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MAIZE RESPONSE TO FALL ARMYWORM (*SPODOPTERA FRUGIPERDA*) AND ASIAN CORN BORER (*OSTRINIA FURNACALIS*) IN THE PHILIPPINES

M.T. CAASI-LIT and A.M.D. MARMETO*

Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines
*Corresponding author's email: admarmeto@up.edu.ph
Email address of co-author: mclit@up.edu.ph

SUMMARY

Philippine traditional maize has long existed for hundreds of years, facing and coping with continuous threats of different pests and diseases, with Asian corn borer and fall armyworm as the most damaging insect pests. Developing resistant varieties can employ rich genetic resources, as these varieties may have likely adapted to these biotic stresses. The development of ACB-resistant varieties proved successful with the Bt corn introduction; however, the attempt to breed for resistance to FAW is still underway. The two insect pests belong under the same Order, and cross-resistance between these pests may be possible. In this study, several traditional maize varieties underwent screening for resistance to ACB and FAW through larval survival on leaf-feeding assay. *Among the accessions observed, three variants of APN 0120 (Silangan) and three other traditional maize (APN 0397, APN 0913, APN 0938) showed intermediate to high resistance toward FAW leaf damage.* Few of these resistant varieties showed negative to Bt introgression. The most notable is the variant SI of APN 0120, with high resistance to ACB and FAW and low larval weight, without the presence of Bt. A previous report stated the original collection of this accession have potential ACB resistance, although Bt-introgressed. On the other hand, APN 0397 (Lagkitan), introgressed with Bt Cry1Ab, expressed dual resistance to ACB and FAW, too. It elucidates the reaction and potential of previously screened ACB-resistant varieties against FAW and the efficiency of Cry1Ab against FAW. It also demonstrates the potential of the traditional maize as source of germplasm to be explored to promote the utilization of open-pollinated varieties with inherent insect resistance. Moreover, this paper establishes mass-rearing procedures and laboratory techniques for effective fall armyworm-resistance screening using excised leaf method.

Keywords: Asian corn borer, bioassay technique, fall armyworm, host plant resistance, insect resistance, Philippine traditional maize

Key findings: Eight variants of Philippine traditional maize APN 0120 and two other conventional varieties showed potential resistance to ACB leaf feeding. In addition, three APN 120 variants and three more traditional maize exhibited possible fall armyworm leaf-feeding resistance. Dual resistance to both insect pests took notice on a few entries.

Abbreviations used: ACB – Asian corn borer, Bt – *Bacillus thuringiensis*, FAW – fall armyworm, CGUARD – Corn Germplasm Utilization Through Advanced Research and Development, DAP – days after planting, DAI – days after infestation, IPB – Institute of Plant Breeding, OPV – open-pollinated variety.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important crops in the Philippines, utilized both as food and feed, with a total production for 2021 reported at 915,592 mt: 22.24% for white corn and 77.76% for yellow corn (PSA, 2022). Production, however, dropped to approximately 681,000 mt of the total harvest in the first quarter of 2022. A survey revealed that in 2009, 71.88% of corn farmers utilized native or traditional open-pollinated varieties (OPV) for the whole country (PSA, 2014).

Traditional maize or native landraces are varieties utilized by local people, especially among small-scale farmers in major corn growing areas, which gained improvement through years of selection for traits suitable for their needs in their localities or through natural selection (Palacios-Pola *et al.,* 2021). Maize is naturally outcrossing, which makes open-pollinated varieties highly heterozygous (Flint-Garcia, 2013). As a result, the genetic variability of traditional varieties is considerably high, especially as the usual planting of different varieties occurred within an area before the introduction of commercial hybrids (Salazar *et al.,* 2016). In Mindanao, most corn farmers still allocate a high portion of their harvests for food and feed for backyard animals, sufficiently using these traditional varieties, even though Mindanao is one of the centers of growing corn hybrids, with Luzon (Gerpacio *et al.,* 2004; Salazar *et al.,* 2016). In a survey conducted by Caasi-Lit *et al.* (2021) among local farmers in Pangasinan, one of the provinces that have greatly adopted hybrid corn farming, farmers still plant traditional corn, mainly for personal consumption due to its good eating quality and low input requirements, such as, fertilizer and irrigation.

