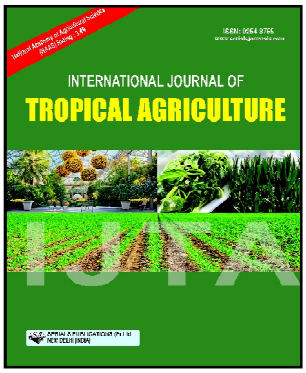

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Selection and inheritance of Cry1Ac resistance in eggplant fruit and shoot borer (*Leucinodes orbonalis*)

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Abstract: Brinjal fruit and shoot borer (*Leucinodes orbonalis*) is one of the most destructive pests of brinjal in India, Bangladesh, and South East Asia. *Bt* brinjal expressing Cry1Ac protein was developed as a viable alternative as the existing management options are being proven ineffective. One of the basic assumptions in release of a *Bt* product is that any resistance inheritance to the *Bt* protein is recessive in nature. In this study we report the results of the selection and inheritance of Cry1Ac resistance in laboratory selected EFSB colony. Selection of *L. orbonalis* was carried out using a laboratory colony on *Bt* brinjal expressing Cry1Ac protein. The inheritance of the selected Cry1Ac resistance in the resistant colony was studied by mating adults as single mating pairs of the Cry1Ac-resistant and laboratory susceptible colonies. The males and females of resistant and susceptible colonies were sexed, separated and allowed to mate in pairs *viz*., susceptible (@&) x Cry1Ac-resistant (B&) and Cry1Ac-resistant (@&)x susceptible (B&). The F1 neonates of these crosses were subjected to bioassay using Cry1Ac protein and observations were recorded after seven days. The LC_{50} values were calculated and were used to estimate the dominance ratio (*D*). The calculated LC_{50} values and estimated degree of dominance (*D*), by Stone's formula, indicated that the resistance to Cry1Ac protein in *L. orbonalis* larvae is an autosomal trait and is inherited in an incompletely recessive manner.

Key words: *L. orbonalis*, selection, inheritance studies

INTRODUCTION

Brinjal, *Solanum melongena* is an important cash crop for small and marginal farmers in India and cultivated in almost all the seasons. Farmers start harvesting fruits at about 60 days after planting and continue to harvest till 120 days, thereby providing a steady

supply of food for the family and stable income for most of the year. India is one of the largest producer of brinjal in the world, where the crop is cultivated in an area of 6.80 lakh hectares and annual production of around 12.55 MT [1]. The major constraints for brinjal fruit production are the insect pests and brinjal fruit and shoot borer, *Leucinodes orbonalis* (Guenee) (Lepidoptera: Pyralidae), is the most serious and destructive pest that inflicts damage by attacking tender shoots in nursery and growing fruits till harvest. The pest is known to cause yield loss of 70-92% [2, 3, 4]. The young larvae bore into the nearest tender shoot, flower or fruit. Soon after boring into shoots or fruits, they plug the entrance hole with excreta. Larval feeding inside the fruit results in destruction of fruit tissues making them unfit for marketing. The management of this pest is through calendar spraying of insecticides irrespective of pest incidence. The extensive and indiscriminate use of conventional insecticide over period of time leads to development of resistance to insecticides [5].

Insect protective transgenic crops that express Cry toxins derived from *Bacillus thuringiensis* (Berliner) (Bt), are being deployed successfully since 1996. During 2016, biotech crops were planted on 185.1 million hectares by ~18 million farmers in 26 countries [6]. In India, Bt cotton (*Gossypium hirsutum* L.) expressing Cry1Ac toxin was introduced in 2002 and Bollgard II expressing two genes viz., Cry1Ac and Cry2Ab was released for commercial cultivation in 2006. Bt brinjal expressing Cry1Ac protein was developed during and initially tested during 2002-2008. It is very effective in controlling *L. orbonalis*, as demonstrated in regulatory trials in India [7]. The control against fruit and shoot borer and benefits of cultivating Bt Brinjal was reported in Bangladesh and Philippines [8]. Consequently, understanding the risks involved in resistance development to the Bt proteins and deciphering the mechanisms of resistance inheritance are important for designing sustainable resistance management plans. Here we present results of a study conducted to evaluate the inheritance of Cry1Ac resistance in *L. orbonalis*.

