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Developmental biology and protein interactions of spotted stem borer, *Chilo partellus* on diverse array of maize genotypes

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Abstract
We studied the developmental biology of *Chilo partellus* on different maize types and total protein content in seedlings of different maize types *vis-à-vis* the *C. partellus* larvae reared on these genotypes to gauge the level of antibiosis in these specialty maize genotypes. The studies suggested significant differences for larval weight, larval survival, larval period, pupal period and adult emergence of *C. partellus* on the test maize types. The larval weight of *C. partellus* was significantly lower on resistant check, CML 345 as compared to other genotypes, being highest on sweet corn and Quality Protein Maize (QPM) genotypes. The larval period and pupal periods were significantly higher on white kernel and yellow kernel genotypes as compared to that on sweet corn and QPM genotypes, and the susceptible check, Basi Local. In general, the larval survival and adult emergence were significantly lower on white kernel and yellow kernel genotypes as compared to that on sweet corn and QPM genotypes including susceptible check, Basi Local. There were significant differences for total protein content in different maize types and the *C. partellus* larvae fed on these genotypes. Protein content in maize seedlings was lower as compare to that in stem borer larvae fed on them. Total protein content was significantly higher in QPM, while lower in susceptible check, Basi Local as compared to other group of maize genotypes, except in few cases. Present studies have implications for understanding the mechanism of resistance and the host plant-insect biochemical interactions.

Keywords: Maize, spotted stem borer, host plant resistance, developmental biology, protein interaction

Introduction
Maize (*Zea mays* L.) is an important staple food for millions of people across the world and is the third most important food crop after rice and wheat. It is a miracle crop with high yield potential. In India, as per the latest report, maize area and production was 9.47 Mha and 28.72 Mt, respectively in 2018 (https://eands.dacnet.nic.in). According to recent estimates, it contributing 49% to poultry feed, 25% to human food, 12% to animal feed, 12% to industrial products mainly the starch, and 1% each in brewery and seed [1].

The grain yields under subsistence farming conditions are quite low (2.56 t/ha) because of several biotic stresses *viz*., insect pests and diseases. Maize is damaged by more than 250 species of insects during different stages of its growth, of which the cereal stem borers [*Chilo partellus* (Swinhoe) and pink stem borer, *Sesamia inferens* Walker in South Asia and eastern and southern Africa, *Busseola fusca* in Africa, *Diatraea* spp. in the America, and *Ostrinia* spp. in the America, Europe, and East Asia] pose a great challenge to increase productivity potential of this crop [2]. Among these, *C. partellus* is one of the most important biotic constraints resulting in 27 to 80% crop losses in India [3], and is a ubiquitous and key pest of maize [4].

Exploitation of Host plant resistance and use of tolerant varieties is one of the components of Integrated Pest Management (IPM) approach and also a comprehensive tool for minimizing crop losses due to insect pests. Evaluation of available maize germplasm has reported low to moderate levels of resistance to spotted stem borer, *C. partellus* [3, 5-7], and several new sources with high levels of stem borer resistance have also been identified and supplemented to the existing resistance sources [8]. The stem borer resistance has been reported due to all the three components of resistance, *viz*., non-preference, antibiosis and tolerance [9]. Maize is a genetically diverse crop having wide genetic variability pertaining to its purpose of use such as for human food, micronutrient enrichment, sweet corn, poultry feed, animal feed, industrial

products, brewery, etc. It is also likely that with the increase in concentration and quality of nutritional compounds like sugar content and particularly the protein in QPM might favor the proliferation of insect pests as they too prefer quality food for their growth and development, and could be major constraints to increasing production and productivity of different specialty maize [2].

Present studies were undertaken to gauge the level of antibiosis mechanism of resistance for *C. partellus* in different specialty maize genotypes. We studied developmental biology of *C. partellus* on different maize types and the total protein content present in seedlings of different maize type *vis-à-vis* the *C. partellus* larvae reared on these genotypes. The present studies are highly important for understanding the nutritional resistance factor and host plant-insect biochemical interactions in different maize types.

