

## Detection of epistatic interaction for susceptibility towards pink borer (*Sesamia inferens* Walker) in maize (*Zea mays* L.)

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### Abstract

Pink borer (*Sesamia inferens* Walker) is a major insect pest of winter maize across the peninsular India. In India winter season maize is more productive than rainy season maize. Thus, control of this insect pest assumes wide importance in ensuring higher maize productivity in India. Among various control measures resistance breeding is one of the most viable and sustainable options to control this insect pest. In the present investigation response of 48 promising maize inbred lines belonging to diverse sources to pink borer was analyzed and reported for the first time the inheritance pattern of resistance against pink borer in maize. Out of the 48 inbred lines screened under artificial infestation following standard technique, eight were found to be resistant to pink borer with leaf injury rating (LIR) score less than 3.0, while 16 were moderately resistant and 24 were highly susceptible. Generation mean analysis of a cross between E 62 and CML 451 revealed presence of negative additive and dominance effects, and positive additive  $\times$  dominance (*i*) and dominance  $\times$  dominance (*l*) epistatic interaction effects. Based on the findings pedigree and population improvement breeding with low selection intensity in early generations are suggested to develop new pink borer resistant lines. Single cross hybrids with lower pink borer susceptibility are feasible to develop with use of at least one of the resistant parent.

**Key words:** Maize, pink borer, insect resistance, inheritance, generation mean analysis

### Introduction

In India, maize production is greatly affected by the infestation of two insect pests, spotted stem borer [*Chilo partellus* (Swinhoe)] and pink borer (*Sesamia inferens* Walker). While stem borer is restricted to the northern part of the country during rainy season, pink borer

causes extensive damage to the crop in the peninsular India throughout the year and across the country during the winter season [1]. Pink borer with more extensive coverage, may cause damage up to 78.9% to the crop [2]. It is a pertinent point to note that productivity of maize in peninsular India is higher than the northern states, and that of winter maize (3.22 t/ha) is higher than the rainy season maize (1.74 t/ha) [3]. Thus to stabilize the maize production effective control of pink borer is of immense importance. The pink borer neonates enter the plant from base of the stem and their feeding results in dead heart symptom. Upon maturation the adults leave by making exit holes on the stem. The typical symptoms of the pink borer are gummy oozing with water soaked lesions at the bottom of leaf sheaths, oblong holes in the unfolded leaves, drying of the central shoots and dead heart in younger plants.

In the context of integrated pest management to control insect pest, growing of resistant cultivars is the most viable and sustainable option. Towards this direction first step is to identify sources of resistance against the target insect and secondly, to study the gene action involved in the expression of the trait so that appropriate breeding strategy for incorporating resistance may be planned [4, 5]. In this regard effective and efficient screening against the target insect is very important. Using the screening technique [6] involving cultured insects and leaf injury rating (LIR), large number of parental lines and experimental hybrids has been evaluated by several authors [1, 7, 8]. LIR is the best parameter to identify resistance level of a genotype [6].

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Generation mean analysis (GMA) has been employed to study genetic effects affecting various trait expression in many crops including maize [9-11]. It is relatively simple and reliable tool, in which first order statistics are used for estimating the genetic effects. Using generation mean analysis, Cartea *et al.* [12] reported additive-dominance model without non-allelic interactions for inheritance of antibiosis to *Sesamia nonagrioides* in two maize crosses, CM109 × EP31 (resistant × moderately resistant) and CM109 × EP42 (resistant × susceptible). Similar results emphasizing additive-dominance model of inheritance for resistance against *S. nonagrioides* have also been reported by Cartea *et al.* [5] and Velasco *et al.* [13]. However, to the best of our knowledge no reports are available in published literature on the inheritance studies against *S. inferens*. The present work was carried out with two objectives: (1) to study the extent of variation for response to the infestation of pink borer among promising maize inbred lines, and (2) to study the genetic effects defining the resistance against the pest using generation mean analysis.

## Materials and methods

### Screening of maize inbred lines against pink borer

Forty eight maize inbred lines (Table 1) were screened in augmented randomized block design in four blocks, using two checks with distinct response to the insect pest, CML 451 (susceptible) and CM 500 (resistant) in each block. The screening was carried out during winter season of 2007-08 at Winter Nursery, Directorate of Maize Research, Hyderabad. Each genotype was grown in two 3 m rows with inter- and intra-row spacing of 75 cm and 20 cm, respectively. Recommended agronomic practices were followed in raising the crop. The pink borer larvae were mass reared on artificial diet [14] and each plant was infested with ten neonate larvae of pink borer using larval dispenser, Bazooka. Leaf injury rating (LIR) on 1-9 scale [6] was recorded 35 days after infestation. Based on the rating scale, the 48 maize genotypes were classified into three distinct groups, using the specific range of values (least susceptible 1-3 score, moderately susceptible 4-6 score and highly susceptible 7-9 score). Square root transformed LIR scores were subjected to augmented analysis using Windostat ver. 7.5 (Indostat Services, Hyderabad).

