

Table S1. Populations of *Spodoptera frugiperda* collected in different countries and years used for genotyping of target-site mutations.

Country	Sample ID	City. State	Year	Host plant
Brazil ¹	Sf_Bra	Unknown, São Paulo	2005	corn
	Sf_Cor	Correntina, Bahia	2016	corn
	Sf_Des	São Desidério, Bahia	2016	corn
	PR-PG	Ponta Grossa, Paraná	2018	corn
	SP-IT	Ituverava, São Paulo	2018	corn
	MS-CS	Chapadão do Sul, Mato Grosso do Sul	2018	corn
	MT-SZ	Sapezal, Mato Grosso	2018	corn
	MT-TS	Tangará da Serra, Mato Grosso	2018	corn
	MT-PL1-2	Primavera do Leste, Mato Grosso	2018	corn
	MT-LV	Lucas do Rio Verde, Mato Grosso	2018	corn
	BA-SD	São Desidério, Bahia	2018	corn
RO-VI	Vilhena, Rondônia	2018	corn	
Indonesia	WS-I	Padang Pariaman, Sumatra	2019	corn
	DS-I	Deli Serdang, Sumatra	2019	corn
	S-I	Simalungun, Sumatra	2019	corn
	WC-I	Waled Cirebon, Java	2019	corn
	BC-I	Babakan Cirebon, Java	2019	corn
	JL-I	Jati Agung, Lampung	2019	corn
	SB-I	Saputih Banyak, Lampung	2019	corn
	K-I	Kediri, Java	2019	corn
B-I	Blitar, Java	2019	corn	
Kenya	EP-K	Eldoret	2019	corn
	KV-K	Kisii	2019	corn
	NJ-K	Nakuru	2019	corn
	MJ-K	Muranga	2019	corn
	MD-K	Mombasa	2019	corn
	KF-K	Kajiado	2019	corn
	BA-K	Bungoma	2019	corn
	NW-K	Narok	2019	corn
Puerto Rico	PR60	Ponce	2017	corn
	PR61	Ponce	2017	corn
	PR62	Ponce	2017	corn
	PR63	Ponce	2017	corn
	PR64	Ponce	2017	corn

¹ Samples were described in Boaventura et al. (2020) [1] and Boaventura et al. (2020) [2].

Table S2. List of primers for pyrosequencing and dual fluorescence probe assay used for the identification of different target-site mutations and *Spodoptera frugiperda* strain identification by RFLP-PCR and Sanger sequencing.