The Asian corn borer, *Ostrinia furnacalis* (Guenée), has long been the most damaging insect pest of corn in the Philippines (Sanchez, 1971; Morallo-Rejesus, 1985). Banks (1906) documented the first observation of ACB damage in several corn farms in Luzon. In the 1980s, the high usage of insecticides coincided with when ACB populations in the country were at their most destructive phase (Caasi-Lit *et al.,* 2017). Extreme reliance of farmers on synthetic pesticides to control this pest, including unauthorized mixing of chemicals and daily application, led to pesticide resistance of ACB. Open-pollinated varieties, including traditional maize, remain generally more susceptible to different insect pests and pathogens than commercial hybrids (Carpenter *et al.,* 2002; Ranilla, 2020). The Corn Germplasm Utilization through the Advanced Research and Development (CGUARD) Program of the Institute of Plant Breeding, UP Los Baños, in collaboration with the Department of Agriculture Bureau of Agricultural Research, aims to conserve and utilize Philippine traditional maize and screen for resistance or tolerance to biotic and abiotic stresses. The Entomology Laboratory of the IPB, part of this program, focuses on laboratory mass rearing and host plant resistance of corn to different lepidopterous pests, such as ACB, corn earworm (*Helicoverpa armigera* Hübner), corn semilooper (*Chrysodeixis eriosoma* Doubleday), common cutworm (*Spodoptera litura* Fabricius), true armyworm (*Mythimna separata* Walker), and most recently FAW and onion armyworm (*Spodoptera exigua* Hübner) (Caasi-Lit *et al.,* 2016).

The use of resistant or tolerant varieties in controlling insect pests emerged as a better alternative to lessen pesticide reliance while limiting the hazard to human health and the environment. Moreover, it would allow corn farmers to boost their income through increased yield and low input costs and utilize white corn as an alternative staple food to rice (Salazar *et al.,* 2016). Bt corn first gained commercialization approval in the Philippines in 2002, specifically, MON810, which expresses the gene *cry1Ab* — the toxin responsible for resistance to ACB (Yorobe and Quicoy, 2006; Mutuc *et al.,* 2012). According to the Bureau of Plant Industry (Alvarez *et al.,* 2021), the BPI approved, as of 2020, a total of 42 GM events for commercial use in the Philippines, either for direct use as food or feed or for commercial planting. GM hybrids in 2019 utilized around 0.943 million ha of land area planted with corn.

In comparison with ACB, the fall armyworm (*Spodoptera frugiperda* Smith) is a relatively new invasive pest of corn, with the first report in the Philippines by Navasero *et al.* (2019) in different municipalities of at least 10 provinces, having the very first record of incidence documented at Piat, Cagayan. This species originated in America and has now become a major pest problem for corn in the country (Adamczyk *et al.,* 1999; Navasero *et* *al.,* 2019; IPPC, 2019). Given its polyphagous nature, it has become a threat not only to the corn industry, but also to other economically important crops, such as, sorghum, cotton, and sugarcane (Parimi, 2019).

Screening for sources of resistance to ACB has already proceeded under one of the projects of the CGUARD Program (Salazar *et al.,* 2016; Caasi-Lit *et al.,* 2018). In one of its studies, selecting several corn accessions occurred with potential for ACB resistance: APN 0036 ('Valencia Orange' from Bukidnon), APN 0042 ('Tiniguib Cotabato' from South Cotabato), APN 0120 ('Silangan' from Pangasinan), and APN 0131 ('Malungun D' from Bukidnon). Confirming the inherent resistance of these accessions through the Bt ELISA test showed the introgression of Cry1Ab in the traditional maize varieties, especially the APN 0120 (Caasi-Lit *et al.,* 2021).

This study aims to evaluate the response of different traditional maize germplasm to leaf feeding by fall armyworm and Asian corn borer, including the different variants of APN 0120, and to determine whether the resistance response of this germplasm correlates to the two related insect pests. It is the first attempt of studying host-plant resistance against FAW in the Philippines, especially among native landraces. The preliminary results of this study will provide baseline information on the potential of Philippine traditional maize as germplasm source in breeding for fall armyworm resistance.