MATERIALS AND METHODS

Insects and Cry1Ac selection: Two colonies of the insect were used in our study *viz*, Cry1Ac-susceptible laboratory colony (S) and a Cry1Ac-resistant colony (BF-RM). The Cry1Ac-resistant BF-RM colony was developed through selection of *L. orbonalis* larvae using Bt brinjal fruits as the source of Cry1Ac protein. Bt Brinjal plants expressing Cry1Ac protein, of the event EE-1, were planted in staggered manner to provide consistent source of Cry1Ac protein and maintained under Greenhouse conditions. Care was taken not to spray any chemical insecticides on these plants. The initial rounds of selection were done by releasing approximately 1300-1500 neonates on Bt Brinjal fruits and the larvae surviving after 3rd or 4th day were transferred to a diet containing no Cry1Ac protein. Subsequently, the surviving larvae from prior round of selection were transferred and fed upon Bt Brinjal fruits for five to six days. In all cases, the surviving larvae were transferred to a diet containing no Cry1Ac protein. The larvae were reared to pupae on diet and the emerging adults are bulk mated in a cage, following standard rearing procedure [5]. During the selection process, every few generations, a portion of the neonates are used in a dose-range assay to estimate the fold resistance in selected colony (BF-RM).

Inheritance studies: The resistance inheritance experiment was conducted twice during the entire study period (2015-16). Initially the third instars of the Cry1Ac-susceptible and BF-RM colony were identified and separated for rearing on artificial diet in separate containers. Pupae were collected separately and the adults of BF-RM and Cry1Ac-susceptible colonies that emerged were allowed to mate as single pairs, resulting in development of two pairs of crosses (reciprocal crosses) *viz.,* Resistant (R)(@&) x Susceptible (SC)(B&) and Resistant

(R)(B&) x Susceptible (SC)(@&). The F1 progeny produced by single pair mating in separate containers were used in the bioassays.

Bioassays: Bioassays involved exposure of neonate larvae to various concentrations of diet incorporated Cry1Ac protein that produced 0-100% mortality. A stock solution (250 µg/ml) of Cry1Ac was made in 0.2% agar solution and dilutions were made in deionized water. The source of Cry1Ac protein used in the bioassays was the commercial formulation, MVP II® (Mycogen Corp., USA), which contained 19.7% (by weight) Cry1Ac protein. Various concentrations of Cry1Ac were later mixed in the semi-synthetic diet and 750 µl of Cry1Ac mixed diet was poured into single well of the bioassay tray. One neonate larva was placed in each well and there were 32 larvae per replication with a total of three replications for each Cry1Ac concentration. Bioassay trays were kept in dark at a temperature of 26±1°C and 55-65% RH. Larval mortality and instar stage of surviving larvae were recorded on 7th day. Larvae that did not move when disturbed were considered to be dead.

Data Analysis: The probit analysis of mortality data from Cry1Ac bioassays was done using POLO-PC [9]. The LC_{50} values and the estimate of dominance (D_{LC}) were calculated. The degree of dominance (*d*) was determined using the formula of Stone (1968) [$d = (2x2 - x1 - x3)/x1 - x3$, where, $x1$, $x2$, and $x3$ are the logarithms of the LC_{50} (concentration for 50 % lethality) values for resistant, F1 hybrid, and susceptible strains, respectively. The estimate of dominance is estimated as $(D_{LC}) = (d+1)/2$.

RESULTS

The LC_{50} values estimated from the dose-range assays demonstrated that the susceptibility value of the resistant colony, BF-RM, increased 28.21-fold compared to the susceptible laboratory colony after 60 generations of exposure to Cry1Ac protein (Fig 1). The LC_{50} value of resistant parent (BF-RM) after 60 generations of exposure was 1.89 ppm where as LC_{50} value at F0 (Initial generation) was 0.067 ppm (Figure 1).

The resistance inheritance studies were done twice during the study period. The LC_{50} values of the assays conducted with the neonates of the reciprocal crosses is presented in Table 1. The mean LC_{50} values of assays done with reciprocal crosses viz., BF-RM (♀) x S (♂) and BF-RM (♀) x S (♂) were estimated to be 0.019 ppm and 0.010 ppm, respectively (Table 2). The LC_{50} value of the susceptible colony was estimated to be 0.002 ppm. The dominance values (D_{LC}) were estimated during 2015 and 2016. During 2015, the dominance values were observed as 0.32 and 0.23 for the crosses BF-RM (♀) x S (♂) and BF-RM (♀) x S (♂), respectively. During 2016, the dominance values were observed as 0.40 and 0.29 for the crosses BF-RM (♀) x S (♂) and BF-RM (♀) x S (♂), respectively

The mean of dominance values were (D_{LC}) estimated and are presented in Table 1. The mean values were estimated as 0.37 and 0.26 for the crosses R (♀) x SC (♂) and R (♂) x SC (@&), respectively.

