yield/ Hectare (Kg)
1.	CM-2100/96	1.55 k	13.24 k	366.7 a	1222.3
2.	CM-4068/97	1.81 joke	14.41 k	330.0 b	1100.0
3.	CM-3021/97	2.62 fg	21.35 hi	250.8 ef	836.0
4.	CM-3000/97	2.74 fg	20.96 hi	243.3 f	811.0
5.	CM-3837/97	2.38 gh	19.89 i	264.2 de	880.6
6.	CM-4212/97	2.82 ef	23.67 fg	203.3 g	677.6
7.	CM-1441/98	2.95 def	22.42 gh	214.2 g	714.0
8.	CM-1223/98	1.96 ij	16.74 j	297.5 c	991.6
9.	CM-1991/94	3.17 cde	26.39 e	180.8 h	602.6
10.	CM-1463-2/94	3.43 c	32.87 c	137.5 i	458.3
11.	CM-2000	2.93 def	24.16 f	198.3 g	661.0
12.	BITTLE-98	3.32 cd	30.49 d	150.0 i	500.0
13.	No. 96052	3.24 cd	31.00 d	150.8 i	502.6
14.	No. 96051	4.18 a	38.00 a	101.7 j	339.0
15.	PBC-2000	3.80 b	36.00 b	116.7 j	389.0
16.	CM-98	2.22 hi	17.98 j	280.8 cd	936.0
	LSD value	0.353	1.409	17.37	

Around the experimental area, *Brassica* species, trees, weeds and wheat were growing, but the eggs of *Helicoverpa* were found on gram plants after 2 week of seedling emergence, and continued till vegetative and flowering stages. Conversely, the scarcity of eggs on the crop was observed after flowering and grain filling stages of the gram, suggesting that it was not much attractive at crop maturity period in relation to the other crops in the study area. After that period, some oviposition activities of moth were observed on *Brassica* crops especially canola. These results showed that this discrepancy attractiveness could be utilized to manage control operation for this pest. Still much work needs to be done to fully understand the observed patterns of host plant selection. Patterns of egg distribution indicated by differential oviposition activity are end results of the host selection process (Fitt, 1991). This host selection process in *Helicoverpa* spp., was influenced by a large number of factors, including plant species, plant height and plant physiological stage (Jallow & Zalucki, 1996). An additional possible cause for the observed oviposition response was the chickpea foliar secretions containing high concentrations of malic acid (Rembold, 1981). The amount of foliar exudates and the concentration of malic acid depend on temperature and growth stage, and have been shown to increase during the reproductive stages of the plant (Koundal & Sinha, 1981). Whilst moths were drawn to chickpea in all growth stages, there was relatively less oviposition activity and damage in resistant cultivars that secrete high concentrations of malic acid (Reed *et al.*, 1987). Similar to our results, Shah & Shahzad (2005) monitored the seasonal changes in the population of *H. armigera*, data revealed that the pest population was low initially during 4 to 6 standard weeks, but increased from 7 standard week to onwards and declined again during 14 standard week. Our results correspond to all these earlier researchers. But, Mandal (2005) observed 5%-15% pod damage due to pod borers than our judgment from 13.24% to 38.00% damage due to variable pest incidence.

These results provide the basis for the selection and incorporation of gram resistance sources and present an optimized blending for developing *H. armigera* resistant plants. The promotion of such resistant lines for general cultivation may also help in improving yield and stability of gram in farmers' fields. Further evaluation of these genotypes under diverse environments, however, is essential to establish the stability of the resistance. New biotechnology tools are providing new levels of protection against certain pests and diseases. Such biotechnology is offering unique opportunities to produce plants with desired resistance traits, which had been difficult to achieve using conventional techniques. Sarmah *et al*., (2004) through polymerase chain reaction confirmed the presence of the transgene (Bt-cry1Ac gene) in primary transgenic plants and in their progenies in chickpea to confer resistance against pod borer. Sharma *et al*., (2005) suggested that wild relatives of chickpea show high levels of antibiosis to *H. armigera* and can be used to introgress diverse resistance genes into cultivated chickpea to increase the levels and diversify the basis of resistance to this insect. So, genetically modified crop can provide substantial benefits to the farmers by providing enhanced protection against such pests.

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(Received for publication 22 April 2009)