MATERIALS AND METHODS

Background of the study

The study took place in the Entomology Laboratory, Institute of Plant Breeding, University of the Philippines Los Baños, from December 2019 to April 2021, under one of the external projects funded by the Department of Science and Technology Grants-In Aid (DOST GIA). Seeds of selected traditional maize varieties utilized came from the CGUARD Collection of the Cereals Breeding Group, IPB-UPLB. Collection of the different variants of APN 120 (Silangan) from local farmers in various municipalities of Pangasinan occurred from February 2017 to March 2019. Open-pollinated varieties, IPB VAR6 (white corn) and IPB VAR13 (yellow), also came from the Cereals Breeding Group, with the Bt corn hybrid NK6410 procured from commercially available supply.

Test plants

Collected variants of APN 120 (Table 1; referred to as Set A) followed planting in double 5-m rows in IPB-UPLB in December 2017, with 50 cm row and 20 cm planting distance, arranged in Alpha Lattice Design. Planting of other traditional maize varieties (Table 1; referred to as Set B) proceeded in April 2021 at a 75- cm row distance. Standard agricultural management of corn was followed, including fertilizer application (14-14-14 and 46-0-0), irrigation, thinning out, and weeding.

Test insects

The FAW and ACB egg masses collection from the field progressed weekly in nearby maize fields of Los Baños, Laguna, then brought to the laboratory for mass rearing in a Petri dish lined with moist filter paper. Upon hatching, transferring neonates continued in rearing pans with the IPB ACB Diet formulated by Caasi-Lit *et al.* (2015), with corn and soybean as the main ingredients and fortified with a vitamin complex, to provide optimum growth and development of larvae.

Transfer of newly hatched ACB larvae to a rearing pan with layer of artificial diet ensued, following the methods of Caasi-Lit *et al.* (2015) for laboratory mass rearing of ACB. These were allowed to grow until the last instar stage before harvesting and transferring to a new rearing pan lined with tissue paper to promote pupation. Pupae separation according to sex succeeded, with the adults allowed to mate in oviposition cages lined with wax paper as an oviposition medium. The ACB adult diet used honey-sugar solution. The harvest of laid egg masses includes cutting these from the wax paper and transferring them to rearing pans with a thin layer of artificial diet until hatching. Five-day-old G1 population larvae underwent bioassay screening.

As for FAW larvae, an individual separation occurred in rearing cups upon reaching the second instar stage to prevent cannibalism and reared until pupation. The artificial diet replenishment or replacement continued whenever necessary or in the presence of molds. Pupae were collected from each cup, separated according to sex, and placed inside an oviposition cage with one pot of 21-day-old susceptible maize plant as an oviposition medium. A honey-sugar solution served as an adult diet. After adult emergence, these were allowed to mate in the dark, with laid egg masses collected two days after mating. These were transferred to a rearing

Table 1. Basic information and response of different traditional maize on Asian corn borer and fall armyworm.

