RESEARCH ARTICLE

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SOURCES OF RESISTANCE TO THE ASIAN CORN BORER [*Ostrinia furnacalis* (Guenee)] IN PHILIPPINE'S TRADITIONAL MAIZE VARIETIES

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SUMMARY

For native or traditional corn there has been no breeding program in the Philippines which included Asian corn borer resistance. With the rich genetic diversity of native corn populations or accessions, sources of resistance may be available. There were 3, 12 and 13 superior traditional varieties identified for field, leaf-feeding and stalk-feeding resistance, respectively. Positive correlation was identified among these 3 insect resistance traits. The resistant materials can be used as donor parents for breeding Philippine's traditional corn for improved resistance.

Key words: Resistance, *Ostrinia furnacalis* G., leaf-feeding, stalk-feeding, field damage, Philippine's traditional maize

Key findings: A number of Philippine's traditional varieties exhibited moderate to high levels of resistance to Asian corn borer; these varieties may be used in native maize breeding as important donors for Asian corn borer resistance.

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INTRODUCTION

The Asian corn borer (ACB) [*Ostrinia furnacalis* (Guenee)] is still the most destructive insect pest of maize in the Philippines. It was first observed in 1906 in several maize farms in Luzon. The larval stage, which has five to six instars, lasts for at least 14 to 20 days (Buligan, 1929; Camarao, 1976; Caasi-Lit and Sapin, 2012). It is the most destructive pest of corn from vegetative to reproductive stage. At very high level of infestation, ACB can damage all parts of the plant resulting in yield losses up to 100% especially during wet season.

Several methods to control ACB were developed. One of them is the use of *Trichogramma* parasitoids which was proven effective for biological control against the ACB, especially in Bukidnon, Mindanao, Philippines (Javier and Rejesus, 2004; Camarao and Rejesus, 2003). ACB populations have also been managed with the use of pesticides. These chemicals particularly a number of systemic insecticides, however, are very hazardous. ACB eventually develops resistance to these chemical sprays, which are commonly applied as cocktail of several synthetic formulations.

Development of resistant varieties is one classical approach to regulate and control ACB. Deployment of resistant varieties is an economically and environmentally safe method of crop protection (Siambi *et al*., 2000). In addition, the use of resistant varieties is regarded as the farmer's first line of defense against pests (Lit, 2009). Traisiri *et al*. (2010) have reported intermediate level of resistance to ACB in elite lines and commercial hybrids of maize in Thailand. However, two traditional maize landraces have recently been reported to exhibit potential corn borer resistance in the country (Salazar *et al*., 2016). Keeping this fact in view, this work included native varieties or landraces. The current study further exploited the field efficacy of selected germplasm collected against ACB along with laboratory assays. In addition, the study aimed to evaluate larger number of traditional maize germplasm collections to identify multiple sources of resistance to ACB in the Philippines.

MATERIALS AND METHODS

Germplasm

In total, 125 native maize varieties from the Cereals Breeding Section, Institute of Plant Breeding (IPB), University of the Philippines Los Baños (UPLB), Laguna - Philippine were obtained for ACB resistance screening in dry and wet seasons during 2014-2016 (Figure 1) (Table 1). Out of these, 120 were tested for leaf-feeding resistance, 73 for field resistance while all accessions were screened for stalk-feeding resistance (Table 2). Bt strip testing were done to detect presence of Bt cry proteins in the leaves (Figure 2). Varieties that were positive for Bt were excluded from further screening for ACB resistance. Bt-maize which served as resistant check was acquired from Syngenta Philippines Inc., while Philippine Super sweet variety (PSS) was used as susceptible check.

Insect mass-rearing

Egg masses of *O. furnacalis* were collected in corn fields 21 – 60 days after planting (DAP) at IPB – Experiment Station, Tranca, Bay, Laguna (14°11′N 121°17′E) during 2014-2016. Neonates were grown in a meridic corn borer diet with corn and soybean as main ingredients in a rearing container (21 × 14 × 6 cm) until late instar. Pupae were collected and transferred to separate container lined with tissue after six days. Adults that have emerged were sexed (1:1 ratio) and transferred to wooden oviposition cages (37 × 30 × 30 cm) with wax paper on top and cotton ball dipped on a 10% honey solution inside. These were mated for 4 days and resulting egg masses were stored

Table 1. Native corn accessions used for corn borer resistance screening from 2014-2016 at Institute of Plant Breeding- CGUARD.

