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Measurement of antibiosis in maize (*Zea mays)* genotypes against pink stem borer (*Sesamia inferens)*

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ABSTRACT

Study of antibiosis is used to identify the sources of resistance in plant germplasm. The quantitative determination of six parameters of antibiosis was carried out in 20 genotypes of maize *(Zea mays* L.*)* against *Sesamia inferens* (Walker). The number of larvae recovered from 30 plants of each genotype at three sampling intervals (7, 14 and 21 days after artificial infestation with neonates) was maximum (39) from WNZPBTL6 and minimum (13) from HKI 1040-11-7 and Basi local. The larval developmental period was found to range from 26-32 days. The expression of antibiosis in terms of prolonged larval period was observed in AEB(Y) C5 55-1 (32 days), PFSR S3 (31 days), CM 202 (31 days), and HKI PC4B (31 days), whereas shortest development period of 26.5 days was recorded in Basi local. Maximum and minimum pupal development period was recorded for AEB(Y)C5 34-7 (10 days) and AEB(Y) C 5-55-1 (7 days) respectively. Strong antibiotic effect of genotype E 30 was observed in terms of the least larval weight (20.53mg). The genotypes Basi local and HKI 164-4-ER-3 did not show much antibiosis as reflected from their maximum larval weight 48.32mg and 46.50mg respectively. The pupal weight ranged from 76.36-120.35 mg in the test genotypes. The leaf feeding symptoms in terms of leaf injury rating (LIR) at 21 days after infestation on a scale of 1 to 9, varied form 4.33-9. Positive correlations were observed between larval weight and LIR (0.52); pupal weight and LIR (0.20); pupal weight and larval recovery (0.27), whereas negative correlation were observed between larval weight and larval period (-0.49); larval weight and larval recovery (-0.46); larval period and pupal weight (-0.40); and LIR and larval recovery (-0.21). The cumulative susceptibility level of genotypes indicated significant variation among genotypes against *Sesamia* which can be used to unravel the basis of resistance.

Key words: Antibiosis, Maize genotypes, *Sesamia inferens,* Susceptibility level,

Pink stem borer [*Sesamia inferens* (Walker)] is the key pest of maize (*Zea mays* L.), which is predominant throughout the year particularly in the peninsular India. It also causes extensive damage to the maize crop in several northern states during *rabi* (winter) season. The losses due to *S. inferens* in *rabi* season varies from 25.7 to 78.9 percent with an estimated annual loss of ₹110.5 million in India (Sekhar *et al.* 2004). Thus the effective control of pink borer might reduce the crop loss significantly in *rabi* maize across the country, and in *kharif* and *rabi* maize in peninsular India. Use of insecticides for stem borer management is uneconomic under subsistence farming, and is largely beyond the means of poor farmers of India. Therefore, host plant resistance (HPR) assumes a pivotal role in limiting the loss caused by stem borer. Breeding for host plant resistance holds immense potential in controlling this pest, in a durable and ecologically sustainable manner. An essential pre-requisite of resistance breeding is characterization of resistance factors and underlying mechanism. Knowledge of resistance mechanism and its associated factors is essential for effective utilization of resistant sources in crop improvement programme.

Antibiosis is one of the major mechanisms of resistance in maize along with antixenosis and tolerance (Painter 1951). In antibiosis, the biology of the insect is affected which leads to reduced longevity, reproduction and increased insect mortality (Munyiri *et al*. 2013). In antibiosis type of resistance, the biology of the insect is affected leading to reduced longevity and reproduction, and increased insect mortality. Antibiosis decreases larval development as well as the number of larvae per plant, thereby decreasing the stem damage levels (Pimentel 2002). All three parameters of resistance have been identified in stem borer resistant maize. Maize inbred lines are an important source for studies in genetics and breeding superior maize hybrids. Maize genotypes have been shown to have the greatest diversity of genetic structure and are thus expected to hold a wide variety of resistance mechanisms (Liu *et al*. 2003). Keeping in view the importance of genotype variability in maize,

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some of the biological parameters related to antibiosis were studied for *S. inferens,* which can be further manipulated and incorporated in the breeding programme. Broadening the genetic pool for maize breeding through identification of sources of resistance will form an important source of diversity for breeding against the pink stem borer damage in *rabi* maize.