Variety	Set	Local Name	Origin of Seeds	Type	MLS (ACB)	LFR (ACB)	LDR (ACB)	MLS (FAW)	LFR (FAW)	MLW (g) (FAW)	MFDR (FAW)	FDR (FAW)	EM (FAW)	Bt Cry1Ab
APN 0120 (SA)	A	Silangan A	Pangasinan	Traditional	79.29% ^{bc}	HS	11.5	93.33% ^{abc}	HS	0.0816 ^{bc}	4.2 ^{ab}	I	-	Positive
APN 0120 (SB)	A	Silangan B	Pangasinan	Traditional	20.11% ^{def}	R	4.9	53.49% ^{f}	I	0.0485 ^{ef}	4.5 ^{a}	I	-	Positive
APN 0120 (SC)	A	Silangan C	Pangasinan	Traditional	67.82% ^{c}	HS	8.5	88.89% ^{abc}	HS	0.0697 ^{cd}	4.5 ^{a}	I	-	Negative
APN 0120 (SD)	A	Silangan D	Pangasinan	Traditional	73.33% ^{c}	HS	9.8	84.10% ^{abc}	HS	0.0517 ^{de}	4.0 ^{abc}	I	-	Negative
APN 0120 (SE-A)	A	Silangan E-A	Pangasinan	Traditional	6.67% ^{efgh}	HR	2.4	60.07% ^{ef}	S	0.0222 ^{g}	3.0 ^{bcdef}	R	-	Positive
APN 0120 (SE-B)	A	Silangan E-B	Pangasinan	Traditional	20.71% ^{de}	R	4.4	65.56% ^{def}	S	0.0307 ^{fg}	3.6 ^{abcd}	I	-	Positive
APN 0120 (SE-C)	A	Silangan E-C	Pangasinan	Traditional	15.56% ^{defgh}	R	4.2	64.98% ^{def}	S	0.0314 ^{fg}	3.7 ^{abcd}	I	-	Positive
APN 0120 (SF)	A	Silangan F	Pangasinan	Traditional	18.89% ^{defg}	R	5.2	90.79% ^{abc}	HS	0.0409 ^{efg}	3.4 ^{abcde}	I	-	Negative
APN 0120 (SG)	A	Silangan G	Pangasinan	Traditional	2.26% ^{gh}	HR	2.9	55.14% ^{d}	I	0.0228 ^{g}	2.1 ^{ef}	R	-	Positive
APN 0120 (SH)	A	Silangan H	Pangasinan	Traditional	3.41% ^{fgh}	HR	3.6	81.11% ^{abcd}	HS	0.0538 ^{de}	3.1 ^{abcde}	R	-	Positive
APN 0120 (SI)	A	Silangan I	Pangasinan	Traditional	3.37% ^{fgh}	HR	1.9	32.72% ^{g}	R	0.0316 ^{fg}	2.1 ^{ef}	R	-	Negative
APN 0120 (SJ)	A	Silangan J	Cavite	Traditional	90.87% ^{ab}	HS	11.0	97.78% ^{a}	HS	0.1111 ^{a}	3.5 ^{abcde}	I	-	Negative
APN 0909	A	Native Yellow	Iloilo	Traditional	26.59% ^{d}	R	5.8	77.36% ^{cde}	S	0.0388 ^{efg}	3.0 ^{bcdef}	R	-	Positive
APN 0941	A	Igod	Antique	Traditional	96.67% ^{a}	HS	10.7	93.26% ^{abc}	HS	0.0958 ^{ab}	2.6 ^{def}	R	-	Negative
APN 0955	A	Bukay	Antique	Traditional	83.03% ^{abc}	HS	11.1	93.10% ^{abc}	HS	0.0687 ^{cd}	2.7 ^{cdef}	R	-	Negative
IPB VAR6	A	White OPV	IPB-UPLB	OPV	91.11% ^{ab}	HS	12.1	89.73% ^{abc}	HS	0.0934 ^{ab}	3.8 ^{abcd}	I	-	Negative
IPB VAR13	A	Yellow OPV	IPB-UPLB	OPV	76.90% ^{bc}	HS	9.9	95.56% ^{ab}	HS	0.0764 ^{bc}	3.5 ^{abcde}	I	-	Negative
BT	A	NK6410	Syngenta Philippines, Inc.	Commercial hybrid	0.00% ^{h}	HR	1.2	97.78% ^{bcd}	S	0.0291 ^{fg}	1.7 ^{f}	HR	-	Positive
APN 0296	B	Zamboanga Mahayag	Zamboanga del Sur	Traditional	93.30% ^{a}	HS	6.8	77.60% ^{ab}	S	-	-	-	3.8	Negative
APN 0397	B	Lagkitan	Batangas	Traditional	10.23% ^{c}	HR	1.5	55.82% ^{abcd}	I	-	-	-	3.4	Positive
APN 0399	B	Lagkitan	Batangas	Traditional	96.51% ^{a}	HS	6.2	82.06% ^{a}	HS	-	-	-	3.6	Negative
APN 0424	B	Mais Na Pula	Quezon Province	Traditional	94.33% ^{a}	HS	6.8	70.77% ^{abc}	S	-	-	-	2.6	Negative
APN 0428	B	Mais Na Pula	Batangas	Traditional	87.19% ^{a}	HS	5.5	69.14% ^{abc}	S	-	-	-	3.1	Negative
APN 0432	B	Sabnit	Batangas	Traditional	92.03% ^{a}	HS	6.5	85.36% ^{a}	HS	-	-	-	3.8	Negative
APN 0445	B	Lagkitan	Batangas	Traditional	93.33% ^{a}	HS	6.1	89.65% ^{a}	HS	-	-	-	4.1	Negative
APN 0612	B	Sinabmit	Palawan	Traditional	87.59% ^{ab}	HS	5.3	63.97% ^{abc}	S	-	-	-	2.7	Negative
APN 0913	B	Native Pula	Capiz	Traditional	94.25% ^{a}	HS	6.1	38.74% ^{cd}	R	-	-	-	3	Negative
APN 0938	B	Pilit	Antique	Traditional	97.78% ^{a}	HS	5.7	41.27% ^{bcd}	I	-	-	-	2.3	Negative
APN 0977	B	Mais Nga Pula	Iloilo	Traditional	88.89% ^{a}	HS	6.0	82.68% ^{a}	HS	-	-	-	2.6	Negative
IPB VAR6	B	White OPV	IPB-UPLB	OPV	95.52% ^{a}	HS	7.1	84.29% ^{a}	HS	-	-	-	1.8	Negative
IPB VAR13	B	Yellow OPV	IPB-UPLB	OPV	98.89% ^{a}	HS	5.9	53.74% ^{abcd}	I	-	-	-	3.3	Negative
BT	B	NK6410	Syngenta Philippines, Inc.	Commercial hybrid	0.00% ^{c}	HR	0.7	24.91% ^{d}	R	-	-	-	2.2	Positive