Target	Mutation	Primers	Sequence (5'- 3')	Annealing Temperature (°C)	Assay	
Ryanodine receptor	G4946E ¹	Sf_G4946_F	GTGATGGGCAACTTCAAC	50	Pyrosequencing	
		Sf_G4946_R.btn	[btn]TTTTCCGTTATGCGTGAC			
		Sf_G4946_F.Seq	ATTTGCTAGATGTCGCT			
	I4790M ¹	Sf_I4790_F.btn	[btn]CGAGGACTTCTTCTACATGG-	50		
		Sf_taq_I4790_R	CACCTTGAGATGATAGTACC			
		Sf_I4790_R.Seq	ATGGTAGTACCCGATGA			
I4790M ¹	Sf_taq_I4790_F	ACGACGATGCACTAGAAG	60.6	Probe assay		
	Sf_taq_I4790_R	CACCTTGAGATGATAGTACC				
	Sf_I4790_HEX	[HEX]TGTCGCTCGCTATACTCATCG[BHQ1]				
Voltage-gated sodium channel	L1014F	Sf_L1014_F	TCTTCCTGGCTACAGTCG	50	Pyrosequencing	
		Sf_L1014_R.btn	[btn]GACAGTAACAGGGCCAAG			
		Sf_L1014_Seq	CAGTCGTCATYGGCA			
	L932F/T929I	Sf_L932_T929_F.btn	[btn]TAATGGGTAGGACAATGG	53		
		Sf_L932_T929_R	AATCCACGTAATTTTCC			
Acetylcholinesterase	F290V	Sf_F290_F	GCATCCGATTAGCAGAAG	52	Pyrosequencing	
		Sf_F290_R	[btn]TATGATGGGCACAAAAGG			
		Sf_L932_F	GAACCTTTGGTATTTGTGA			
		Sf_taq_F290_F	CCAGATGAACTAGTCAATAATG			
	A201S / G227A	Sf_taq_F290_R	GGAACGAACCATCTATGA	60	Probe assay	
		Sf_F290_FAM	[FAM]TATTTGTGAATTCCTTTTGTGCC[BHQ1]			
		Sf_F290_mut_HEX	[6HEX]TATTTGTGAAGTTCCTTTTGTGCC[BHQ1]			
		Sf_A201S_G227A_F	TTGATACCCCTGATGTACC			
ATP-binding cassette transporter subfamily C2	GY deletion ²	Sf_A201S_G227A_R	[btn]AATGAAACCGAACTGCTC	53	Pyrosequencing	
		Sf_A201S_Seq	TAACATTATTCGGTGAGTC			
		Sf_G227A_Seq	GGCGATAATGCAGTCA			
	GC insertion ³	Sf_788-Gydel_F	[btn] CCGACTACTGGCTTAGTTT	50		Pyrosequencing
		Sf_788-Gydel_R	GCTCGCATAGTCATCACT			
Mitochondrial cytochrome oxidase subunit I	JM76 ⁵	Sf_788-GYdel_seq	CTTCGGGTAAAGTTTGT	60	PCR-RFLP	
		Sf_ABCC2_F	TGGAGGCCGAAGAGAGACA			
		Sf_ABCC2_R	AGGAGTTGACTGACTTCATGTACCT			
Mitochondrial cytochrome oxidase subunit I	JM76 ⁵	SfABCC2mut allele	[HEX]AAGCACATCGCCCACTT[BHQ1]	60	Probe assay	
		SfABCC2	[6FAM]CCAAGCACATCCCACTT[BHQ1]			

	JM77 ⁵	ATCACCTCCWCCTGCAGGATC		
	891F_COI ⁵	TACACGAGCATATTTTACATC	52	
	c1303R_COI ⁵	CAGGATAGTCAGAATATCGACG		
Triosephosphate isomerase	TpiE4 ⁶	CCGGACTGAAGGTTATCGCTTG	56	PCR-Seq
	850R ⁶	AATTTTATTACCTGCTGTGG		

Primers described by ¹Boaventura et al. (2020) [2]; ²Boaventura et al. (2020) [1]; ⁴Banerjee et al. (2017) [3]; ⁵Nagoshi et al. (2017) [4]; ⁶Nagoshi et al. (2019) [5].

