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Photosynthetic pigments in maize (*Zea mays*) *vis-à-vis* biological performance and host selection by *Sesamia inferens*

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ABSTRACT

In the present study, host selection behaviour and biological performance of *Sesamia inferens* on different maize (*Zea mays* L.) genotypes was investigated in 2018–19, and constitutive and insect damage-induced levels of various photosynthetic pigments were determined. There were significant differences in larval period, larval survival, larval weight, pupal period, pupal weight, adult emergence, and fecundity of *S. inferens* on the test maize genotypes. The *S. inferens* that fed on maize genotypes, viz. CPM 2, CPM 4, CPM 8, CPM 15 and CML 345 showed significant increase in developmental period, decrease in larval weight, and reduced larval survival, adult emergence and fecundity as compared to other test genotypes. The *S. inferens* larval recovery and preference were significantly lower, while the larvae took longer time to establish in the whorls of CPM 2, CPM 15 and CML 345 as compared to other test maize genotypes. The chlorophyll A, chlorophyll B and total chlorophyll content varied significantly in the seedlings of different maize genotypes, under healthy and *S. inferens* damaged conditions (except, chlorophyll B), while the genotype × treatment interactions were non-significant. The differences for total carotenoids were non-significant. The *S. inferens* infestation reduced these photosynthetic pigments in the seedlings of all test maize genotypes, except Basi Local, with lowest reduction in CPM 2. The study suggests that the maize genotypes, viz. CPM 2, CPM 4, CPM 8, CPM 15 and CML 345 have greater detrimental effects on the development, survival and fecundity of *S. inferens*, and can be used in maize improvement program.

Keywords: Maize, Photosynthetic pigments, Pink stem borer, *Sesamia inferens*, *Zea mays*

Maize (*Zea mays* L.) is an important cereal crop occupying third global rank in area and production (Kumar *et al.* 2014). In India, maize is grown on 9.18 million ha with production of 27.23 million tonnes and productivity of 2965 kg/ha (ASG 2019). The pink stem borer, *Sesamia inferens* Walker poses a serious challenge to the productivity of maize crop (Dhillon *et al.* 2014). Several management strategies including crop rotation, field sanitation, biological control agents and synthetic pesticides have been recommended for the control of *S. inferens*, but none of these have been found effective particularly when the larvae enter inside the stalks. Therefore, host plant resistance could be one of the most effective means of minimizing these problems. Breeding for stem borer resistance has become a major research focus in most of the maize growing countries of Asia, Africa and America, however, its success is largely dependent on the identification and deployment of sources of insect resistance.

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The antibiosis and antixenosis mechanisms of resistance are operational against *S. inferens*. The antixenosis mechanism interferes in larval orientation, settling and feeding response, while antibiosis affects the growth, development and survival due to several biochemical and morphological factors. Furthermore, the feeding of herbivorous insects induces biochemical and physiological changes in the host plants, affecting the life processes of host plants such as photosynthesis. The photosynthetic pigments in the plant tissues are one of the primary biochemicals involved in host plant-insect interaction. Studies investigating plant resistance mechanisms and exploring photosynthetic pigments as markers for identifying different kinds of chlorosis-causing insects can be helpful (Huang *et al.* 2014). Considering importance in pest management, present study was conducted on host selection behaviour and biological performance of *S. inferens* on different maize genotypes, to determine constitutive and insect damage-induced levels of various photosynthetic pigments.

MATERIALS AND METHODS

Sesamia inferens culture and test plant materials: The *S. inferens* larvae were collected from maize crop in the experimental fields of ICAR-Indian Agricultural Research Institute, New Delhi and reared on maize stalks under

laboratory conditions at 27±2°C, 80±5% RH and 12L:12D. The food was changed on alternate days until pupation. The pupae, thus obtained, were collected and kept on butter paper in separate plastic jars (12 cm height and 8 cm diameter). The adults that emerged were transferred to oviposition cages provided with butter paper at bottom (16 cm height and 8 cm diameter) in the batches of 5–7 pairs/jar for egg laying. The eggs were collected daily and kept in another jar (250 ml capacity). To create appropriate humidity, a moist blotting paper was placed over the wire-mesh fitted lid of the egg jar till the eggs hatched. The neonate larvae of *S. inferens* obtained from this culture were used for the experiments.

The experimental material consisted of ten maize genotypes, viz. CPM 2, CPM 4, CPM 8, CPM 9, CPM 13, CPM 15, CPM 18, CPM 19, CML 345 (resistant check), and Basi Local (susceptible check). Each genotype was sown in 2 row plots of 4 m row length, and 75 cm distance between the rows during 2018–19. Test maize genotypes were raised by following the recommended agronomic practices except insecticide spray, and used for biological studies on *S. inferens*.