### Inheritance of pink borer resistance

Among the identified least pink borer susceptible lines, E 62 is agronomically most superior and registered donor for pink borer resistance with National Bureau of

Plant Genetic Resources (India) (INGR No. 10077). On the other hand the highly susceptible check, CML 451 has profound pink borer susceptible expression across environments and is an inbred line. E 62 and CML 451 also have synchrony for flowering. Hence, these two lines were involved in crossing to develop six generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BCP_1$  and  $BCP_2$ ) to carry out generation mean analysis (GMA). The initial cross (E 62 × CML 451) was made in 2007-08 winter season, while  $BCP_1$ ,  $BCP_2$  and  $F_2$  were developed during 2008 rainy season. Six generations were evaluated during 2008-09 winter season in a randomized complete block design with two replications. Crossing and evaluation were carried out at the Winter Nursery, Directorate of Maize Research, Hyderabad. Non-segregating generation ( $P_1$ ,  $P_2$  and  $F_1$ ) were grown in two rows, while segregating generations ( $BCP_1$ ,  $BCP_2$  and  $F_2$ ) were grown in 10 rows. Row length was 3 m in 75 cm × 20 cm plant geometry, with approximately 15 plants per row. Each plant was artificially infested as described earlier. In each replication among the parental lines and  $F_1$  hybrids data were recorded on 10 randomly infested individuals totaling 20 plants per non-segregating generation, while in  $F_2$  and each backcross generation respectively 70 and 60 random infested plants were considered per replication leading to analysis of 140  $F_2$  plants and 120  $BCP_1$  and  $BCP_2$  plants each. GMA scaling test [15] was performed to test the adequacy of additive-dominance model. Four scaling tests were carried out as follows: A =  $2BCP_1 - P_1 - F_1$ ; B =  $2BCP_2 - P_2 - F_1$ ; C =  $4F_2 - 2F_1 - P_1 - P_2$ ; and D =  $2F_2 - BCP_1 - BCP_2$ . As inadequacy of the additive-dominance model was observed, the six parameter model of GMA [16] was employed to estimate various genetic components, viz.,  $m$  (mean),  $d$  (additive effect),  $h$  (dominance effect),  $i$  (additive × additive gene interaction),  $j$  (additive × dominance gene interaction) and  $l$  (dominance × dominance gene interaction). Joint scaling test was carried out according to Cavalli [18]. Three parameters  $m$ ,  $d$  and  $h$  were calculated and used to estimate the generation means of six generations subjecting the estimates to goodness of fit test. Significance of  $\chi^2$  values suggested again inadequacy of additive-dominance model. Subsequently, the data were subjected to step-down elimination of one component at a time and re-estimation of the remaining components and testing of the same. Finally model with all significant components were tested for goodness of fit using  $\chi^2$  test. Non-significance of  $\chi^2$  values suggested fitting of the model. Additive variance ( $\sigma^2_A$ ) and dominance variance ( $\sigma^2_D$ ) were calculated as follows:  $\sigma^2_A = 2\sigma^2_{F_2} - \sigma^2_{BCP_1} - \sigma^2_{BCP_2}$  and  $\sigma^2_D = \sigma^2_{BCP_1} + \sigma^2_{BCP_2} - \sigma^2_{F_2} - \sigma^2_E$ , where  $\sigma^2_{F_2}$ ,  $\sigma^2_{BCP_1}$

#Y – yellow; W – white; O – orange; F – flint; SF – semi flint; D – dent; <sup>s</sup>Values in parenthesis are transformed LIR score; CD at 5%: C<sub>i</sub>-C<sub>j</sub> (between checks) 0.07; B<sub>i</sub>V<sub>i</sub>-B<sub>j</sub>V<sub>j</sub> (between varieties within a block) 0.14; B<sub>i</sub>V<sub>i</sub>-B<sub>j</sub>V<sub>j</sub> (between varieties in different blocks) 0.17; C<sub>i</sub>-V<sub>j</sub> (between check and variety) 0.14