MATERIALS AND METHODS

The nucleus culture of *S. inferens* was collected from National Dairy Research Institute, Karnal, and village Atterna near Sonepat, Haryana, India. The culture was multiplied in the Entomology Laboratory, Indian Institute of Maize Research. It was maintained at a temperature of 26 ± 2.0°C and relative humidity of 65 ± 5%. The field collected larvae were reared on fresh maize stalk and baby corn till pupation. After emergence, the male and female adults in equal numbers were released on potted maize plants (7-12 day old), kept in versatile insect rearing cage (VIRC) (Kumar *et al*. 2011) for egg laying. The larvae were reared on artificial diet (Siddiqui *et al*. 1977) from second generation onward.

Maize genotypes

The following twenty maize genotypes were procured from Winter Nursery Centre, Hyderabad to study their antibiotic effects on *S. inferens*.

Code No.	Pedigree	Code No.	Pedigree
1	E4-C	11	HKI PC4B
2	E5-O	12	HKI 1040-5
3	E9-B	13	HKI 1040-11-7
4	E 30	14	Hyd05R/2-1
5	E37-A(O)	15	HKI C323
6	E57(0)	16	HKI 164-7-4 ER-3
7	WNZPBTL 6	17	PFSR-S3
8	E 60(FC)O	18	PFSR-R9
9	AEB(Y)C5 55-1	19	Basi local
10	AEB(Y)C5 34-1	20	CM-202

The experiment consisted of 20 treatments/genotype replicated thrice and five plants constituted one replication. Plants were grown under greenhouse conditions (28°C/24°C day/night and 16 hr/8 hr light/dark) with five plants per pot (30 cm top diameter, 28 cm height, 14.5 L volume) in soil, coco peat, vermiculite 2:1:1mixture with no additional fertilizer. Twelve days after germination, five neonates were released on each plant into the the first leaf sheath simulating natural infestation. Plants were sampled on 7, 14 and 21 days after infestation (7, 14, 21 DAI) to recover the larvae. The number of recovered larvae and their weight was recorded. The number of larvae recovered on dissection at 7th and 14th day after infestation were weighed and recorded. A few plants died before taking second observation on 14 DAI. Leaving 12 plants/genotype, for third observation, remaining plants were dissected on 14 DAI. Visual grading based on 'Leaf Injury Rating' (LIR) was done in the 1-9 scale (1implies - healthy plant, and 9 a dead heart) on 21DAI as standardized by Rao *et al*. (1983). After taking observations for LIR, the plants were dissected in the laboratory to recover the developing larvae. The recovered larvae were collected and weighed individually. These larvae were then reared on baby corn till pupation and further observations on larval period, larval weight, pupal period and pupal weight were recorded. The data was computed for only ten plants for each sampling interval since the number of plants in each sampling was not uniform. The data on larval recovery from infested plants, larval weight, leaf injury rating, larval and pupal development period, pupal weight and pupal period were transformed on common scale of 0-100 using the formula [{(X-Xmin)/(Xmax-Xmin)}*100]. The Pearson's correlation coefficients of the above parameters were determined using SPSS 16. Relative susceptibility of all the genotypes was calculated by aggregating the weighted value (0-100 scale) for all the parameters except pupal period, as this parameter did not show significant correlation with the other parameters. The value of larval development was subtracted from 100 to obtain reverse trend as this parameter showed negative correlation with other parameters. The cumulative weightage was again brought to scale of 0-100 using the same formula. All the genotypes were then arranged in ascending order of the relative susceptibility level.

The experiment was conducted in a completely randomized design (CRD). The data of all the parameters was subjected to Analysis of Variance (ANOVA) using SPSS16.

RESULTS AND DISCUSSION

The results of the investigation carried out on the genotype susceptibility level of 20 maize genotypes based on each parameter are as below.

Leaf injury rating (21 DAI)

It is evident from Table 1 that the differences due to LIR among 20 genotypes were statistically significant. LIR ranged from 4.33-9 among different genotypes. The maximum LIR recorded in the genotype PFSR R9 (9.0) was statistically at par with HKI 1040-5 (8.7), AEB(Y) C5 34-1(7.8), HKI PCB4 (7.5), HYD05R/2-1(8.3) HKI 164-7-4 ER-3(8.1), Basi local (7.3) and CM 202 (6.3). The least LIR was observed in the genotype E4-C (4.33) which was statistically at par with E5-0, E9-B, E30, E37-A (O), E57 (O), WNZP8TL 6, E 60(FC) O, AEB(Y) C555-1, HK 1 1040-11-7, PFSR S3 and CM 202. The LIR of rest of the genotypes varied from 4.75 to 6.17.