*Letter of significant difference from Tukey's LSD Test (α=5%)

MFDR – mean field damage rating
FDR – field damage response
EM – egg mass count
MLS – mean larval survival
LFR – leaf feeding response
MLW – mean larval weight
LDR – leaf damage rating

Resistance Response:
HR – highly resistant
R - resistant
I – intermediate
S – susceptible
HS – highly susceptible

Table 2. Rating scale used to assess feeding damage of ACB in leaf feeding assay.

Score	Description
0	No visible feeding damage
1	Few pinholes
2	Few elongated lesions
3	Fifty percent (50%) of leaf area has lesions
4	Seventy-five percent (75%) of leaf area has lesions
5	Leaves totally consumed and need replacement

Table 3. Rating scale for fall armyworm damage (Prasanna *et al.,* 2018).

Score	Description
1	No visible leaf-feeding damage
2	1-2 older leaves with pinholes
3	Shot-hole injuries on less than 5 leaves; Small circular hole damage to leaves
4	Shot-hole on 6-8 leaves; Small circular lesions and few small elongated lesions
5	Elongated lesions on 8-10 leaves; Few small- to mid-sized holes (basement membrane consumed)
6	Large, elongated lesions on 6-8 leaves; Large uniform to irregular-shaped holes
7	Many elongated lesions of all sizes on several leaves; Several mid- to large-sized uniform to irregular-shaped holes
8	Many elongated lesions of all sizes on several leaves; Many mid- to large-sized uniform to irregular-shaped holes
9	Whorl and furled leaves almost destroyed and plant dying

pan with an artificial diet and allowed to hatch under optimum conditions. Second instar larvae served as test insects for laboratory assay.

ACB resistance screening

Evaluation of the response of the maize accessions to leaf feeding by the Asian corn borer used the bioassay screening methods of Caasi-Lit *et al.* (2018). At 25 to 30 DAP, maize whorls underwent harvest from at least 10 plants per replicate, cutting into leaf discs with a 16.52 cm^2 area. Two leaf discs got placed in a small plastic Petri dish lined with moist filter paper and infested with one vigorous four-day-old larva, with 30 samples for each of the three replicates. Survival was observed daily for five days.

Another parameter considered was the feeding damage through the leaf area consumed. Characteristics of leaf damage rating used the description in Table 2. Cumulative feeding damage was taken at the end of the observation period for all samples.

Testing for presence of Bt protein

Determining the purity of the maize accessions from possible introgression of Bt genes used AgraStrip® Cry1Ab Seed and Leaf (Romer Labs, Austria). Leaf samples from all test plants were collected and macerated with distilled water using a plastic pestle in a microcentrifuge tube. After standing for around 1 min each tube received a Bt Cry1Ab strip until the plant extract reached the top of the strip through capillary action. Strips with one line were interpreted as negative to Cry1Ab protein, while strips with two lines were as positive.