and  $\sigma^2_{BCP_2}$  are the variances in  $F_2$ ,  $BCP_1$  and  $BCP_2$  generations respectively, and  $\sigma^2_E$  is the environment variance.  $\sigma^2_E$  was calculated as follows:  $\sigma^2_E = (\sigma^2_{P_1} + \sigma^2_{P_2} + \sigma^2_{F_1})/3$ , where  $\sigma^2_{P_1}$ ,  $\sigma^2_{P_2}$  and  $\sigma^2_{F_1}$  are variance in  $P_1$ ,  $P_2$  and  $F_1$  generations, respectively. The calculated values were used to estimate heritability in broad sense ( $h^2_{bs}$ ) and in narrow sense ( $h^2_{ns}$ ) as follows:  $h^2_{bs} = (\sigma^2_A + \sigma^2_D) / (\sigma^2_A + \sigma^2_D + \sigma^2_E)$ , and  $h^2_{ns} = (\sigma^2_A) / (\sigma^2_A + \sigma^2_D + \sigma^2_E)$ . The data were analyzed using 'generation mean analysis' sub-command of Advanced Biometrics module of Windostat ver. 7.5 (Indostat Services, Hyderabad).

## Results and discussion

### Resistance response of lines against pink borer

The LIR scores of the lines are presented in Table 1. Mean squares due to checks, varieties and check vs. varieties were highly significant with  $P$  values of 0.00003, 0.0003 and 0.0007, respectively. This suggested significant variation among the lines under investigation in terms of pink borer resistance. Among the 48 lines investigated eight proved to be least susceptible to pink borer with LIR score less than 3.0, while 16 lines found to be moderately susceptible with LIR score ranging from 3.0 to 6.9. Remaining 24 lines were highly susceptible to pink borer registering LIR scores between 7.0 and 9.0. Further, nine of these lines showed more susceptibility than the susceptible check, CML 451. Of the eight lines found to be least susceptible to pink borer, seven (E63, E4, E5, E9, E 37, E 57, E62) had significantly lower LIR scores than the resistant check, CM 500. In a similar experiment Sekhar et al. [1] failed to identify any resistant or least susceptible line upon screening of 28 exotic lines. They reported four moderate susceptible and 24 highly susceptible lines. Among the resistant or least susceptible group identified in the present study all but HKI 1128 have been developed through a cyclic improvement programme [8]. As all these lines are agronomically desirable with good seed yield, they can potentially be used in breeding programme. The other resistant line, HKI 1128 has been used as male parent in single cross hybrid, HM 9 [19]. As the line showed pink borer resistance, it may further be used in development of other hybrids. Identified pink borer resistant inbred lines can be useful for analyzing and ascertaining biochemical and molecular mechanism of resistance response to the pink borer. Further, populations generated from inter-mating among the resistant genotypes could be appropriately handled for deriving new lines and even might be screened against the infestation of stem borer, another important pest

relevant to Indian conditions to identify multiple insect resistant lines. Selected least susceptible lines may be involved in chain cross to develop pink borer resistance gene pool for extraction of new inbred lines.

### Genetics of pink borer resistance

The best performing pink borer resistant or least susceptible line, E 62 was crossed with the susceptible inbred line, CML 451 to generate six generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $BCP_1$ ,  $BCP_2$  and  $F_2$ ) for generation mean analysis. The resistant parent, E 62 registered LIR of  $2.00 \pm 0.30$ , while the susceptible parent, CML 451 recorded average LIR of  $8.08 \pm 0.15$ . Consistency of the LIR score of resistant and susceptible parents in two replications indicates robustness of the screening method. The  $F_1$  hybrid revealed an intermediate LIR score of  $6.83 \pm 0.17$ . This comes in susceptible range, suggesting partial dominance of resistance over susceptibility with degree of dominance of 1.52. The mid-parent heterosis was highly significant at 20.65%. The mean LIR scores of  $F_2$ ,  $BCP_1$  and  $BCP_2$  were  $4.00 \pm 0.48$ ,  $3.17 \pm 0.53$  and  $3.33 \pm 0.45$ , respectively. Data suggested inbreeding depression of 23.09% and heritability in broad sense ( $h^2_{bs}$ ) and narrow sense ( $h^2_{ns}$ ) of 0.81 and 0.22, respectively. Values for the scaling test parameters, A, B, C and D were  $-0.52 \pm 0.27$ ,  $-1.79 \pm 0.22$ ,  $-1.57 \pm 0.47$  and  $0.37 \pm 0.28$ , respectively. Among the four scaling tests, B and C were highly significant with  $P$  values of 0.000 and 0.002, respectively. This indicated inadequacy of additive-dominance model and presence of additive  $\times$  dominance ( $j$ ) and dominance  $\times$  dominance ( $l$ ) interactions. Data were further fitted to three parameter model of joint scaling test [17]. However, fitting of the model with the estimated  $m$ ,  $d$  and  $h$  values gave a highly significant  $\chi^2$  value of 78.03 ( $P = 0.000$ ) (Table 2). This indicated absence of additive-dominance model in this cross. Several models with various interactions were tested and step-wise elimination revealed existence of additive ( $d$ ), dominance ( $h$ ), additive  $\times$  dominance ( $j$ ) and dominance  $\times$  dominance ( $l$ ) gene interactions (Table 2). The  $\chi^2$  value of the model (1.71) was insignificant ( $P = 0.191$ ). Inadequacy of additive-dominance model and frequency distribution pattern in the segregating generations suggested that pink borer resistance in maize was governed by more than one gene in this cross. Similar results were reported for downy mildew resistance in muskmelon [20]. This finding contradicts the earlier reports [5, 12, 13] where the absence of non-allelic interactions was observed towards resistance against another species, i.e., *S. nonagrioides* in maize. Genetics of resistance against these two different