Number of larvae recovered

Out of 75 larvae released in each genotype, maximum number of larvae (35) was recovered from genotype PFSR S3, while minimum number of larvae (11) was recovered from HKI-1040-11-7, after seven days of infestation (DAI) (Table 2). At 14 DAI, larval recovery was maximum (15) in WNZPBTL6, while no larva was recovered in HKI 1040-11-7 and Basi local. The genotype E4-C supported

Table 1 Antibiosis parameters of antibiosis against *Sesamia inferens* in maize germplasm

Germplasm	LIR	Av. larval weight(mg)	Larval period (days)	Pupal weight (mg)	Pupal Period (days)
E4-C	4.33±0.94a	27.75±3.76ab	29.2±0.08abc	92.13±4.28abc	8.8±0.73ab
E5-O	6.00±1.01abcdef	30.20±5.25abc	30.3±1.66abc	112.62±11.87bc	8±0.57ab
E9-B	4.75±0.90abc	34.87±5.66abcd	30.5±1.5abc	101.20±3abc	8.5±1.5ab
E30	6.17±0.69abcdef	20.53±0a	0	0	0
E37-A(O)	4.50±0.94ab	31.98±4.86abcd	30.5±0.86abc	104.75±6.78abc	8.75±0.75ab
E57(O)	5.92±0.90abcdef	32.70±3.35abcd	30.5±1.5abc	86.53±2.2a	9±1.0a
WNZPBTL6	6.00±0.97abcdef	30.97±3.15abc	29.83±1.3abc	107.51±6.89abc	7.83±0.40ab
E60(FC)O	5.75±0.93abcde	37.96±4.85bcd	30.75±1.25abc	76.36±20.32a	8±0.40ab
AEB(Y)C5 55-1	6.17±1.01abcdef	31.17±2.63abc	32±0a	99.25±10.05abc	7±0.0b
AEB(Y)C5 34-1	7.80±0.77defg	28.51±0ab	29±0abc	106.70±0abc	10±0.0a
HKI PC4B	7.55±0.76cdefg	40.07±7.85bcd	31±1ab	103.70±8.44abc	9±0.57a
HKI 1040-10-5	8.70±0.15fg	0	0	0	0
HKI 1040-11-7	5.89±1.04abcdef	34.38±4.31abcd	28.4±1.16abc	116.97±4.91bc	9±0.94a
Hyd 05R/2-1	8.30±0.26efg	0	0	0	0
HKI C323	5.22±0.92abcd	33.77±4.14abcd	28±0.57abc	120.35±4.64c	9±0.70a
HKI164-7-4 ER3	8.11±0.51defg	46.50±6.43cd	27.67±1.33bc	119.57±6.95c	8.5±1.5ab
PFSR S3	6.18±0.76abcdef	26.91±5.55ab	31±1ab	109.17±4.9bc	8.3±0.88ab
PFSR R9	9.00±0g	0	0	0	0
Basi Local	7.33±0.66bcdefg	48.32±3.38d	26.5±0.95c	98.85±5.59abc	7.75±0.75ab
CM 202	6.33±0.92abcdefg	38.22±4.49bcd	31±1ba	91.03±6.69ab	8.33±0.88ab

maximum (8) larvae till 21 DAI, while no larva was recovered from the genotype HKI 1040-10-5, Hyd 0 5 R/ 2-1 and PFSR R9.

The cumulative larval recovery from three sampling intervals (7, 14 and 21 DAI), showed a maximum number of larvae (39) in WNZPBTL6, while the minimum number of larvae (13) were recovered in HKI 1040-11-7 and Basi local (Table 2).

Larval weight

The larval weight varied significantly among all the test genotypes. Seven days after infestation, the maximum larval weight (5.83mg) was recorded in the genotype Basi local, and the minimum (2.81mg) was recorded in E9-B (Fig 1). At 14 DAI, maximum larval weight was observed in AEB(Y)C5 55-1 (20.73mg), while minimum in WNZPBTL6 (8.20mg). The maximum larval weight (48.32 mg) was recorded in case of Basi local at 21 DAI, which was statistically at par with the weight of larvae recovered from HKI 164-7-4-ER-3, HKI PC4B, CM202, E 60(FC)O, E9-B, HKI 1040-11-7, HKI C323, E 57(O) and E37-(A)O. The minimum larval weight was recorded in E 30 (20.53mg).