Acc#	Designation	Variety Name	Source	Acc #	Designation	Variety Name	Source
1	CGUARD N1	Tiniguib A	Unknown	41	CGUARD N41	LawaanBukidnon	Bukidnon
2	CGUARD N2	Basay	Unknown	42	CGUARD N42	TiniguibCotabato	S. Cotabato
3	CGUARD N3	Batik	Unknown	43	CGUARD N43	Tiniguib Quezon	Bukidnon
4	CGUARD N4	Toledo	Cebu	44	CGUARD N44	Senorita Busco	Bukidnon
5	CGUARD N5	Memis A	N.Occidental	45	CGUARD N45	Cebu Red	Cebu
6	CGUARD N6	Manaka	Unknown	46	CGUARD N46	Baga-baga	N.Occidental
7	CGUARD N7	Pastilan	N. Oriental	47	CGUARD N47	Calimpus Negros	N.Occidental
8	CGUARD N8	Banlon	Bukidnon	48	CGUARD N48	Abra Glutinous	Abra
9	CGUARD N9	Tiniguib B	Unknown	49	CGUARD N49	Diket Sur	Ilocos Sur
10	CGUARD N10	Calimpus A	Unknown	50	CGUARD N50	Lagrimas	Cagayan
11	CGUARD N11	Kabagtik A	Bukidnon	52	CGUARD N52	Acorda	Cagayan
12	CGUARD N12	Lakha Red A	Bukidnon	53	CGUARD N53	Munaw Cagayan	Cagayan
13	CGUARD N13	Lakha Red B	Unknown	41	CGUARD N41	LawaanBukidnon	Bukidnon
14	CGUARD N14	Tiniguib C	Unknown	42	CGUARD N42	TiniguibCotabato	S. Cotabato
15	CGUARD N15	Tiniguib D	Unknown	43	CGUARD N43	Tiniguib Quezon	Bukidnon
16	CGUARD N16	Lawaan A	Unknown	44	CGUARD N44	Senorita Busco	Bukidnon
17	CGUARD N17	Bulldog A	Unknown	45	CGUARD N45	Cebu Red	Cebu
18	CGUARD N18	Senorita A	Unknown	46	CGUARD N46	Baga-baga	N.Occidental
19	CGUARD N19	FarmerVar.White	Unknown	47	CGUARD N47	Calimpus Negros	N.Occidental
20	CGUARD N20	FarmerVar.Orange A	Unknown	48	CGUARD N48	Abra Glutinous	Abra
21	CGUARD N21	FarmerVar.Orange B	Bukidnon	49	CGUARD N49	Diket Sur	Ilocos Sur
22	CGUARD N22	FarmerVar.Red A	Unknown	50	CGUARD N50	Lagrimas	Cagayan
23	CGUARD N23	FarmerVar.Red B	Bukidnon	52	CGUARD N52	Acorda	Cagayan
24	CGUARD N24	FarmerVar.Red C	Unknown	53	CGUARD N53	Munaw Cagayan	Cagayan
25	CGUARD N25	C.Compostela	C.Valley	72	CGUARD N69	Odiongan	NPGRL
26	CGUARD N26	Cotabato White	South Cotatbato	74	CGUARD N70	TiniguibLingayo	NPGRL
27	CGUARD N27	Compostela White	Comp.Valley	75	CGUARD N71	TiniguibSibagat	NPGRL
28	CGUARD N28	Bukidnon WR	Bukidnon	76	CGUARD N72	TiniguibLibas	NPGRL
29	CGUARD N29	Poblacion White	Bukidnon	77	CGUARD N73	M.Glutinous Flint	NPGRL
30	CGUARD N30	TiniguibCompostela	Comp.Valley	78	CGUARD N74	Kabagtik 10560	NPGRL
31	CGUARD N31	Bukidnon Red	Bukidnon	79	CGUARD N75	Matatais	NPGRL
32	CGUARD N32	Kitaotao Red	Bukidnon	80	CGUARD N76	Pacing	NPGRL
33	CGUARD N33	Manggahan White	Bukidnon	81	CGUARD N77	Bukidnon C2	NPGRL
34	CGUARD N34	San Jose White	Bukidnon	82	CGUARD N78	Sultan	NPGRL
35	CGUARD N35	TiniguibMaramag	Bukidnon	83	CGUARD N79	Kalinpos	NPGRL
36	CGUARD N36	Valencia Orange	Bukidnon	84	CGUARD N80	Red Batuan	NPGRL
37	CGUARD N37	Senorita Pangantukan	Bukidnon	85	CGUARD N81	Maguprak	NPGRL
38	CGUARD N38	RedhorseBukidnon	Bukidnon	86	CGUARD N82	Ballunggay	NPGRL
39	CGUARD N39	CalimpusBukidnon	Bukidnon	88	CGUARD N84	Malagkit Salt	Palawan
40	CGUARD N40	Musuan White	Bukidnon	89	CGUARD N85	Batik Palawan	Palawan

Table 1 (cont'd).

Acc#	Designation	Variety Name	Source	Acc #	Designation	Variety Name	Source
90	CGUARD N86	Takurong	Quezon	126	CGUARD N114	CMU Kayuta	Bukidnon
91	CGUARD N87	DiketAbra A	Abra	127	CGUARD N115	TiniguibGlorybell B	Bukidnon
92	CGUARD N88	Pukek	Abra	128	CGUARD N116	Dlastillo	Bukidnon
93	CGUARD N89	DiketAbra B	Abra	129	CGUARD N117	Malungun C	Bukidnon
94	CGUARD N90	DiketAbra C	Abra	131	CGUARD N119	Malungun D	Bukidnon
95	CGUARD N91	DiketAbra D	Abra	132	CGUARD N120	B.M. Campilan	Bukidnon
96	CGUARD N92	Senorita Camiguin	Camiguin	133	CGUARD N121	TiniguibGlorybell A	Bukidnon
100	CGUARD N93	Cebu WR	Cebu	134	CGUARD N122	Tiniguib N Doding	Bukidnon
101	CGUARD N94	Tiniguib Mindanao	Unknown	135	CGUARD N123	TiniguibPilos B	Bukidnon
102	CGUARD N95	ManoloFortich	Bukidnon	136	CGUARD N124	Malungun B	Bukidnon
107	CGUARD N96	Marinduque Yellow	Marinduque	137	CGUARD N125	Senorita CMU	Bukidnon
108	CGUARD N97	Malibago	Marinduque	138	CGUARD N126	D.rosario	Bukidnon
110	CGUARD N98	Bukidnon White	Bukidnon	139	CGUARD N127	TiniguibGlorybell C	Bukidnon
111	CGUARD N99	TiniguibAdtuyon A	Bukidnon	140	CGUARD N128	Katagbo	Bukidnon
112	CGUARD N100	TiniguibAdtuyon B	Laguna	141	CGUARD N129	Kabang C Citoy	Bukidnon
113	CGUARD N101	TiniguibPadada	DDS	142	CGUARD N130	Malungun G	Bukidnon
114	CGUARD N102	MaisEdung	Palawan	143	CGUARD N131	Malungun F	Bukidnon
115	CGUARD N103	MaisBundok	Palawan	144	CGUARD N132	CMU Glutinous	Bukidnon
116	CGUARD N104	TiniguibLingayao	Ag.delNorte	145	CGUARD N133	Claveria	Bukidnon
117	CGUARD N105	CalimpusAgusan	Ag. del Norte	146	CGUARD N134	Rarap	Bukidnon
118	CGUARD N106	TiniguibAgusan	Ag. del Sur	147	CGUARD N135	Malungun E	Bukidnon
120	CGUARD N108	Silangan	Pangasinan	148	CGUARD N136	Jerome	Bukidnon
121	CGUARD N109	Calimpus Leyte	Leyte	149	CGUARD N137	Jarap	Bukidnon
122	CGUARD N110	Senorita Baldina	Bukidnon	150	CGUARD N138	Malungun H	Bukidnon
123	CGUARD N111	Malungun A	Bukidnon	151	CGUARD N139	JarapUnduyUnduy	Bukidnon
124	CGUARD N112	TiniguibPlanta J	Bukidnon	-	Check 1	Bt/Gt	-
125	CGUARD N113	Calimous M Bangud	Bukidnon	-	Check 2	Phil. Supersweet	-

***** Acc # is Accession number, does not come in particular sequence since not all were available during the screenings conducted.