Materials and methods

Plant material

The experimental material consisted of 10 different maize types QPM (HKI 161, HKI 163 and HKI 193-2), sweet corn type (SK 37228 and SU 37242), white kernel type (CPM 2, CPM 4 and CML 345), and yellow kernel type (CPM 8 and Basi Local). These genotypes were sown in a randomized complete block design (RCBD) with three replications and crop was maintained by following all the agronomic practices recommended for maize cultivation, except insecticide spray.

Developmental biology of *C. partellus*

The nucleus culture of *C. partellus* was collected from the field and reared on fresh maize stalk till pupation. Further the culture was multiplied and maintained on artificial diet under laboratory conditions at 27 ± 2 ºC and relative humidity of 65 ± 5% [10]. The developmental biology experiment was carried out at 27 ± 2 ºC temperature, 65 ± 5% relative humidity and 12L:12D photoperiod under controlled conditions in the laboratory. The seedlings (14 days after germination) of the test maize genotypes raised under field conditions were used for *C. partellus* biological studies. Three seedling stems near central whorl (about 5 cm) of each of the above mentioned test maize genotypes were placed on 3% agar-agar solution in individual plastic jars (10 × 8 cm). Ten neonate *C. partellus* larvae were released on each seedling stem, and the experiment was replicated three times. The food was changed on alternate days, and every day whenever required. Each experiment was repeated two times. The larval mortality was recorded at every food change and the dead larvae were recorded and discarded from the experimental jars. Observations were recorded on larval weight, larval period, larval survival, pupal period, pupal weight, and adult emergence.

Larval weight was recorded for individual larvae at 30 days after release. The larval period was recorded separately for each insect, and the mean larval period per replication was calculated for the surviving larvae. The data on the number of larvae survived was expressed as percentage larval survival. The pupal period was recorded separately for each insect, and the mean pupal period per replication was calculated for the surviving pupae. The pupal weight was measured for individual pupa within 24 h of pupation. The data on number of adults emerged from each replication was expressed in percentage adult emergence based on the number of larvae released per replication.

Collection of maize seedling and larval samples

The seedlings of test maize genotypes were raised in 10 pots for each genotype under glasshouse conditions on the potting mixture. The 14-days old seedlings of each genotype from designated 5 pots were harvested separately and immediately transferred to ice-box. After complete harvesting, the seedling samples were stored at -20 ºC.

The 14-days old seedlings of each genotype in the remaining 5 pots were infested with neonate *C. partellus* larvae. Larvae were recovered by dissecting the infested plants after 20 days of release. After starvation of 4 h, larvae from each genotype were stored in the glass vials at -20 ºC in the refrigerator for protein estimation.

Protein estimation

The test maize seedling samples stored at -20 ºC were powdered in liquid nitrogen and 500 mg each was homogenized in mortar and pestle with 5 ml phosphate buffer (pH 7.5). Homogenate was centrifuged at 10,000 rpm for 10 min at 4 ºC. Supernatant was collected and transferred in 2 ml eppendorf tubes and stored at -20 ºC for further analysis.

In case of insects, the larval samples weighing around 50 mg each were selected for sample preparation. The larval samples were homogenized in 1.5 ml eppendorf tube with sterilized disposable homogenizer in 200 ul of phosphate buffer and 200 ul of MgCl2 (24 mM). Homogenate was centrifuged at 10,000 rpm for 10 min at 4 ºC. Supernatant was collected and stored in 2 ml eppendorf tubes for further analysis.

Total protein content of the plant and insect samples were estimated by Bradford's method [11] using BSA as standard. BSA stock solution was prepared in the concentration of 1 mg/ml. Known concentration of BSA @ 2, 4, 6, 8, 10, 15, 25 and 50 mg/ml were prepared using BSA stock. To these concentration 1 ml of Bradford reagent were added to 2-50 ml of BSA stock solution and final volume was made to 1100 µl by adding 0.1N NaCl. Blank solution was prepared by adding 1 ml Bradford reagent and 100 µl of 0.1 NaCl solution.