Pupal weight

Maximum pupal weight (120.35 mg) was recorded in HKI C323 which was statistically at par with pupal weight recovered from all other genotypes except CM 202, E 57(O) and E 60(FC)O. Lowest pupal weight (76.36mg) was observed in E60(FC)O (Table 1). The single larva recovered in E 30 did not pupate, while in genotype HKI 1040-10-5, Hyd 0 5 R/2-1 and PFSR R9 no larva was recovered at 21 DAI.

Larval and pupal period

The larval period was shortest in Basi local (26.5 days), which was statistically at par with larval period in all other genotypes, except AEB(Y) C5 55-1, HKI PC4B, PFSR S3 and CM202 (Table 1) and longest in AEB(Y) C5 55-1 (32 days) (Table 1).

The moths started emerging after seven days in genotype AEB(Y)C5 55-1, while it took 10 days in genotype AEB(Y) C5 34-1, which was statistically at par with all other genotypes, except AEB(Y)C5-55-1 (Table 1).

Correlation among different parameters of antibiosis

Pearson's correlation among all six parameters of antibiosis was computed (Table 3). Positive correlations were observed between larval weight and LIR (0.52); pupal weight and LIR (0.20); pupal weight and larval recovery (0.27), whereas negative correlation were observed between larval weight and larval period (-0.49); larval weight and larval recovery (-0.46); larval period and pupal weight (-0.40); and LIR and larval recovery (-0.21). The correlation of LIR with number of larvae recovered, larval weight and larval period suggests that these factors are of significance in determining the antibiosis. The poor correlation of pupal period with other parameters indicated that pupal period is not the true indicator of antibiosis.

Relative susceptibility of genotypes

Table 4 reveals that by aggregating five parameters of antibiosis, viz. LIR, larval recovery, larval weight, larval period, and pupal weight, E 30 is least susceptible while HKI 164-7-4 ER-3 is most susceptible among all the twenty genotype. PFSR R9, E 60 (FC) O, and AEB(Y)C5 55-1 are least susceptible genotype, whereas Basi local and AEB (Y)