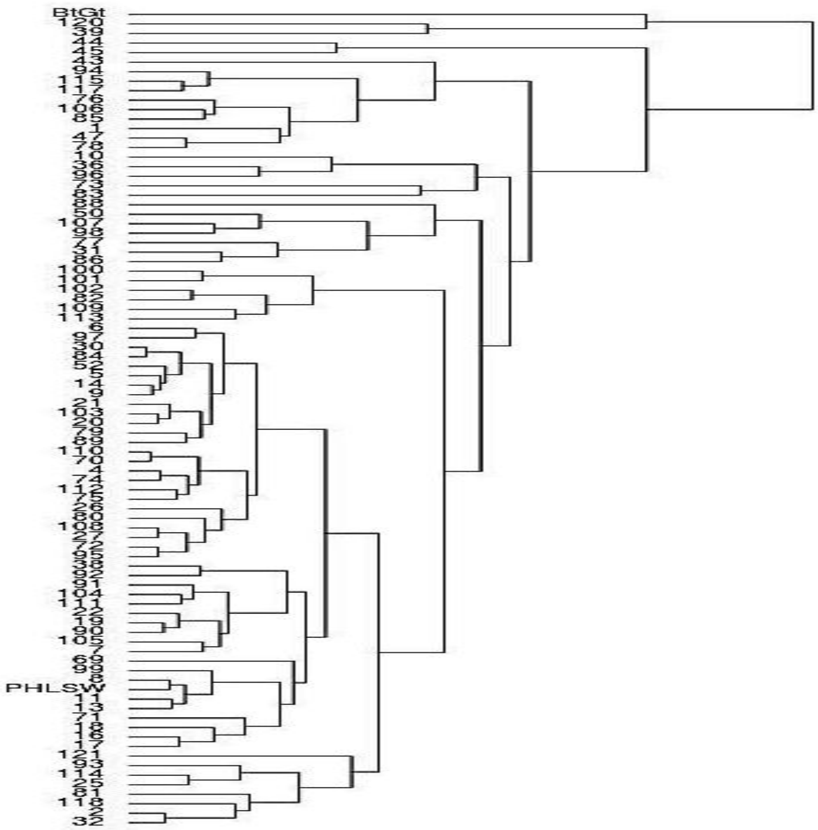

Figure 1. Dendogram depicting relationship among traditional landraces based on MLS, MTL and MFDR due to ACB from 2014-2016.

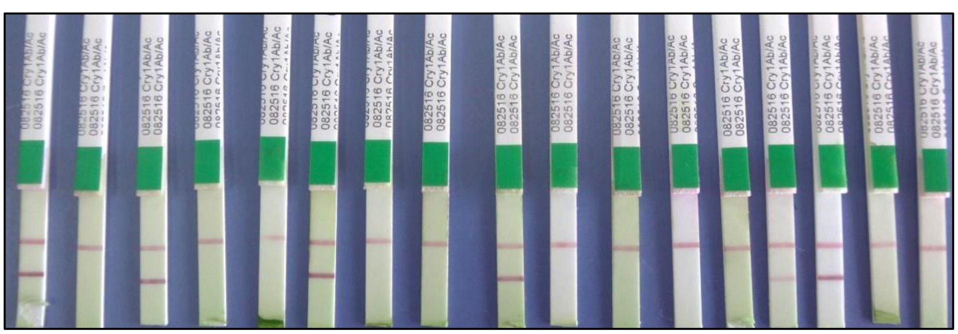

Figure 2. Bt ELISA strip test used to verify purity of native corn: double band [with Bt toxin (+)]whereas single band [no Bt toxin (-)]

Table 2. Mean values of resistance variables against Asian corn borer of selected Philippine native corn germplasm, Institute of Plant Breeding-University of the Philippine Los Baños, College, Laguna, Philippines, 2014-2016.