**Table 2.** Joint scaling test and goodness-of-fit test for pink borer tolerance in different generation

| Parameter       | Three parameter model |         | Best fit model |         |
|-----------------|-----------------------|---------|----------------|---------|
|                 | Estimate              | P value | Estimate       | P value |
| m (mean)        | 2.18±0.04             | 0.000   | 2.24±0.05      | 0.001   |
| d (additive)    | 0.72±0.04             | 0.000   | -0.69±0.05     | 0.002   |
| h (dominance)   | 0.48±0.06             | 0.000   | -1.59±0.30     | 0.000   |
| i (add. × add.) | -                     | -       | -              | -       |
| j (add. × dom.) | -                     | -       | 1.30±0.34      | 0.005   |
| l (dom. × dom.) | -                     | -       | 2.06±0.29      | 0.001   |
| $\chi^2$        | 78.03                 | 0.000   | 1.71           | 0.191   |

species may differ as the resistance mechanisms are likely to be different.

Generation mean analysis assumes unidirectional distribution of genes between two parents [20]. However, detection of epistatic interactions contradicts this assumption. The overlapping nature of the BCP<sub>1</sub> and BCP<sub>2</sub> is indicative of existence of epistatic interaction [21]. Such interactions make the partitioning of genetic variance in the segregating generations into additive or dominance components extremely biased. Thus, only the detected broad sense heritability ( $h^2_{bs}$ ) of 0.81 makes relevance in the study. This suggested that transfer of resistance factor from donor parent to recipient parent is a practical proposition for pink borer tolerance in maize. In our study we failed to obtain transgressive segregants in F<sub>2</sub> or BC generations, suggesting absence of resistance factors in the susceptible line.

In the current study both the additive and dominance effects were negative, while the epistatic interaction effects ( $j$  and  $l$ ) were positive. This indicated that for pink borer resistance, the additive ( $d$ ) and dominance ( $h$ ) effects contributed towards reducing the LIR score, with higher effect of dominance component. On the other hand, the additive × dominance ( $j$ ) and dominance × dominance ( $l$ ) epistatic interactions increased the susceptibility towards pink borer. This suggested that though the resistant alleles contributed towards reduction of the LIR, internal cancellation by opposite  $j$  and  $l$  type epistatic interactions enhanced susceptibility. In muskmelon, positive additive and dominance effects, and negative additive × dominance and dominance × dominance interactions was recorded for downy mildew resistance [20]. We have observed higher magnitude of dominance components ( $h$ ) towards negative side suggesting larger role of dominance gene

effects compared to that of additive gene effects in inheritance of pink borer resistance in this cross. The study also revealed that the fixable gene effect ( $d$ ) was lower than the non-fixable ( $h$ ,  $j$ ,  $l$ ) effects indicating greater role of non-additive effects in inheritance of resistance to pink borer. Existence of significant additive and non-additive effects towards pink borer resistance suggested utility of pedigree and population improvement (reciprocal recurrent selection) breeding in developing new pink borer lines involving E 62 and may be other lines. However, presence of dominance and epistasis likely to retard the pace of progress of incorporation of the trait through early generation selections. A lenient selection pressure may be necessary in early generations, which may be intensified as the lines get fixed slowly [21]. Though development of pink borer resistant lines through pedigree breeding or through population improvement may be time consuming, development of hybrids with reduced pink borer susceptibility is feasible provided one of the parents is pink borer resistant. It may be noted that gene effects are cross specific. Thus, to make a generalized prediction on the mode of gene effects in controlling pink borer resistance more number of crosses involving identified resistant lines may be used in future. However, in view of current findings using one pink borer resistant line single cross hybrids with reduced pink borer susceptibility may be developed, which will have immense practical and economic utility.

This work is a first report on genetics of pink borer resistance in maize. The study has suggested presence of non-allelic interactions in susceptibility against pink borer. However, since gene actions are cross specific the finding needs to be extended involving more number of crosses. The generated information suggested genetic dissection of the quantitative resistance against

pink borer using molecular markers. Identification of linked markers can be used to identify rare combinations containing favourable alleles.

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