From the insect and plant samples, 10 µl of sample was taken in 2 ml tube for protein estimation. To this tube 1 ml of Bradford reagent was added and final volume was made to 1100 µl by adding 0.1N NaCl. From this solution 200 µl of prepared sample was loaded in 96 well microtiter plate, similarly 200 µl of standard prepared of different concentrations and blank were loaded. Each sample and standard was replicated three times. Absorbance was recorded at 595 nm wavelength in ELISA Reader. The data on protein content in different test samples were expressed as µg/mg.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and the significance of differences between the genotypes was tested by *F*-tests, while the treatment means were compared by least significant differences (LSD) at $P = 0.05$ using the statistical software SAS® version 9.2.

Results and Discussion

Developmental biology and survival of *Chilo partellus* on different maize genotype

The studies on developmental biology of *C. partellus* on different maize types resulted in significant effects on the larval weight at 30 DAF ($F_{9,18} = 37.97$; $P = < 0.001$), larval survival ($F_{9,18} = 12.45$ $P = < 0.001$), larval period ($F_{9,18} = 5.39$; $P = < 0.001$), pupal period ($F_{9,18} = 3.48$; $P = < 0.001$) and adult emergence ($F_{9,18} = 8.99$; $P = < 0.001$). The larval weight

of *C. partellus* was significantly lower on white and yellow kernel maize genotypes including resistant check (CML 345) and susceptible check (Basi Local) as compared to that on QPM and sweet corn maize genotypes (Table 1). Furthermore, the resistant check, CML 345 and the white kernel genotype CPM 4 had significantly lower weights as compared to susceptible check and the other group of maize genotypes. It was higher on sweet corn SK 37228 and SU 37242, and QPM genotypes HKI 161 and HKI 163 (Table 1). The larval period was higher on white kernel type genotypes CPM 2 and CPM 4 and being lower on sweet corn genotypes SU 37242 and SK 37228 and susceptible check, Basi Local (Table 1).

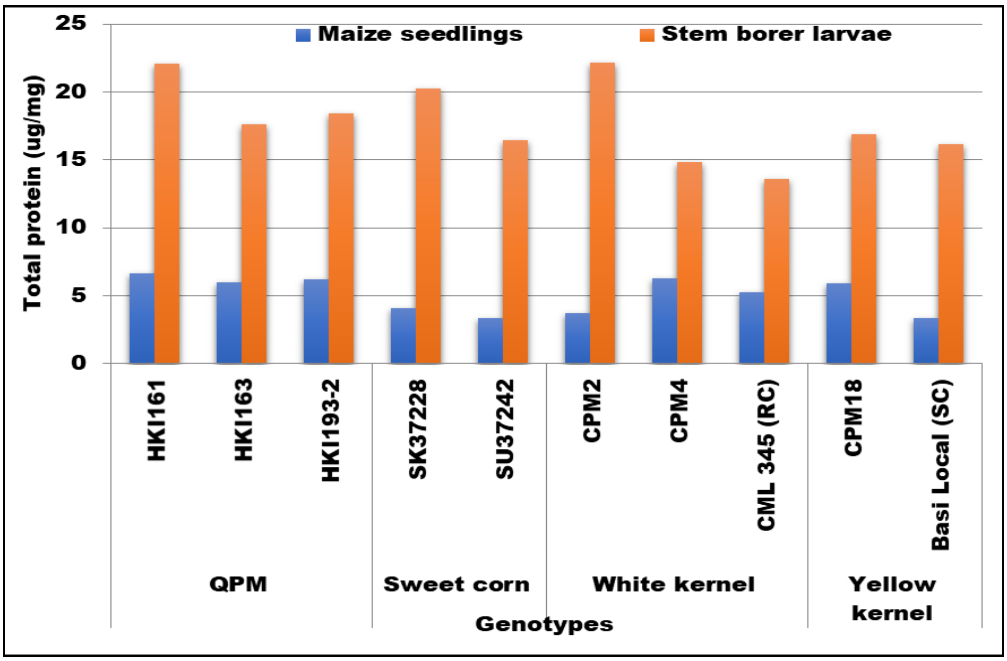
Larval survival was significantly lower on white kernel genotypes CPM 2, CPM 4 and CML 325; and yellow kernel genotype CPM 18 as compared to sweet corn and QPM genotypes (Table 1). However, pupal period was significantly higher on resistant check, CML 345 than all other genotypes. Adult emergence was significantly higher on QPM and sweet corn maize genotypes including susceptible check, Basi Local as compared to white and yellow kernel maize genotypes (Table 1).