ACCESSION	MTL		MLS		MFDR	
CGUARD N1	16.75 ± 1.46	*t*	58.77 ± 2.38	*a-e*	3.86 ± 0.27	*n*
CGUARD N2	15.36 ± 1.12	*t*	54.40 ± 2.02	*z*	2.85 ± 0.25	*a-b*
CGUARD N3	15.68 ± 2.48	*o*	58.37 ± 4.57	*b-c*	-	-
CGUARD N4	13.58 ± 1.06	*r*	60.18 ± 1.92	*a-g*	3.17 ± 0.24	*a-b*
CGUARD N5	12.01 ± 0.99	*m*	52.35 ± 1.87	*b-c*	3.08 ± 0.23	*a-b*
CGUARD N6	12.25 ± 1.07	*q*	51.20 ± 2.17	*b-c*	3.24 ± 0.25	*a-b*
CGUARD N7	14.24 ± 0.95	*s*	50.62 ± 1.81	*b-c*	3.36 ± 0.22	*a-c*
CGUARD N8	12.64 ± 1.06	*p*	53.06 ± 1.93	*b-c*	3.36 ± 0.23	*a-c*
CGUARD N9	11.97 ± 1.28	*p*	58.97 ± 2.48	*a-e*	3.19 ± 0.27	*a-b*
CGUARD N10	12.07 ± 1.12	*q*	56.36 ± 2.07	*a-c-d*	2.48 ± 0.26	*d*
CGUARD N11	12.30 ± 1.03	*q*	56.36 ± 2.07	*a-c-d*	3.70 ± 0.26	*m*
CGUARD N12	10.20 ± 1.79	*n*	67.88 ± 3.88	*a-l*	-	-
CGUARD N13	11.79 ± 1.03	*m*	56.16 ± 2.27	*a-c*	3.68 ± 0.25	*m*
CGUARD N14	12.99 ± 1.37	*d*	54.33 ± 2.08	*z*	2.99 ± 0.24	*a-b*
CGUARD N15	18.33 ± 2.49	*o*	68.38 ± 5.31	*a-m*	-	-
CGUARD N16	13.42 ± 1.28	*o*	49.44 ± 2.81	*x*	3.55 ± 0.26	*k*
CGUARD N17	12.35 ± 1.03	*q*	48.97 ± 2.33	*w*	3.59 ± 0.25	*l*
CGUARD N18	14.05 ± 1.03	*s*	50.60 ± 2.18	*b-c*	3.36 ± 0.23	*a-c*
CGUARD N19	14.20 ± 1.16	*s*	55.02 ± 2.45	*z*	3.47 ± 0.27	*a-b*
CGUARD N20	17.71 ± 1.03	*a-e*	54.08 ± 1.87	*z*	3.22 ± 0.23	*a-b*
CGUARD N21	12.20 ± 1.07	*q*	55.54 ± 2.02	*a-c*	3.13 ± 0.23	*a-b*
CGUARD N22	14.12 ± 1.16	*s*	51.06 ± 2.30	*b-c*	3.25 ± 0.26	*a-b*
CGUARD N23	10.98 ± 2.04	*d*	37.34 ± 4.57	*j*	-	-
CGUARD N24	14.74 ± 2.04	*o*	62.47 ± 4.57	*z*	-	-
CGUARD N25	13.13 ± 1.12	*d*	50.44 ± 2.29	*b-c*	2.95 ± 0.27	*a-b*
CGUARD N26	12.22 ± 1.06	*q*	59.44 ± 2.16	*a-f*	2.87 ± 0.27	*a-b*
CGUARD N27	11.90 ± 1.04	*m*	53.09 ± 2.07	*b-c*	3.02 ± 0.24	*a-b*
CGUARD N28	13.57 ± 2.48	*o*	44.52 ± 4.57	*r*	-	-
CGUARD N29	10.63 ± 1.77	*d*	47.76 ± 3.44	*v*	-	-
CGUARD N30	12.40 ± 0.94	*q*	49.67 ± 2.17	*b-c*	3.17 ± 0.23	*a-b*
CGUARD N31	15.51 ± 1.03	*t*	61.45 ± 2.17	*a-j*	3.52 ± 0.23	*l*
CGUARD N32	15.50 ± 1.11	*t*	52.33 ± 2.52	*b-c*	2.87 ± 0.25	*a-b*
CGUARD N33	12.69 ± 1.80	*o*	50.95 ± 3.44	*x*	-	-
CGUARD N34	10.23 ± 2.04	*d*	41.96 ± 4.57	*c-d*	-	-
CGUARD N35	14.23 ± 2.04	*o*	54.78 ± 4.57	*x*	-	-
CGUARD N36	11.82 ± 1.08	*m*	45.86 ± 2.02	*t*	2.65 ± 0.27	*f*
CGUARD N37	15.76 ± 1.76	*o*	34.18 ± 3.88	*i*	-	-
CGUARD N38	14.39 ± 1.28	*s*	45.51 ± 2.07	*t*	3.17 ± 0.25	*a-b*
CGUARD N39	9.44 ± 1.28	*l*	30.32 ± 2.48	*g*	2.41 ± 0.30	*c*
CGUARD N40	12.93 ± 2.49	*o*	28.89 ± 3.76	*f*	-	-
CGUARD N41	17.07 ± 1.77	*t*	47.69 ± 5.31	*r*	-	-
CGUARD N42	6.47 ± 2.04	*f*	28.38 ± 5.31	*e*	-	-
CGUARD N43	16.05 ± 1.59	*s*	59.84 ± 3.46	*a-c*	3.00 ± 0.28	*a-b*
CGUARD N44	10.10 ± 1.77	*m*	38.48 ± 3.44	*k*	2.26 ± 0.30	*b*
CGUARD N45	19.28 ± 1.16	*w*	39.69 ± 2.87	*l*	2.46 ± 0.27	*d*
CGUARD N46	13.62 ± 2.04	*o*	54.55 ± 3.88	*w*	-	-
CGUARD N47	14.94 ± 1.46	*s*	57.31 ± 2.68	*a-c*	3.44 ± 0.30	*a-b*
CGUARD N48	9.76 ± 1.77	*g*	55.84 ± 3.92	*b-c*	-	-
CGUARD N49	19.60 ± 2.49	*t*	38.92 ± 4.31	*m*	-	-
CGUARD N50	10.41 ± 1.80	*n*	60.57 ± 3.43	*a-c-d*	2.99 ± 0.30	*a-b*
CGUARD N52	14.15 ± 1.21	*s*	53.39 ± 2.44	*b-c*	3.16 ± 0.23	*a-b*
CGUARD N53	4.86 ± 2.48	*b*	65.34 ± 9.00	*v*	-	-
CGUARD N58	3.66 ± 3.49	*a*	-	-	-	-
CGUARD N67	15.21 ± 1.61	*o*	-	-	2.78 ± 0.27	*a-b*
CGUARD N68	15.66 ± 2.48	*o*	-	-	-	-
CGUARD N69	12.32 ± 1.22	*p*	55.39 ± 2.52	*z*	2.88 ± 0.23	*a-b*
CGUARD N70	13.34 ± 1.16	*d*	57.20 ± 2.47	*a-f*	3.20 ± 0.24	*a-b*
CGUARD N71	13.65 ± 1.11	*o*	57.17 ± 2.52	*a-f*	3.25 ± 0.23	*a-b*
CGUARD N72	17.60 ± 1.77	*t*	50.21 ± 3.50	*x*	3.20 ± 0.33	*a-b*
CGUARD N73	14.66 ± 1.77	*o*	64.86 ± 3.43	*a-j*	3.49 ± 0.30	*a-b*

Table 2. (cont'd.)