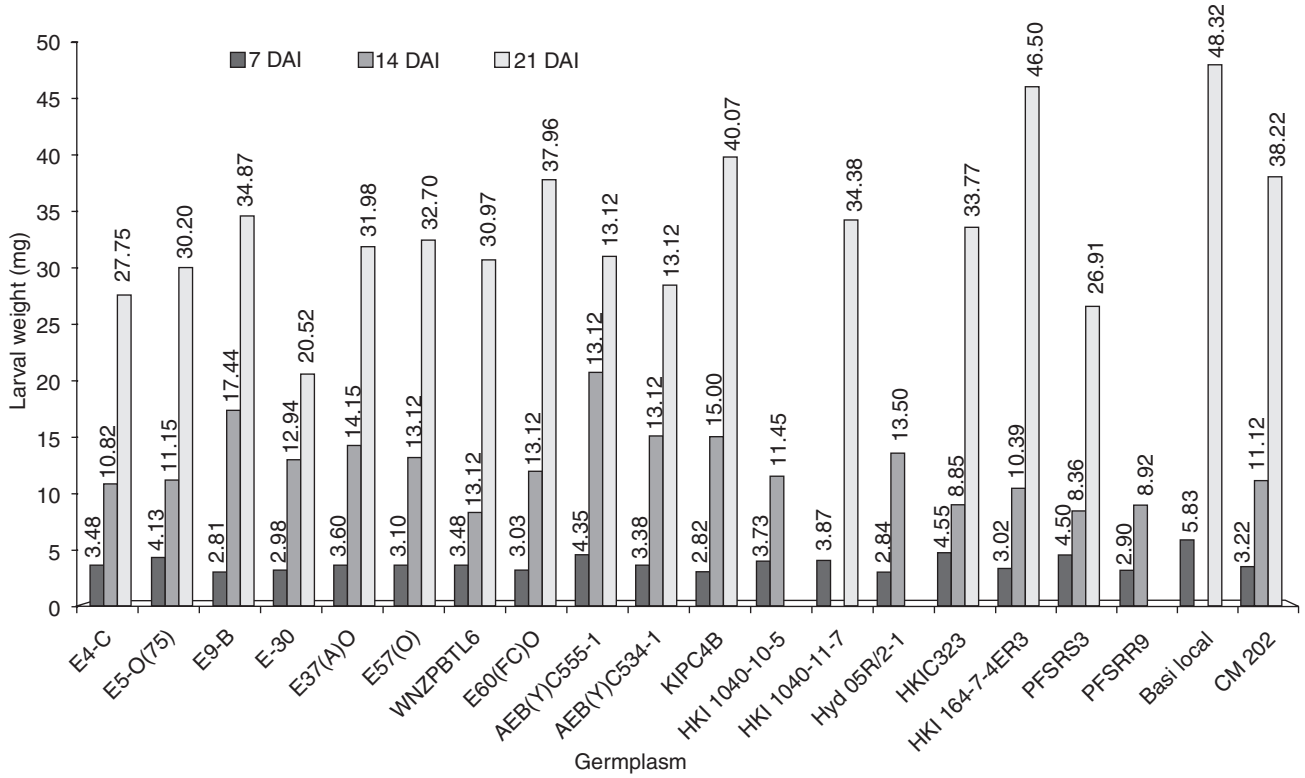

Fig 1 Larval weight of *Sesamia inferens* in maize germplasm at 7, 14 and 21 days after infestation

Table 2 Number of larvae of *S. inferens* recovered from 30 plants each of maize germplasm at 7, 14 and 21 days after infestation

Germplasm	7 days		14 days		21 days		Number of larvae/ 30 plants
	No. of larvae	No. of plants	No. of larvae	No. of plants	No. of larvae	No. of plants	
E4-C	16	15	11	10	8	12	28
E5-O	21	15	12	9	5	12	32
E9-B	24	15	9	10	5	12	29
E30	22	15	10	9	1	12	27
E37-A(O)	21	15	9	11	6	12	27
E57(O)	21	15	11	11	3	12	27
WNZPBTL6	30	15	15	11	7	12	39
E60(FC)O	16	15	13	10	4	12	27
AEB(Y)C5 55-1	15	15	11	10	2	12	23
AEB(Y)C5 34-1	29	15	4	10	1	12	24
HKI PC4B	22	15	6	10	6	12	26
HKI 1040-10-5	16	15	8	9	0	10	20
HKI 1040-11-7	11	15	0	10	5	9	13
Hyd 05R/2-1	27	15	9	9	0	10	28
HKI C323	23	15	10	9	5	9	32
HKI164-7-4 ER3	23	15	12	11	3	9	30
PFSR S3	35	15	7	9	6	11	37
PFSR R9	23	15	2	9	0	9	18
Basi Local	14	15	0	9	4	12	13
CM 202	14	15	3	9	3	9	16

C5-34-1 are the most susceptible genotype (Fig 2).

Antibiosis refers to adverse biological consequences on the well being of an insect due to feeding on a resistant host. The effects may be insect mortality, small size, reduced weight and fecundity, extended life cycle, and/ or abnormal behavior (Sandoya *et al*. 2010). In the present antibiosis studies of maize genotypes, significant differences in biological parameters of *S. inferens* indicates differential reaction of the test genotypes, thus gives strong clue in determining susceptibility level. The trend of larval weight

Table 3 Pearson's correlation coefficient of antibiosis parameters

	LIR	Larval recovery	Larval weight (mg)	Larval period (days)	Pupal weight (mg)	Pupal period (days)
LIR	1	-0.21	0.52*	-0.28	0.20	0.07
Larval recovery	-0.21	1	-0.46	0.27	0.27	-0.04
Larval weight (mg)	0.52*	-0.46	1	-0.49	0.08	-0.18
Larval period (days)	-0.28	0.27	-0.49	1	-0.40	-0.25
Pupal weight (mg)	0.20	0.27	0.08	-0.40	1	0.12
Pupal period (days)	0.07	-0.04	-0.18	-0.25	0.12	1

*Correlation coefficient significant at 0.05 level

Table 4 Parameters of antibiosis (0-100 scale) and Susceptibility index of maize germplasm against *Sesamia inferens*