The inheritance of Cry1Ac resistance as calculated from the LC_{50} values was found to be an incompletely recessive trait (Table 1). Also, the susceptibility values and the inheritance of the resistance indicated that the Cry1Ac resistance in *L. orbonalis* is autosomal in nature and is not sex-linked (Figure 2).

DISCUSSION

There have been no earlier reports of resistance inheritance studies conducted with *L. orbonalis* colonies. In our study inheritance of the Cry1Ac resistance in *L. orbonalis* has been found to be incompletely recessive trait. [10], conducted inheritance study using Cry1F against European corn borer, *Ostrinia nubilalis*, (Family: Crambidae), indicated

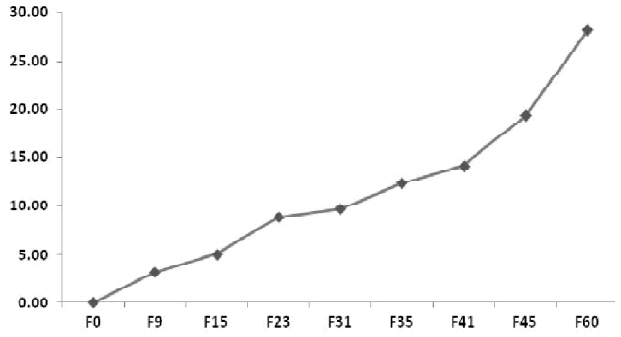

Figure 1: Fold increase at LC50 value exhibited by BF-RM colony at different generations

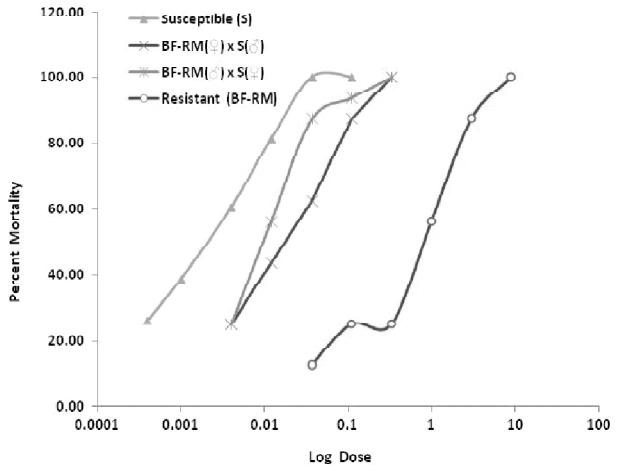

Figure 2: Log dose mortality exhibited by resistant, susceptible, crosses of resistant and susceptible colony

Table 1
Susceptibility responses in parents and crosses and the heritability values, estimated through diet incorporated Cry1Ac assays

Colony	*n*	LC_{50} *(ppm)*	D_{LC} *value*
Resistant (BF-RM)	128	0.954	-
Susceptible (S)	128	0.002	-
BF-RM (♀) x S (♂)	128	0.019	0.37
BF-RM (♂) x S (♀)	128	0.010	0.26

that the resistance is autosomal and recessive. Similar kinds of results were also observed by Alves [11] and Sandhya [12] in insect species *Plutella xylostella*. The calculated LC_{50} values of the crosses DB-RM (♀) x S (♂) and DB-RM (♂) x S (♀) and the estimated dominance values indicate that the resistance to Cry1Ac protein in *P. xylostella* larvae is an autosomal trait that is inherited in an incompletely recessive or partially recessive manner. Similar, results were observed in *Plutella xylostella* by Hama [13], Tabashnik [14], Martínez-Ramírez [15], Tabashnik [16], Tang [17] and Sayyed [18]. In most of the studies related to heliothines, resistance to Bt toxin was inherited as recessive or incompletely recessive trait [19, 20, 21, 22, 23]. In certain other colonies resistance was reported to be more dominant than recessive [24, 25, 19, 26, 23, 20]. Reciprocal genetic crosses between Cry1Ac-reselected and susceptible population (ROTH insects) in Diamondback moth indicated that resistance was autosomal and at the highest dose of Cry1Ac tested, resistance was recessive while at the lowest dose it was almost completely dominant [18]. Similar results were also reported by [34, 35] from their resistance inheritance studies with Cry1Ie and Cry1F proteins in *O.furnicalis*. Although we did not select the Cry1Ac-resistant *L. orbonalis* colony at different selection concentrations, the selected concentration of 40 to 46 ug/g (fresh weight basis) is considered a high dose in Bt Brinjal event, EE-1. Accordingly, the results obtained in our studies corroborates to previously reported results in genera from same family, as discussed above.