ACCESSION	MTL		MLS		MFDR	
CGUARD N74	18.42 ± 1.59	*u*	54.28 ± 3.43	*b-c*	3.51 ± 0.33	*a-b*
CGUARD N75	10.85 ± 1.59	*n*	56.04 ± 4.63	*w*	3.14 ± 0.30	*a-b*
CGUARD N76	12.13 ± 1.03	*q*	60.48 ± 2.27	*a-i*	2.97 ± 0.24	*a-b*
CGUARD N77	18.54 ± 1.30	*v*	53.36 ± 2.27	*b-c*	3.01 ± 0.24	*a-b*
CGUARD N78	13.18 ± 1.36	*o*	38.72 ± 3.02	*l*	3.42 ± 0.24	*a-c*
CGUARD N79	10.20 ± 1.78	*n*	58.00 ± 3.43	*z*	2.74 ± 0.33	*g*
CGUARD N80	13.29 ± 1.08	*d*	50.20 ± 2.35	*b-c*	3.15 ± 0.23	*a-b*
CGUARD N81	20.25 ± 1.36	*y*	56.80 ± 2.52	*a-c*	3.39 ± 0.23	*a-c*
CGUARD N82	15.55 ± 1.28	*t*	58.86 ± 3.43	*z*	3.60 ± 0.24	*l*
CGUARD N84	8.25 ± 1.77	*j*	54.00 ± 3.43	*b-c*	3.74 ± 0.30	*k*
CGUARD N85	12.68 ± 1.16	*d*	52.39 ± 2.55	*b-c*	3.31 ± 0.24	*a-b*
CGUARD N86	17.25 ± 1.28	*u*	53.74 ± 2.53	*b-c*	3.30 ± 0.24	*a-b*
CGUARD N87	16.99 ± 1.16	*u*	49.88 ± 2.52	*w*	3.40 ± 0.23	*a-c*
CGUARD N88	16.24 ± 1.07	*t*	49.52 ± 2.63	*w*	3.27 ± 0.23	*a-b*
CGUARD N89	13.24 ± 1.11	*d*	44.30 ± 2.53	*q*	2.89 ± 0.24	*a-b*
CGUARD N90	17.65 ± 1.82	*t*	60.28 ± 3.43	*a-h*	2.89 ± 0.30	*a-b*
CGUARD N91	10.79 ± 1.59	*n*	47.61 ± 3.45	*v*	2.91 ± 0.24	*a-b*
CGUARD N92	11.56 ± 1.11	*m*	46.56 ± 2.62	*u*	2.50 ± 0.23	*e*
CGUARD N93	11.21 ± 1.07	*i*	41.16 ± 2.28	*n*	3.30 ± 0.23	*a-b*
CGUARD N94	12.58 ± 1.01	*q*	42.25 ± 2.28	*o*	3.39 ± 0.23	*a-c*
CGUARD N95	13.38 ± 1.24	*o*	33.07 ± 2.92	*h*	3.45 ± 0.24	*k*
CGUARD N96	10.65 ± 1.59	*m*	62.86 ± 3.43	*a-k*	3.29 ± 0.30	*a-b*
CGUARD N97	13.22 ± 1.11	*d*	54.90 ± 2.27	*z*	3.04 ± 0.23	*a-b*
CGUARD N98	13.59 ± 1.16	*o*	56.93 ± 2.09	*a-c-d*	3.09 ± 0.23	*a-b*
CGUARD N99	16.25 ± 1.28	*t*	53.53 ± 2.28	*b-c*	3.42 ± 0.23	*k*
CGUARD N100	13.03 ± 1.46	*o*	54.52 ± 2.87	*b-c*	3.09 ± 0.24	*a-b*
CGUARD N101	12.96 ± 1.60	*o*	42.05 ± 4.51	*c-d*	3.26 ± 0.26	*a-b*
CGUARD N102	15.96 ± 1.21	*t*	49.97 ± 2.28	*b-c*	2.78 ± 0.25	*h*
CGUARD N103	17.86 ± 1.36	*u*	55.43 ± 3.43	*b-c*	3.06 ± 0.25	*a-b*
CGUARD N104	13.26 ± 1.60	*o*	64.99 ± 4.52	*a-c-d*	-	-
CGUARD N105	16.76 ± 1.77	*s*	58.08 ± 3.45	*z*	2.84 ± 0.30	*a-b*
CGUARD N106	16.08 ± 1.28	*t*	51.74 ± 3.04	*b-c*	2.95 ± 0.24	*a-b*
CGUARD N107	16.28 ± 1.78	*s*	-	-	-	-
CGUARD N108	6.89 ± 1.46	*i*	21.94 ± 3.74	*b*	2.98 ± 0.26	*a-b*
CGUARD N109	16.32 ± 2.04	*o*	54.74 ± 4.36	*w*	2.91 ± 0.31	*a-b*
CGUARD N110	17.21 ± 2.52	*o*	35.34 ± 9.00	*c-d*	-	-
CGUARD N111	15.10 ± 2.48	*o*	47.34 ± 9.00	*c-d*	-	-
CGUARD N112	19.73 ± 2.48	*t*	27.34 ± 9.00	*c-d*	-	-
CGUARD N113	14.54 ± 3.48	*o*	55.34 ± 9.00	*c-d*	-	-
CGUARD N114	20.20 ± 2.48	*x*	67.34 ± 9.00	*v*	-	-
CGUARD N115	9.90 ± 2.06	*d*	41.34 ± 9.00	*c-d*	-	-
CGUARD N116	5.76 ± 2.48	*c*	63.34 ± 9.00	*r*	-	-
CGUARD N117	17.80 ± 2.48	*o*	47.34 ± 9.00	*c-d*	-	-
CGUARD N118	13.81 ± 2.06	*o*	-	-	-	-
CGUARD N119	10.50 ± 2.48	*d*	47.34 ± 9.00	*c-d*	-	-
CGUARD N120	23.83 ± 2.48	*a-c*	51.34 ± 9.00	*c-d*	-	-
CGUARD N121	24.99 ± 3.49	*z*	41.34 ± 9.00	*c-d*	-	-
CGUARD N122	11.73 ± 2.48	*o*	65.34 ± 9.00	*v*	-	-
CGUARD N123	17.20 ± 2.48	*o*	35.34 ± 9.00	*c-d*	-	-
CGUARD N124	19.76 ± 2.48	*t*	25.34 ± 9.00	*c-d*	-	-
CGUARD N125	29.60 ± 3.48	*a-d*	29.34 ± 9.00	*c-d*	-	-
CGUARD N126	15.96 ± 2.48	*o*	45.34 ± 9.00	*c-d*	-	-
CGUARD N127	7.94 ± 3.48	*d*	23.34 ± 9.00	*c*	-	-
CGUARD N128	21.53 ± 2.48	*z*	23.34 ± 9.00	*c*	-	-
CGUARD N129	11.69 ± 2.52	*o*	55.34 ± 9.00	*c-d*	-	-
CGUARD N130	10.94 ± 3.48	*o*	43.34 ± 9.00	*c-d*	-	-
CGUARD N131	6.13 ± 2.48	*e*	27.34 ± 9.00	*c-d*	-	-
CGUARD N132	19.86 ± 3.49	*o*	33.34 ± 9.00	*c-d*	-	-
CGUARD N133	8.40 ± 2.48	*k*	37.34 ± 9.00	*c-d*	-	-
CGUARD N134	16.59 ± 2.52	*o*	25.34 ± 9.00	*c-d*	-	-
CGUARD N135	13.73 ± 2.48	*o*	25.34 ± 9.00	*c-d*	-	-
CGUARD N136	5.87 ± 3.48	*d*	35.34 ± 9.00	*c-d*	-	-
CGUARD N137	13.26 ± 3.49	*o*	43.34 ± 9.00	*c-d*	-	-

Table 2. (cont'd.)