FAW resistance screening

Assessment of damage to natural FAW infestation on traditional maize used the rating scale in Table 3 by Prasanna *et al.* (2018) at the peak of the infestation (Set A: 25 DAP). Insects observed on all rows, including their stages, were also noted. For Set B, FAW egg masses survey and collection followed at six to 14 DAP every two days.

Observing the leaf-feeding response of FAW on the different maize accessions went on at 35 DAP. Maize whorls collected from the field received cutting into 2.54 cm (1-in) leaf sections, then placed in assay cups, with each cup infested with one five-day-old larva using a fine camel hair brush, with three replicates per accession and 30 samples per replicate. Survival of the test insects assessed daily went on over a 10-day observation period. Leaves, replenished whenever necessary, supported the growth of the larvae. Measuring the larval weight at the end of the observation period used Mettler Toledo Analytical Balance (Mettler-Toledo International Inc., USA). Grouping of potential resistance on FAW of the

Table 4. Classification key for potential fall armyworm resistance (Caasi-Lit *et al.,* 2018).

Scale	Classification	Category					
		Leaf-feeding Mean Larval Survival (%)			Leaf Damage Mean Field Rating		
1	Highly Resistant	0.00	-	20.00	1.0	-	1.4
2	Resistant	20.01	-	40.00	1.5	-	3.4
3	Intermediate	40.01	-	60.00	3.5	-	5.4
4	Susceptible	60.01	-	80.00	5.5	-	7.4
5	Highly Susceptible	80.01	-	100.00	7.5	-	9.0

maize accessions progressed according to percent larval survival, as shown in Table 4.

Experimental design and statistical analysis

All laboratory and field experiments employed a completely randomized design (CRD). Means comparison used Fisher's Least Significant Difference (LSD) Test at α = 5%, while statistical analyses used R Packages v. 4.2.0 (R Core Team, 2022).

RESULTS AND DISCUSSION

Asian corn borer resistance

Most accessions, introgressed with Bt Cry1Ab, were rated resistant to highly resistant in terms of ACB leaf feeding (Table 1), including the Bt corn hybrid for both sets, which remained highly resistant. However, one variant of APN 0120 revealed susceptibility to ACB, although positive for Bt strip test. The low concentration of Bt toxin present in this variety explains this possibility.. According to Alcantara *et al.* (2011), the median lethal concentration (LC_{50}) of Bt Cry1Ab for ACB populations in the Philippines ranged from 0.42 to 2.37 ng/cm^2 and 0.76 to 2.11 ng/cm^2, specifically for Laguna population, which the study used. The commercial Bt strip used provided only qualitative data on the presence of the Bt protein; however, a possibility shows the detected protein has lower concentration than the median lethal dose. On the other hand, the susceptible check IPB VAR6 remained highly susceptible (Figure 1), as well as IPB VAR13. Interestingly, the APN 0120 variants, SF and SI, were rated as resistant and highly resistant, respectively, even in the absence of the Bt gene. Although APN 0120 has been previously reported to exhibit high resistance to ACB, mainly due to the Bt *cry* gene in the genome (Caasi-Lit *et al.,* 2021), some strains remain susceptible to ACB. APN 0909 was also resistant, and Bt strip testing showed that this accession was positive for Bt-introgression.

Expected high mortality of ACB in Bt hybrid already remains since this contains the toxin *cry1Ab* (Alcantara *et al.,* 2011). As such, the same reaction of ACB can result in Bt-introgressed varieties incorporated with the same genes from transgenic pollen. As maize is naturally outcrossing, and with the large proportion of corn field areas planted with Bt corn, there exists a high possibility for nearby

Figure 1. Feeding damage of ACB larva on susceptible maize leaf disc (IPB VAR 6) at two days after infestation.

OPVs' pollination by surrounding transgenic plants (Lin and Pan, 2016). The detection of Bt genes in traditional maize first gained reported by Quist and Chapela (2001) in Oaxaca, Mexico, and recently by Caasi-Lit *et al.* (2021) in Philippine varieties. Non-Bt corn may acquire resistance at par with Bt hybrid through introgression, which may fortify the defense of local traditional maize (Chilcutt and Tabashnik, 2004).