In our study found 28-fold of resistance in *L. orbonalis* after 60 generations of selection. Slower rate of resistance development has been found. Gould et al.[19] found a drastic increase only between 12th and 19th episodes of selection after exposing a maximum of 1760 neonates of *H. virescens* for 30 generations. Kranthi et al. [27] found no apparent change in susceptibility of *H. armigera* in initial 4-5 generations of selection. However, an increased resistance of 76-fold was observed at the end of 10th generation. Further, Wu and Guo [28] also found slow resistance development in *H. armigera* reaching only 6-fold after 15 generations of selection.

Slower rate of resistance may observed due to fitness costs which occur throughout three distinct phases in different insects [29]. In the first phase, we assumed that only physiological adaptations (a common phenomenon in arthropods to any hazard that causes no obvious fitness costs) were involved and the fitness cost increased very slowly. In a second phase, we assumed that selection of genetic mutations conferring high level of resistance was involved. The regulation of resistance genes, such as over-expression of detoxification enzymes [30, 31]. Change in hormones induced by detoxification enzymes, and structural modification of targeted genes has been considered the most important factor producing fitness cost [32]. In a third phase, the fitness cost reached a plateau. This might result from new mutations providing higher resistance level but fewer pleiotropic effects, or from compensatory mutations (other mutations) that might have been selected. Alternatively, new mutations conferring higher resistance levels but lower fitness cost could have been selected and have taken over resistance alleles that caused high fitness costs.

The results presented here could have significant implications for resistance management strategies, especially for the high-dose/ refuge strategy. The results indicated that the presence of Cry1Ac may significantly slow the development of resistance.

CONCLUSIONS

This study indicates that laboratory selected *L. orbonalis* developed 28 fold resistance when compared with laboratory susceptible colony. It was observed that resistance of the BF-RM to Cry1Ac is inherited as recessive or incompletely recessive. The recessive form of inheritance fits into the basic assumptions of insect resistance management strategy for Bt crops. When the resistance inheritance is recessive in nature, the progeny from mating of resistant and susceptible insects will die on Bt crops, substantially slowing the evolution of resistance [33].

REFERENCES

National Horticulture Board (2014). Indian Horticulture Database, Ministry of Agriculture, Government of India, 85, Institutional Area, Sector-18, Gurgaon-122 015, www.nhb.gov.in

Mall N , Pandey R , Singh S and Singh K (1992). Seasonal incidence of insect pests and estimation of the losses caused by shoot-and-fruit borer on brinjal. *Indian J. of Ent.* 54: 241–247.

Rahman A (1997). Screening of 28 brinjal line for resistance/tolerance against the brinjal shoot and fruit borer. *Annual Res. Report*. Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh 32–35.

Roy C and Pande D (1994). Damage to brinjal by *L. orbonalis* (Lepidoptera:Pyralidae) and economic of its insecticidal control. *Indian J. Agr. Res.* 28:110–120.

Shirale D K, Patil M U, and Srinivas Parimi (2017). Insecticide resistance in field populations of *L. orbonalis* (Lepidoptera: Crambidae) in India. *Candian Entomol.*, 149 : 399-407.

ISAAA (2016). International service for acquisition of agri-biotech applications. http://www.isaaa.org/resources/publications/pocketk/16/

ISAAA (2009). The International Service for the Acquisition of Agri-biotech Applications (ISAAA). The development and regulations of Bt Brinjal in India report by Bhagirath Chodhary and Kadambmini Gaur. Brief No.38.

Shelton T and Boersma H (2016). Talking Biotech: GMO Bt eggplant helps poor Bangladesh farmers cut insecticide use. Genetic Literacy Project. August 29, 2016. https://geneticliteracyproject.org/2016/08/29/talking-biotech-gmo-bt-eggplant-helps-poor-bangladesh-farmers-cut-insecticide-use

Finney D J (1971). Probit Analysis, Cambridge Univ. Press, Cambridge, UK.

Pereira E J G, Storer N P and Siegfried B D (2008). Inheritance of Cry1F resistance in laboratory-selected European corn borer and its survival on

transgenic corn expressing the Cry1F toxin. *Bulletin of Entomol Res.* 98 (06): 621-629.

Alves A P, Spences T A, Tabashnik B E and Siegfried B D (2006). Inheritance of Resistance to the Cry1Ab *Bacillus thuringiensis* Toxin in *Ostrinia nubilalis* (Lepidoptera: Crambidae). *J. Econ. Entomol.* 99(2): 494-501.

Sandhya S, Mandlik S, Shirale D, Patil M and Parimi S (2015). Selection and inheritance of Cry1Ac resistance in Diamondback moth Proceedings of The Sixth International. Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests. AVRDC, Taiwan. 57.