ACCESSION	MTL		MLS		MFDR	
CGUARD N138	21.66 ± 2.53	*a-b*	61.34 ± 9.00	*c-d*	-	-
CGUARD N139	11.53 ± 2.53	*o*	27.34 ± 9.00	*c-d*	-	-
PHL SWT	12.53 ± 2.48	*p*	52.68 ± 1.99	*b-c*	3.42 ± 0.25	*a-c*
BT 11 x GA21	6.78 ± 3.49	*h*	21.62 ± 2.16	*a*	1.31 ± 0.24	*a*
p value	< 0.000		< 0.000		< 0.000	
F-value	7.48		4.76		7.51	

Note: Mean ± standard error of mean followed by different letter/s are significantly different at 5% significance level by Tukey's-HSD; MTL - mean tunnel length in mm, MLS – mean larval survival in % and MFDR – mean field damage rating from 1-5 rating scale.

at 10-14 °C until the onset of laboratory and field efficacy assays. Newly molted 2nd instar larvae from the homogenized populations were used in the experiments.

Laboratory screening

A. Leaf feeding assay

Foliage feeding resistance to ACB was tested for the different varieties of native maize under laboratory conditions at the Entomology Laboratory of the Institute of Plant Breeding, UPLB during 2014-2016. Leaves were collected from 30-45 DAP maize plants and brought to the laboratory. These were cut into leaf discs (area = 6.54 cm^2) (n = 30). Newly molted 2nd instars were seeded onto each leaf sample. After seeding, it was placed in a small Petri dish or assay cup, moistened with 0.30 mL distilled water, cling-wrapped with transparent plastic, and placed in the Bioassay Room. Leaf samples were replaced every day. Larval survival was recorded for 5 consecutive days and computed as percentage of larvae that survived over the total number of larvae seeded.

B. Stalk-feeding assay

Stalk-feeding assay was conducted at 45 DAP, 60 DAP and 75 DAP. Maize stalks were collected and brought to the laboratory. Cleaned stalks were cut into small cross sections for each variety (2.54 cm long) and seeded with 2nd instar larvae. Each stalk was placed in a plastic assay cup (3.81cm in diameter) laid with tissue paper on top and sealed with a plastic cover with screen at the center for adequate ventilation. Cups were placed in the Insect Bioassay Room. Tunnel lengths (in mm) in the stalk due to larval boring were measured after five days.

Field screening

The experimental design for ACB field experiments was Alpha Lattice Design (ALD). There were three replicates for each field trial. Each plot consisted of three 3-meter rows spaced 0.75m apart keeping inter plant (hill) distance of 0.25m, and seeding rate of two seeds per hill. Buffer rows at each side in between replicates were planted with IPB Var 6. Check varieties were Bt/Gt maize hybrid (resistant check) and Philippine Super sweet (susceptible check). Thirty (30) laboratory-reared 2nd instar larvae were artificially infested onto each variety 25 days after planting (25 DAP) during dry and wet season in 2014-2016. Using a fine Camel™ hair brush, ACB larvae were introduced on the whorl of the inner or middle row plants. Field efficacy against ACB of each variety or treatment was assessed a week after infestation based on nature of leaf and stalk damage (Table 1).

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) and mean comparisons were done using Tukey's HSD. Pearson's product moment correlation was used to determine relationships among resistance traits. Cluster analyses were done using hierarchical agglomerative clustering via Euclidian's distance and unweighted pair group method with arithmetic mean and principal component analysis (PCA). All statistical analyses were performed using R Version 3.4.0. (R Core, 2016).

RESULTS

Evaluation via no-choice tests in the laboratory by leaf feeding method showed significant variation in terms of mean percentage of 2nd instar larval survival (Table 3). 12 traditional varieties viz., CGUARD N40 (28.89%), CGUARD N42 (28.38%), CGUARD N108 (21.94%), CGUARD N112 (27.34%), CGUARD N124 (25.34%), CGUARD N125 (29.34%), CGUARD N127 (23.34%), CGUARD N128 (23.34%), CGUARD N131 (27.34%), CGUARD N134 (25.34%), CGUARD N135 (25.34%) and CGUARD N139 (27.34%) exhibited resistance to leaf-feeding ACB, while 11 namely CGUARD N23 (37.34%), CGUARD N37 (34.18%), CGUARD N44 (38.48%), CGUARD N45 (39.69%), CGUARD N49 (38.92%), CGUARD N78 (38.72%), CGUARD N95 (33.07%), CGUARD N110 (35.34%), CGUARD N123 (35.34%), CGUARD N132 (33.34%) and CGUARD N133 (37.34%) were classified as moderately resistant. Out of 120 varieties screened for ACB leaf-feeding resistance, 98 were rated susceptible to ACB damage. Leaf-feeding response of different native varieties was significantly different at 30 DAP and 45 DAP (Table 3). The number of larvae that survived in resistant varieties decreased as older stage of maize leaves was tested.

No-choice test revealed that 13 accessions had stalk-feeding resistance on ACB larvae. These were CGUARD N39 (9.44 mm), CGUARD N42 (6.47 mm), CGUARD N48 (9.76mm), CGUARD N53 (4.86 mm), CGUARD N58 (3.66 mm), CGUARD N84 (8.25 mm), CGUARD N108 (6.89 mm), CGUARD N115 (9.90 mm),CGUARD N116 (5.76 mm), CGUARD N127 (7.94 mm), CGUARD N131 (6.13 mm), CGUARD N133 (8.40 mm) and CGUARD N136 (5.87 mm). On the other hand, 22 varieties in total were moderately resistant, and 90 were susceptible to stalk damage. ACB larval tunnel length differed significantly at 45, 60 and 75 DAP (Table 3). As the age of maize stalks used increased, the tunnel lengths also increased.

Screening of selected native corn germplasm in the study showed variable reaction when exposed to ACB attack based on choice (MFDR) (Table 2). Accessions such as CGUARD N39 (2.41), CGUARD N44 (2.26) and CGUARD N45 (2.46) had scored lower than other corn genotypes such as CGUARD N1 (3.86), CGUARD N11 (3.70), CGUARD N13 (3.68), CGUARD N17 (3.59), CGUARD N73 (3.49), CGUARD N74 (3.51), CGUARD N82 (3.60) and CGUARD N84 (3.74) under artificial field infestation. From these three entries, CGUARD N44 (2.26)

Table 3. Pooled over-all response of native corn after ACB infestation.

Leaf-feeding larval survival	
30 DAP	53.49 ± 35.00a
45 DAP	45.13 ± 29.81b
Mean	49.86
C.V. (%)	65.88
Stalk-feeding tunnel length	
45 DAP	9.08 ± 9.00a
60 DAP	12.71 ± 10.80b
75 DAP	16.41 ± 17.87c
Mean	13.51
C.V .(%)	104.07

Means followed by the same letter are not significantly different at 5% by Tukey's HSD; DAP - days after planting; C.V.- coefficient of variation.

Table 4. Correlation analyses of ACB resistance variables for 120 Philippine maize varieties from 2014-2016.