Germplasm	LIR	Larval recovery	Larval weight (mg)	Larval period (days)	Pupal weight (mg)	Susceptibility index
E4-C	0	57.69	25.98	50.91	35.85	170.43
E5-O	35.76	73.08	34.8	30.91	82.43	256.98
E9-B	8.99	61.54	51.6	27.27	56.47	205.87
E30	39.4	53.85				93.25
E37-A(O)	3.64	53.85	41.2	27.27	64.54	190.50
E57(O)	34.05	53.85	43.79	27.27	23.12	182.08
WNZPBTL6	35.76	100.00	37.57	39.45	70.81	283.59
E60(FC)O	30.41	53.85	62.72	22.73	0	169.71
AEB(Y)C5 55-1	39.4	38.46	38.29	0.00	52.03	168.18
AEB(Y)C5 34-1	74.3	42.31	28.72	54.55	68.97	268.85
HKI PC4B	68.95	50.00	70.31	18.18	62.15	269.59
HKI 1040-10-5	93.58	26.92				120.50
HKI 1040-11-7	33.4	0.00	49.84	65.45	92.32	241.01
Hyd 05R/2-1	85.01	57.69				142.70
HKI C323	19.06	73.08	47.64	72.73	100	312.51
HKI 164-7-4 ER3	80.94	65.38	93.45	78.73	98.23	416.73
PFSR S3	39.61	92.31	22.96	18.18	74.59	247.65
PFSR R9	100	19.23				119.23
Basi Local	64.24	0.00	100	100.00	51.13	315.37
CM 202	42.83	11.54	63.66	18.18	33.35	169.56

with other parameters shows that larval weight increases as the susceptibility of the genotype increases. The trend of larval recovery and larval period suggests that susceptible genotypes support less number of larvae but hastens their development than the resistant genotypes.

It has been assumed that except pupal period, all other five parameters of antibiosis have equal significance in imparting resistance to the genotype, and have linear relationship. The host plant resistance to the pest may change with the age of plant. The larval survival at 7, 14 and 21 days after infestation, showed that a susceptible plant is more palatable and results in heavier larvae, but supports less number of larvae. This trend is further supported by negative correlation coefficient, observed between larval weight and larval recovery (-0.46). The genotype that showed aversion to pest (antixenosis) might have induced migration of larvae to other genotypes, and the remaining less number of larvae gained more weight in the absence of competition. Lack of antibiosis in such genotypes help the larvae to complete their larval development faster, which is evident from the significant negative correlation coefficient between larval weight and development period (-0.49). Whereas antibiosis is the cause of reduced larval survival and prolongation of post-embryonic development period in case of *C. partellus* as reported by Woodhead and Taneja (1987) and Van den Berg and Van der Westhuizen (1997). Similarly *C. partellus* larvae showed less feeding and low survival on CM 500 as compared to JH 3459 because of growth inhibiting antibiotic factors present in CM 500 (Jindal and Hari 2010). The shortest larval (26.5 days) and pupal (7.75 days) development period observed in Basi local will tend to increase the number of generations produced within a given period of time, as compared to the genotype AEB(Y)C5 55-1(32 days) that delays the development of larvae. The reduced survival and establishment of insect on some of the genotypes has been found to reduce the insect population and the resultant crop damage (Kumar *et al*. 2006). Further, a good feeding will result in more damage symptoms, which is reflected in significant positive correlation between larval weight and LIR (0.52). The