Biological performance of S. inferens: The seedlings of above mentioned field grown maize genotypes at 14 days after germination were used to carry out developmental biology of *S. inferens* at 27±2°C, 70±5% RH and 12L:12D under laboratory conditions. A cohort of 25 neonate *S. inferens* was inoculated in each jar containing leaf whorl cuttings (4–5 cm long) of each test maize genotype, and there were five replications for each genotype in a completely randomized design. The food was changed on alternate days from the respective test maize genotypes. The observations were recorded on larval period (days), larval survival (%), larval weight at 21 days after release (mg/larva), pupal period (days), pupal weight at 24 h after pupation (mg/pupa) and adult emergence (%). The five pairs of adults obtained from each test genotype were paired in individual mating jars, and eggs laid by each female were recorded separately, and expressed as number of eggs/female.

Larval behaviour of S. inferens on test maize genotypes: The leaf discs from the whorl leaves of 15 days old seedlings of test maize genotypes were prepared for no-choice (3 cm diameter) and multi-choice (2.5 cm diameter) assays. There were five replications for each treatment in a completely randomized design. Ten newly hatched larvae were released in petri dish having leaf disc of each genotype separately. Number of surviving larvae on each genotype were recorded at 4 days after release, and expressed as larval recovery (%). In multi-choice assay, leaf disc of each genotype was placed equidistant on the periphery, and 50 neonates were released in center of Petri dish. Number of larvae settled in leaf disc of each genotype were counted separately at 4 days after release, and expressed as larval preference (%).

For larval settlement behavior, test maize genotypes were grown on potting mixture of soil and manure (2:1 ratio) having five plastic pots for each genotype. At 15 days old seedlings, one neonate *S. inferens* larva were released each on the 1st and 3rd leaf from the top in each test genotype, and there were five replications for each treatment. Time taken by the larvae to settle on the whorl of test maize genotypes from the site of release was recorded in minutes.

Estimation of various photosynthetic pigments in healthy and S. inferens damaged maize: The test maize genotypes were grown on potting mixture of soil and manure (2:1 ratio) in plastic pots (1000 ml capacity) under net-house conditions. There were 4 pots for each test maize genotype, out of which two were inoculated with *S. inferens* larvae and two were kept as control. Two *S. inferens* larvae (9 to 10 days old) were inoculated in the central whorl of each plant in the treatment designated pots 15 days after germination. At 24 h after exposure, the *S. inferens* inoculated and the counterpart healthy seedlings were collected from each test maize genotype separately and processed for estimation of different photosynthetic pigments. The test samples from the damaged and counterpart healthy maize seedlings weighing 2 g were separated, and crushed in liquid nitrogen. In each crushed sample 10 ml of 50 Mm phosphate buffer (*p*H 7.8) was added and again macerated in mortar and pestle, the slurry was transferred to centrifuge tube and centrifuged at 12000 rpm for 20 min at 4°C. The supernatant was collected and stored in Eppendorf tube at -20 °C for estimation of various photosynthetic pigments. Photosynthetic pigments, viz. chlorophyll A, chlorophyll B, total chlorophyll and total carotenoids in the test plant samples were measured by using the method given by Nayek *et al*. (2014), and expressed as µg/ml.

Statistical analysis: The biological and behavioural data of *S. inferens* on the test maize genotypes were analyzed using one-way analysis of variance (ANOVA), while for various photosynthetic pigments in the seedlings of healthy and *S. inferens* damaged test maize genotypes, and genotype × treatment interactions were subjected to factorial analysis. The significance of differences were tested by *F*-test, and the treatment means and their interactions were compared by least significant differences at P=0.05 using statistical software SAS® version 9.2.

RESULTS AND DISCUSSION

Biological performance of S. inferens on different maize genotypes: The host plants having xenobiotic traits negatively impact the insect pest biology in terms of reduced growth and development, increased mortality and reduced fecundity (Smith 2005). The *S. inferens* biological performance studies revealed significant differences for larval period, larval survival, larval weight, pupal period, pupal weight, adult emergence, and fecundity on the test maize genotypes. The larval period of *S. inferens* was significantly higher and larval weight lower on CPM 2, CPM 4, CPM 8 and CML 345 (resistant check), while reverse was the case for CPM 18 and Basi Local. The pupal period was significantly longer and pupal weight lower on CPM 2 and CML 345, while reverse was the case for CPM 18 and Basi Local (Table 1). The larval survival and adult emergence of *S. inferens* were significantly lower

Table 1 Biological performance of *Sesamia inferens* on different maize genotypes