	MLS	MTL	MFDR
MLS		0.35^{**}	0.35^{**}
MTL			$0.16^{n.s.}$
MFDR			

Note: **significant- (P=0.001), $^{n.s.}$-not significant

exhibited the lowest MFDR, and appeared significantly different from accessions CGUARD N4 (3.17), CGUARD N5 (3.08), CGUARD N6 (3.24), CGUARD N18 (3.36), CGUARD N27 (3.02), CGUARD N36 (2.65), CGUARD N52 (3.16), CGUARD N95 (3.42), CGUARD N102 (2.78), and CGUARD N108 (2.98). Severe ACB damage in the field was observed in several entries such as CGUARD N7 (3.36), CGUARD N18 (3.36), CGUARD N81 (3.39), CGUARD N87 (3.40) and CGUARD N94 (3.39) which did not differ significantly from the susceptible check, PSS (3.42).

Significant positive correlations between larval survival, tunnel length and field damage score were observed based on Pearson's product moment correlation (Table 4). Correlation values for larval survival and mean tunnel length and mean field damage and 2nd instar ACB survival were the same (r = +0.35). While a lower correlation coefficient value was observed for mean tunnel length and damage in the field (r = +0.16). For cluster analyses, both the hierarchical agglomerative clustering and PCA bi-plot showed that the putatively traditional maize varieties group closely associated with the positive check (Bt/Gtcorn: NK8840) (Figures 3 and 4).

DISCUSSION

Insect pest survival has been commonly used as the criteria for germplasm screening in maize. This parameter is useful in quantifying insect resistance (Tollefson, 2007). In this study, it was shown that leaf-feeding resistant native corn varieties were able to decrease the survival of 2nd instar. Leaf-feeding resistance has been commonly attributed to both physical and biochemical factors.

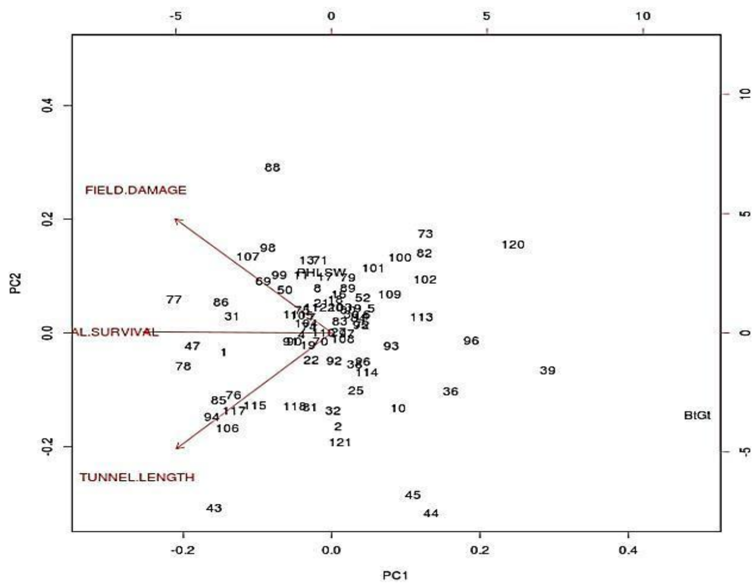

Figure 3. Principal component analysis biplotamong traditional landraces based on MLS, MTL and MFDR due to ACB from 2014-2016.

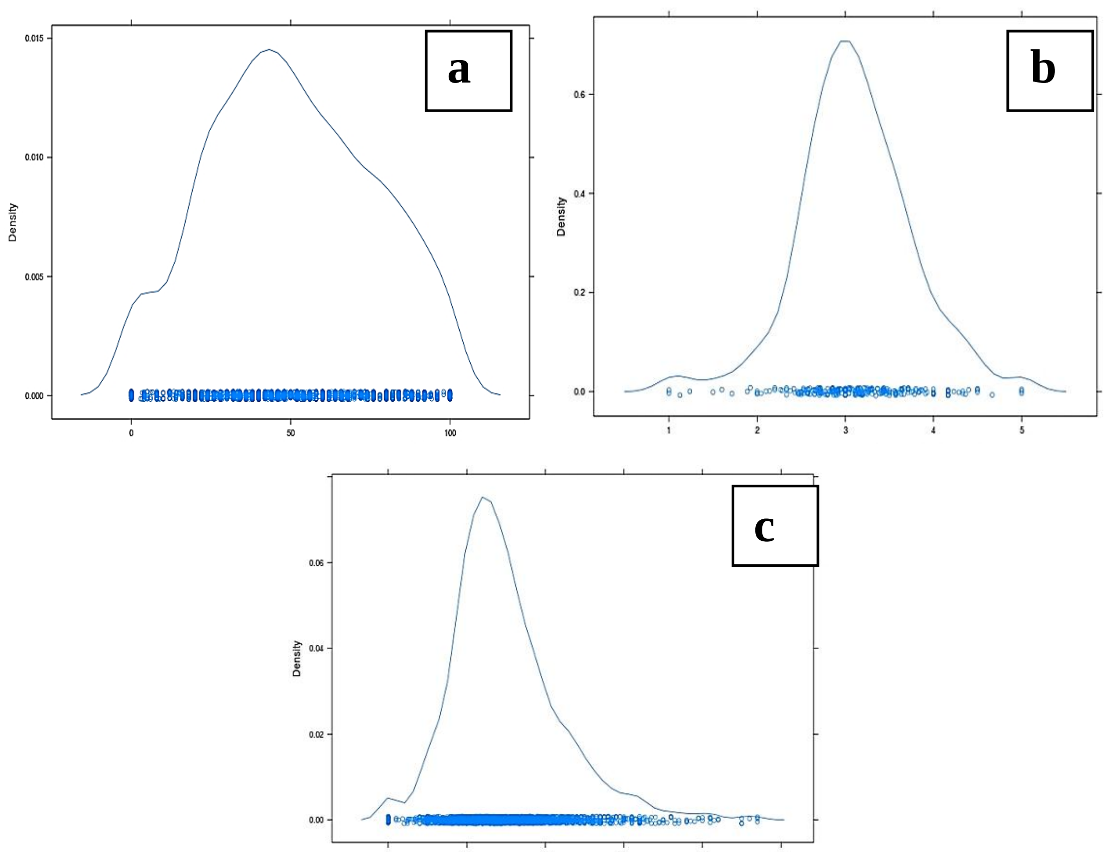

Figure 4. Density plots of percent larval survival (a), field damage (b) and tunnel length (c) due to ACB pressure.