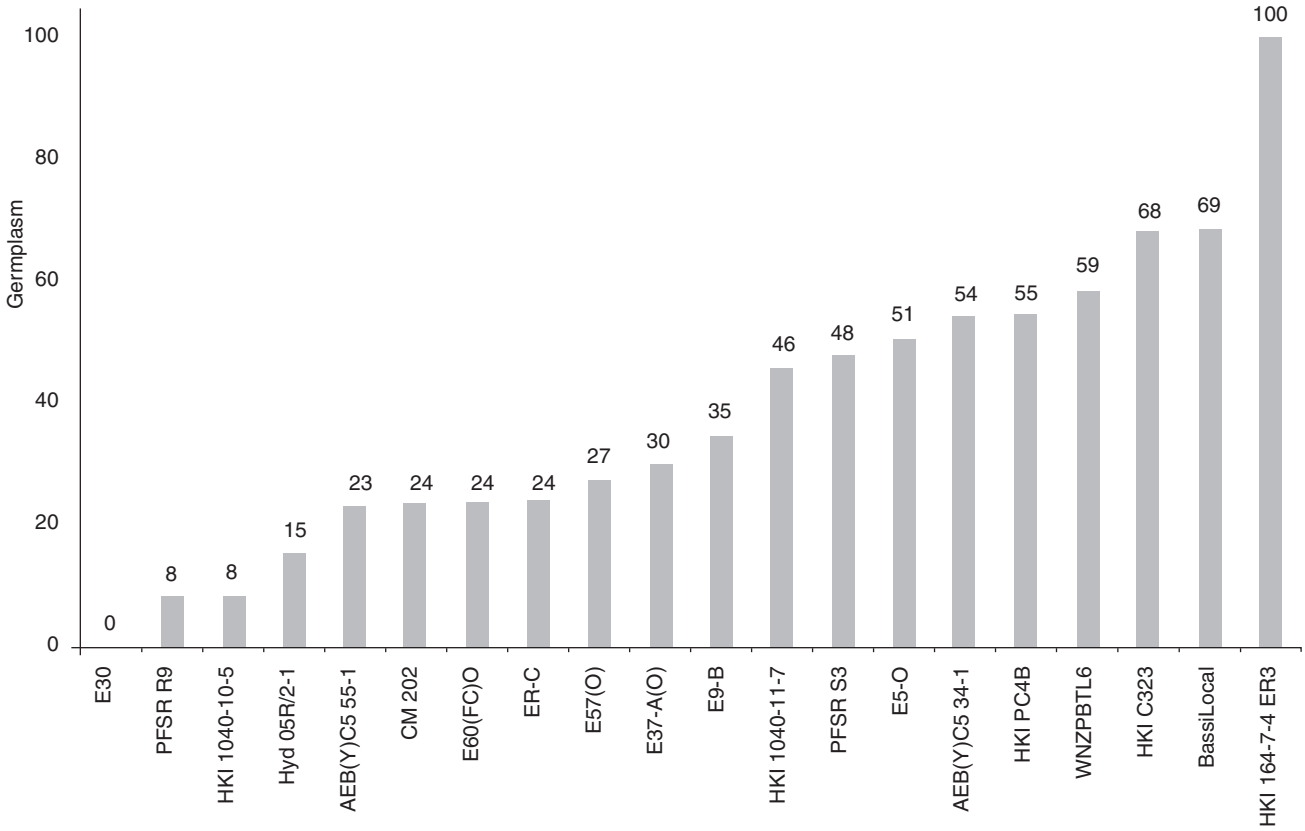

Fig 2 Relative susceptibility of maize germplasm against *Sesamia inferens*

genotypes, harbouring more number of larvae, resulted in underweight larvae because competition did not allow them to feed well and inflicted less leaf injury, which is evident from the negative correlation (-0.21) between LIR and larval recovery. However, the results do not corroborate with Durbey and Sarup (1984), who reported lower larval recovery and percent pupation of *C. partellus* on resistant varieties (Antigua Gp I and Mex-17) than on susceptible varieties, Basi local and Vijay composite of maize. Similarly significant differences were observed between percent larval survival, percent pupation, pupal weight and pupal period of *C. partellus* on two varieties CM137 and HY4642 (Arabjafari and Jalali 2007). Pupal weight depends on how well fed the insect was in its larval stage. Since there is negative correlation between larval period and larval weight, the same correlation is reflected in larval period and pupal weight (0.40). The higher pupal weight, 120.35mg in HKI C323 and 119.57mg in HKI 164-7-4-ER-3 indicates higher susceptibility level for *Sesamia*. The higher pupal weight recorded on genotype HKI C323 in spite of the lower larval weight recorded on it (Table 1) may be due to the fact that larvae remained underdeveloped and underweight due to antibiosis in genotypes, but on getting suitable natural food (baby corn), they fed well and developed into heavier pupae. The trend of correlation among five parameters of antibiosis in twenty genotypes and aggregation of their weightage on 0-100 scale provides a sound inference for quantifying susceptibility and grading the genotypes based on the level of susceptibility. Accordingly, the genotypes E 30, PFSR R9, HKI 1040-10-5 were least susceptible while HKI 164-7-4 ER-3, Basi local, HKI C323 and WNZPBTL6 were more susceptible.

The antibiosis parameters of maize genotype against *S. inferens* display differential reaction to the insect. The cumulative impact of these parameters on the pest's well being consequently determines its population on maize genotype and damage caused to it. Therefore, it can be used for identifying resistant sources in maize germplasm, which could be used in the maize breeding programmes for developing pest resistant cultivars.

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