Genotypes	Larval period (days)	Larval weight (mg/larva)	Pupal period (days)	Pupal weight (mg/pupa)	Larval survival (%)	Adult emergence (%)	Fecundity (eggs/ female)
CPM 2	27.7	67.9	11.7	84.5	31.2	25.6	52.0
CPM 4	27.2	68.8	10.7	116.6	49.6	35.2	93.2
CPM 8	27.7	83.5	10.3	121.6	32.8	25.2	68.8
CPM 9	26.0	108.8	9.6	116.5	56.8	46.4	107.1
CPM 13	26.1	107.2	8.9	123.0	50.8	43.2	70.3
CPM 15	26.9	109.6	9.2	109.6	32.4	28.6	74.0
CPM 18	25.4	120.1	9.5	118.7	64.0	54.4	117.2
CPM 19	25.4	102.5	10.5	104.7	53.8	45.4	110.8
CML 345	28.7	62.6	12.5	76.5	32.8	28.0	45.9
Basi Local	22.7	121.5	8.9	136.8	70.4	61.6	169.7
LSD (P=0.05) (P<0.001)	0.96	12.32	0.49	13.01	8.24	6.84	28.89

on CPM 2, CPM 4, CPM 15 and CML 345, while higher on CPM 18 and Basi Local as compared to other maize genotypes. The fecundity of *S. inferens* females obtained from the CPM 2, CPM 8, CPM 13, CPM 15 and CML 345 fed larvae was significantly lower, while higher in CPM 18 and Basi Local compared to other genotypes (Table 1). Some of these maize genotypes have also been reported to result in adverse effects on certain biological parameters of *Chilo partellus* (Dhillon and Gujar 2013, Dhillon and Chaudhary 2015).

Larval behaviour of S. inferens on test maize genotypes: There were significant differences in larval recovery and larval preference by *S. inferens* among the test maize genotypes (Fig 1). The larval recovery and larval preference were significantly lower for CPM 2, CPM 9, CPM 15 and CML 345, while higher in CPM 19 and Basi Local (Fig 1). These findings suggest that the neonate *S. inferens* larvae take longer time to establish and recovered less in numbers on the resistant as compared to the susceptible maize genotypes. Some of these maize genotypes like CPM 15, CPM 18 and CML 345 were also found less preferred by *C. partellus* (Bhoi *et al.* 2017).

There were significant differences in time taken by the neonate *S. inferens* to establish in the central leaf whorl of different maize genotypes when released on the tip of top 1st and 3rd leaves (Fig 1). The neonate *S. inferens* larvae released on the tip of top 1st and 3rd leaf of resistant check, CML 345, took more time to establish in the whorl, while on susceptible check, Basi Local, they took less time than on other test maize genotypes (Fig 1). Furthermore, the *S. inferens* neonates released on the tip of top 1st and 3rd leaf of CPM 2, CPM 15, CPM 19 took longer time, while that on CPM 4 and Basi Local less time to establish in their whorls as compared to other test maize genotypes (Fig 1). The *C. partellus* larvae were also found to take longer time to establish in the whorls of CPM 15 and CML 345 (Bhoi *et al.* 2017).

Various photosynthetic pigments in healthy and S. inferens damaged maize seedlings: The levels of different photosynthetic pigments change during different plant growth stages as well as in response to a wide variety of stresses including biotic stresses such as insect feeding and pathogen infections (Heng-Moss *et al.* 2003, Goławska *et al.* 2010). The chlorophyll A, chlorophyll B and total chlorophyll content varied significantly in the seedlings of different maize genotypes, under healthy and *S. inferens*

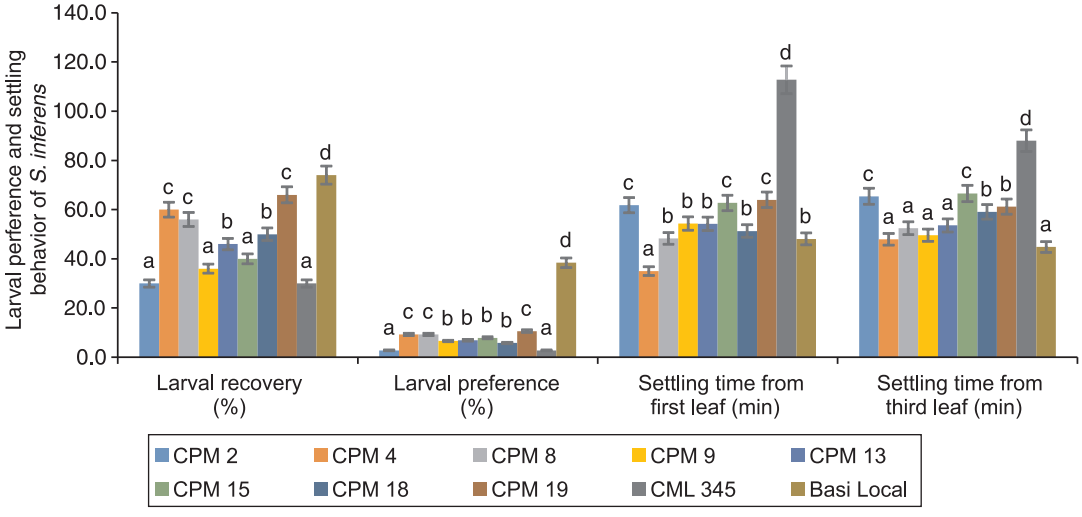

Fig 1 Establishment and feeding behaviour of neonate *Sesamia inferens* on different maize genotypes.