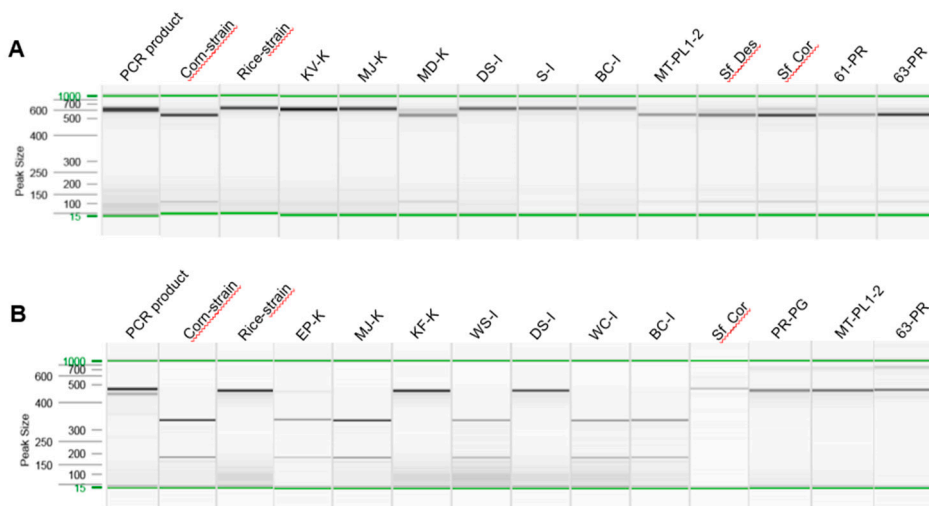


Figure S1. Automated analysis of DNA fragments showing *COI* polymorphism in *Spodoptera frugiperda*. (A) PCR product containing a strain specific *MspI* site that was amplified using the JM76 and JM77 primers (Table S2) followed by products obtained after the digestion with FastDigest *MspI*. Corn-strain is cut and rice-strain remains uncut as it does not have the *MspI* site. (B) PCR product amplified with the primers 891F_COI and c1303R_COI (Table S2) that contains a *EcoRV* strain specific site. After digestion with *EcoRV* the corn-strain amplicon remains uncut whereas it is cut in the rice-strain. Details about samples, see Table S1.

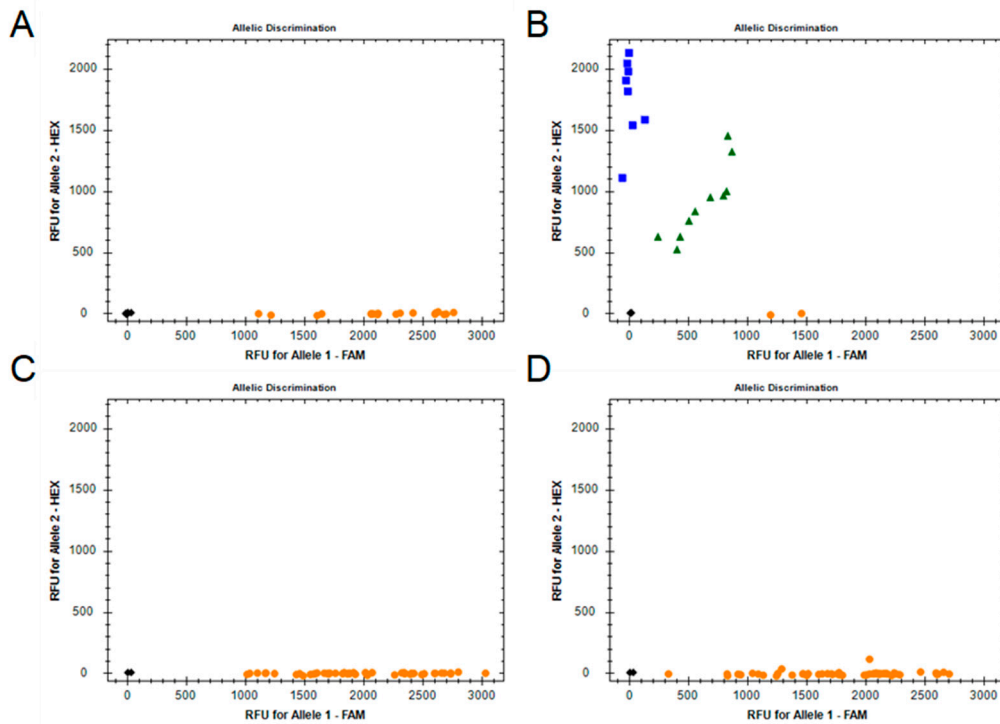


Figure S2. Detection of GC insertion allele at the ATP-binding cassette subfamily C2 (ABCC2) conferring resistance to *Bacillus thuringiensis* Cry1F toxin using PCR fluorescent probe assay described by Banerjee et al [3]; Blue squares represent mutant ABCC2 homozygotes for the GC insertion, orange circles ABCC2 wildtype SS homozygotes, and green triangles SR representing heterozygotes. Analysis of fall armyworm field samples collected in (A) Brazil, (B) Puerto Rico, (C) Kenya, and (D) Indonesia.

References

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