Davis *et al*. (1995) have provided evidence that resistance of some inbred lines screened for leaf feeding South-western corn borer [*Diatraea grandiosella* (Dyar)] and Fall army worm [*Spodoptera frugiperda* (J.E. Smith)] was correlated with anatomical characters of the inner whorl leaf tissue, particularly the upper and lower cell wall complexes. Leaf-feeding resistance in European corn borer (*Ostrinia nubilalis*) was attributed to secondary metabolites, DIMBOA (Campos *et al*., 1988; Campos *et al*., 1989). Papst *et al*. (2003) found that there is a tendency of lower content of DIMBOA in susceptible maize. Guiterrez *et al*. (1988) showed induced resistance in a maize variety with high DIMBOA content to the maize borer (*Sesamia nonagrioides*). In the Philippines, Santiago and Mendoza (1983) also observed that DIMBOA levels increased in ACB resistant varieties. Although the degree of leaf damage especially during vegetative stage is also dependent on larval density, it was confirmed that the traditional varieties of corn tested have varied reaction after artificial infestation. Manifestation of insect resistance during the early vegetative stage of corn development can help reduce potential damage of ACB. Meanwhile, it was observed that the number of larvae that survived from 30 DAP to 45 DAP decreased among varieties. Possibly, changes in concentration of phyto-derived chemicals in maize leaves have caused the decline. Santiago *et al*. (2017) reported the antibiosis levels, i.e., larval survival differed significantly from early to later stages of leaf bioassay in Mediterranean corn borer (*Sesamia nonagrioides* Lef.).

Variability in tunnel measurements due to ACB feeding on corn stalks was also observed in this study. Stalk tunnelling is an important resistance trait for borers since it often leads to stalk breakage and ear-dropping in maize thus affecting yield. In this study, the resistant test varieties had also the least tunnel length as compared to the highly susceptible ones, suggesting that this character is also useful in determining ACB resistance. The values for tunnel length in the best performing populations in this study were relatively at par with the values obtained by Bohn *et al*. (2003) for European corn borer (ECB) after screening certain fractions of S_1 maize lines (0.1-0.9 cm). The mean tunnel length for potential sources of ACB in this study was 0.65 cm. In the findings of Papst *et al*. (2003), data from the former author were cited, where the mean tunnel length for resistant group was 2.34 cm among a population of 230 F_{2-3} lines. Barry and Darrah (1994) found that the average tunnel length per plant due to ECB feeding for selected commercial hybrids was 0.38 cm. The reaction of promising native corn observed from the stalk feeding experiments might be due to innate inimical structural defense against ACB (e.g. stalk tensile strength and fiber content). Papst *et al*. (2003) proposed that increased resistance seems to be associated with stalk toughness and lower digestibility. Fiber traits such as digestibility, lignin content, cellulose, silica content, tensile strength and stalk toughness are important factors that will be useful in determining the mechanism of ACB stalk resistance. Rojanaridpiched *et al*. (1984) found that increased leaf and stem silica content contribute to ECB resistance in some maize varieties. It was proposed that the shorter the tunnel length, the more resistant a variety was and vice versa. During the experiment, developmental effects on the ACB larvae such as explicit reduction in size were observed in stalk-resistant entries. This might be due to the previously mentioned associated stalk resistance parameters in corn borers. Furthermore, high mortality of 2nd instar larvae infested was also observed in some varieties akin to the results observed for Bt check variety. In general, dead larvae in resistant varieties were paler in color, weaker and with darkened anus. Moreover, it was observed that ACB stalk damage was directly proportional to the age of corn stalks (45, 60, 75 DAP) mostly in susceptible varieties.

Whole plant damage (i.e., stalk and leaf) on test varieties after corn borer infestation was assessed from post-vegetative to pre-tasseling stage of corn using a 1-5 scale (Table 5). Visual injury rating scales of 1-5 or 1-9 have been widely used for evaluating leaf-feeding injury by Lepidopteran pests (Ortega *et al*., 1980). According to Kreps *et al*. (1998), stalk damage ratings are highly correlated with grain yield. However, Traisiri *et al*. (2010) used leaf feeding damage ratings as the sole resistance indicator of maize plants against ACB. In this study, it was found out that the field damage ratings were consistently high on the susceptible varieties and low in resistant or tolerant ones. In addition to the field tests, non-preference might be possible in native corn since ears harvested for some entries classified as resistant were comparable to resistant check variety. This means that these varieties could be damaged by ACB; however, later on these (varieties) were able to overcome borer pressure. Based on the results of the study, antibiosis and non-preference levels were exhibited by the best performing native corn varieties tested. The former resistance was shown in the two laboratory tests *viz.*, leaf feeding and stalk feeding assays, whereas the latter was observed for varieties with least field damage. Furthermore, this study has shown that the three resistant traits can be used to effectively screen Philippine native corn varieties for ACB resistance. Further selections will then be carried out from the best performing populations to develop ACB resistant inbred lines for their utilization in maize breeding program.

Table 5. Damage rating scale used in field screening for ACB resistance.

Score	Description
1	No damage on leaves and stalk
2	Top 1 or 2 leaves of the plant showing leaf feeding damage; internodes below or above the ears showing stalk feeding damage
3	About 3-5 leaves from the top showing feeding damage; at least 3 internodes showing stalk feeding damage
4	Almost 75% of the leaves showing feeding damage in the lower leaves; almost 75% of the stalks showing feeding damage
5	Severely damaged; all the leaves showing feeding damage; all the stalks showing feeding damage

Table 6. Classification key for Asian corn borer resistance in native varieties of corn in the Philippines.

Classification	Category		
	Leaf-feeding (% survival)	Stalk-feeding (mm)	Field (rating)
Highly Resistant	0.00 - 15.00	0.30 - 1.00	1.00 - 1.31
Resistant	16.00 - 30.00	1.00 - 10.00	2.00 - 2.50
Moderately Resistant	31.00 - 40.00	11.00 - 12.00	2.51 - 2.80
Susceptible	45.00 - 60.00	13.00 - 15.00	2.81 - 4.00
Highly Susceptible	60.00 - 100.00	16.00 - 30.00	4.10 - 5.00

CONCLUSION

A total of 125, 120, and 73 Philippine's traditional corn varieties were screened for Asian corn borer stalk-feeding, leaf-feeding and field resistance, respectively (Table 6). Out of these, several varieties were identified as candidate sources of resistance *viz.*, 3, 12 and 13 for field, leaf-feeding and stalk-feeding resistance, respectively. These materials can be utilized in the traditional maize breeding work of the country.

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