Hama H, Suzuki K and Tanaka H (1992). Inheritance and stability of resistance to *Bacillus thuringiensis* formulations of the Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 27: 355–362.

Tabashnik B, Schwartz J, Finson N And Johnson M (1992). Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J Econ. Entomol.* 85:1046–1055.

Martinez A, Escriche B, Real M, Silva F and Ferre J (1995). Inheritance of resistance to a *Bacillus thuringiensis* toxin in a field population of Diamondback moth (*Plutella xylostella*). *Pestic. Sci.,* 43:115–120.

Tabashnik B, LiuY, Finson N, Masson L and Heckel D (1997) One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proc. Natl. Acad. Sci.,* 94:1640–1644.

Tang J, Gilboa S, Roush R and Shelton A (1997). Inheritance, stability, and lack-of-fitness costs of field-selected resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae) from Florida. *J. Econ. Entomol.,* 90:732–741.

Sayyed A, Ferre J and Wright D (2000). Mode of inheritance and stability of resistance to *Bacillus thuringiensis* var *kurstaki* in a Diamondback moth (*Plutella xylostella*) population from Malaysia. *Pest Manag. Sci.,* 56:743–748.

Gould F, Anderson A, Reynolds A, Bumgarner L and Moar W (1995). Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera:Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J. Econ. Entomol.*, 88:1545–1559.

Akhurst J, James W, Bird L and Beard C (2003). Resistance to the Cry1Ac delta-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.,* 96:1290–1299.

Bird L and Akhurst J (2004). Relative fitness of Cry1A-resistant and susceptible *Helicoverpa armigera* (Lepidoptera:Noctuidae) on conventional and transgenic cotton. *J. Econ. Entomol.,* 97: 1699–1709.

Xu X, Yu L and Wo Y (2005). Disruption of a cadherin gene associated with resistance to Cry1Ac d-endotoxin of *Bacillus thuringiensis* in *Helicoverpa armigera*. *Appl. Environ. Microbiol.,* 71:948–954.

Liang G, Tan J and Guo Y (2000). Study on screening and inheritance mode of resistance to *Bt* transgenic cotton in cotton bollworm. *Acta Entomol. Sinica.,* 43:57–62.

Huang F, Buschman, L, Higgins A and McGaughey H (1999) Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science*, 284: 965–967.

Liu Y and Tabashnik B (1997). Inheritance of resistance to the *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. *Appl. Environ. Microbiol.*, 63:2218–2223.

Daly J and Olsen K (2000). Genetics of Bt resistance. In Proceedings 10th Australian Cotton Conference 16-18 August 2000 Brisbane Australia. Australian Cotton Growers' Research Association Narrabri Australia 185–188.

Kranthi K, Kranthi S, Ali S and Banerjee S (2000). Resistance to Cry1Ac dendotoxin of *Bacillus thuringiensis* in a laboratory selected strain of *Helicoverpa armigera* (Hübner). *Curr. Sci.,* 78 1001–1004.

Wu K and Guo Y (2004). Changes in susceptibility to conventional insecticides of a Cry1Ac-selected population of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Pest Manage. Sci.,* 60: 680–684.

Liu N N, Zhu F, Xu Q, Pridgeon J W and Gao X W (2006). Behavioral change, physiological modification, and metabolic detoxification: mechanisms of insecticide resistance. *Acta. Entom. Sin.*, 49:671-679.

Gunning R V, Dang H T, Kemp F C, Nicholson I C and Moores G D (2005). New resistance mechanism in *Helicoverpa armigera* threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin. *Appl. Environ. Microbiol.*, 71:2558-2563.

Caccia S. (2012). Association of Cry1Ac toxin resistance in *Helicoverpa zea* (Boddie) with increased alkaline phosphatase levels in the midgut lumen. *Appl. Environ. Microbiol.,* 78:5690 –5698.

Li X, Schuler M A, and Berenbaum M R (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Ann. Review of Entomol.*, 52:231-253.

Tabashnik B E, Van Rensburg J B J, and Carriere Y(2009). Field-evolved insect resistance to Bt crops: definition, theory, and data. *J. Econ. Entomol.* 102: 2011–2025.

Wang Y, Wang Y, Wang Z, Bravo A, Soberón M, He K (2016) Genetic Basis of Cry1FResistance in a Laboratory Selected Asian Corn Borer Strain and Its Cross-Resistance to Other *Bacillus thuringiensis* Toxins. *PLoS ONE*, 11(8).

Wang Y, Yang J, Quan Y, Wang Z, Cai W and He K(2017). Characterization of Asian Corn Borer Resistance to Bt Toxin Cry1Ie. *Toxins, 9(186).*