Table 2 Effect of *Sesamia inferens* damage on various photosynthetic pigments of different maize genotypes

Genotype	Chlorophyll A (µg/ml)		Chlorophyll B (µg/ml)		Total chlorophyll (µg/ml)		Total carotenoids (µg/ml)	
	Healthy	Damaged	Healthy	Damaged	Healthy	Damaged	Healthy	Damaged
CPM 2	10.22	10.05	3.81	3.33	13.85	13.56	1.29	1.46
CPM 4	15.88	13.78	6.31	6.2	22.19	20.49	1.93	1.93
CPM 8	9.91	11.09	5.1	3.83	15.01	14.93	1.33	2.22
CPM 9	11.75	8.44	4.61	3.84	16.36	12.28	1.49	1.81
CPM 13	14.04	11.59	4.17	3.62	18.21	15.21	2.19	2.48
CPM 15	11.42	10.84	4.58	3.03	16	13.87	1.45	2.15
CPM 18	13.82	11.06	6.19	4.42	20.01	15.47	2.12	2.11
CPM 19	14.66	8.25	5.24	2.85	19.9	11.1	1.77	2.43
CML 345	15.78	14.34	5.74	4.49	21.52	18.83	2.26	2.49
Basi Local	10.88	11.78	6.11	7.47	16.99	19.25	1.62	2.74
For comparing	P-value	LSD	P-value	LSD	P-value	LSD	P-value	LSD
Treatment (T)	0.03	1.18	0.06	NS	0.02	0.85	0.21	NS
Genotype (G)	0.05	2.98	<0.001	0.18	0.02	1.2	0.19	NS
G × T	0.59	NS	0.48	NS	0.5	NS	0.36	NS

NS, Nonsignificant at P=0.05; LSD, Least significant difference at P=0.05.

damaged conditions (except chlorophyll B), while the genotype × treatment interactions were non-significant (Table 2). However, there were no significant differences in amount of total carotenoids in the seedlings of different maize genotypes, among healthy and *S. inferens* damaged conditions, and genotype × treatment interactions (Table 2). Across maize genotypes, chlorophyll A, chlorophyll B and total chlorophyll content were higher in the healthy as compared to *S. inferens* infested maize seedlings, except Basi Local. Genotypes CPM 2, CPM 8, CPM 9, CPM 15 and Basi Local were found to have comparatively lower chlorophyll A, chlorophyll B and total chlorophyll content under healthy and *S. inferens* damaged conditions, except in a few cases. Furthermore, the *S. inferens* damage resulted in significantly lower reduction in chlorophyll A content in CPM 2, CPM 15 and CML 345, chlorophyll B content in CPM 2, CPM 4 and CML 345, and total chlorophyll content in CPM 2 and CPM 8 as compared to other genotypes (Table 2). Leaf chlorophyll correlated with resistance to *Sesamia cretica* Lederer in maize, with the anticipation that increase in chlorophyll content in maize leaves promote resistance to this pest (Metwally 2015). However, Nagaraj *et al.* (2002) recorded significant reduction in both photosynthetic rate and chlorophyll content due to greenbug, *Schizaphis graminum* (Rondani) feeding on sorghum. Bhoi *et al.* (2021) found that some of these test maize genotypes also impart resistance against *C. partellus* through activation of enzymatic and nonenzymatic antioxidant defense system. In general, the foliage feeders result in loss of photosynthetic tissues and remaining tissues see fluctuating photosynthesis (Nabity *et al.* 2009, Velikova *et al.* 2010, Koch *et al.* 2016). Present studies suggest that the *S. inferens* damage results in chlorophyll A, chlorophyll B and total chlorophyll reduction, resulting in poor plant photosynthetic activity, and have implications for other plant defense systems and yield potential of the maize.

These study revealed that the maize genotypes, viz. CPM 2, CPM 4, CPM 8, CPM 15 and CML 345 have varying levels of constitutive and *S. inferens* damage induced photosynthetic pigments, and resulted in greater detrimental effects on the development, survival and fecundity of pink stem borer, thus the findings can be used in *S. inferens* resistance breeding program.

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