Total Protein estimation from maize seedlings and the *C. partellus* larvae reared on these genotypes

The amount of protein differed significantly in maize genotypes ($F_{9,18}$ = 14.470; P = < 0.001) and in stem borer larvae fed on these genotypes ($F_{9,18}$ = 43.360; P = < 0.001). Protein amount was significantly higher in QPM maize genotypes *viz*., HKI 161 and HKI 163 and HKI 193-2 as compared to other group of maize genotypes, except in few cases (Graph 1). However, protein content in susceptible check Basi Local was significantly lower than other group of maize genotypes. Protein content in the stem borer larvae fed on white kernel genotypes CPM 4 and the resistant check, CML 345 was significantly lower than those fed on other group of maize genotypes (Graph 1).

Table 1: Expression of antibiosis mechanism of resistance to stem borer, *Chilo partellus* in different maize types

Genotypes	Different biological attributes					
	Larval weight at 30 days (mg/larva)	Larval period (days)	Larval survival (%)	Pupal weight (mg/pupa)	Pupal period (days)	Adult emergence (%)
QPM genotypes						
HKI 161	92.8	34.3	73.3	84.7	9.0	63.3
HKI 163	92.9	35.0	66.7	87.1	8.1	53.3
HKI 193-2	74.1	33.8	56.7	94.9	7.9	53.3
Sweet corn genotypes						
SK 37228	99.3	31.3	66.7	84.3	8.7	56.7
SU 37242	98.2	31.1	63.3	90.3	8.4	56.7
White kernel genotypes						
CPM 2	73.4	35.6	43.3	55.7	9.2	40.0
CPM 4	61.9	38.8	40.0	66.7	8.2	36.7
CML 345 (RC)	68.8	33.4	43.3	54.8	10.4	43.3
Yellow kernel genotypes						
CPM 18	72.1	36.5	46.7	65.5	8.7	36.7
Basi Local (SC)	79.7	31.7	76.7	75.7	8.3	60.0
P-value	<0.001	0.001	<0.001	<0.001	0.012	<0.001
LSD (0.05)	6.46	3.13	11.39	10.40	1.15	9.85
RC = Resistant check. SC = Susceptible check.						



Graph 1: Amount of total protein in seedlings of different maize types and the stem borer, *Chilo partellus* larvae fed on these genotypes

Host plant resistance is one of the effective means of minimizing losses due to insect pests. All the three mechanisms of resistance (non-preference, antibiosis and tolerance) have been reported to be operational in maize for resistance to *C. partellus* [9]. Adverse effects of different conventional maize genotypes on different life attributes have also been reported earlier by several workers [12, 13, 7, 14]. The results revealed that the larval and pupal weights, larval survival and adult emergence were significantly higher and larval and pupal periods were lower on QPM and sweet corn maize genotypes as compared to yellow and white kernel genotypes, except in few cases. Total protein content differed significantly in maize genotypes and the spotted stem borer larvae fed on these genotypes, which was found lower in maize seedlings than in spotted stem borer larvae fed on them. The lower amount of total protein content in maize genotypes could be because of more energy investment in synthesizing some essential metabolic compounds and the anti-nutritional proteins which might not have accounted for in estimation of total soluble proteins. The higher amount of total protein content in QPM genotypes favored *C. partellus* insect growth and development, which could be because of synthesis of more nutritional proteins in comparison to non-nutritional proteins present in other maize types. Present studies also indicated that the total protein content was lower in sweet corn genotypes which could be because of more investment of biochemical energy in production of carbohydrates than the nutritional proteins, again found favoring the growth and development of *C. partellus* on sweet corn genotypes. In general, the protein content in larvae fed on maize genotypes showed reverse trend with that present with high protein content, which was found to be low could be because of easy access to nutritional protein availability of high protein genotypes. These studies suggest that the specialty corn genotypes favor insect growth and development, could be because of higher amount of nutritional factors in comparison to anti-nutritional factors in QPM and sweet corn maize genotypes.

Conclusion

From the present studies it can be concluded that the survival and development of *C. partellus* on white and yellow kernel maize genotypes was significantly poor than that on QPM and sweet corn genotypes indicating their resistance to spotted stem borer..

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