Interest in APN 0120 variant SI increased, which responded with only 3.37% ACB larval survival and only an average of 1.9 leaf damage rating or a few elongated lesions (Table 1). It showed the lowest %MLS and LDR among the varieties screened without the presence of Bt. It indicates that larvae feeding on this variant will not survive long and will cause minimal damage to the plant. The resistance mechanism against ACB of this variant needs more exploration, together with the other varieties exhibiting inherent resistance.

Fall armyworm resistance

Observing the incidence of FAW infestation for Set A began at 16 DAP (Figure 2), with different larval stages observed on all plants. A negligible amount of egg mass occurred in this trial. From the mean-field damage rating (MFDR) in Table 1, Bt had the lowest damage, although a few feeding sites showed and were not entirely damage-free, followed by APN 0120 variants SI and SG. Consequently, three traditional maize (APNs 0909, 0941, 0955) and two other APN 0120 variants have shown potential field resistance to FAW. Field damage was not taken in Set B accessions as infestation and damage occurred at an earlier stage of the plant (6-12 DAP). The available rating scale is not suitable for the early vegetative stage of corn.

Confirmation of resistance through laboratory leaf feeding assay (%MLS) showed few accessions with potential resistance to leaf feeding (Figure 3). For Set A, three APN 0120 variants showed intermediate to resistant response: SB, SG, and SI. Among these three, SB and SG are Bt-introgressed, while SI is negative and has the lowest larval survival. Six other accessions observed exhibited lower survival as well in comparison with Bt maize. Although Bt only had 2.22% larval mortality, the average weight of surviving larvae after a 10-day observation period weighed only 0.0291 g, which is significantly lower than the susceptible IPB VAR6 (0.0934 g). A reduction in larval weight indicates the antibiosis mechanism of Bt, which affects the growth and development of larvae (Sharmad *et al.,* 2005).

Figure 2. Damage on maize plants incurred from fall armyworm infestation on January 2020 at IPB Complex, UPLB.

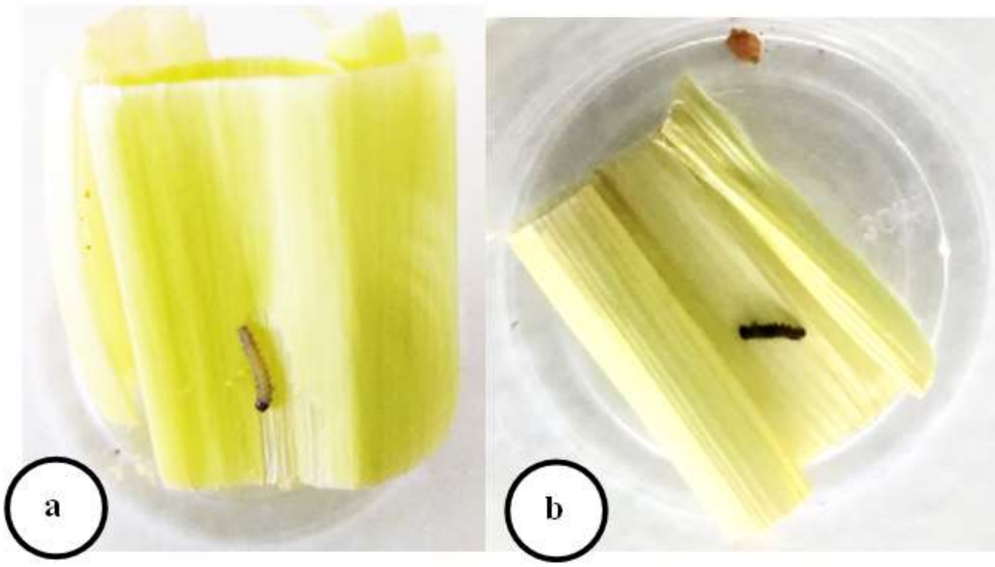

Figure 3. Leaf-feeding assay of fall armyworm: (a) larva feeding on maize whorl and (b) dead larva after feeding on maize whorl (Bt).

This developmental lag among larvae fed with Bt resulted in other accessions with high mortality, such as, APN 0120 strains SI (0.0316 g) and SG (0.0228 g). Other traditional maize, APNs 0909, 0941, and 0955, were included in this study due to their potential for FAW resistance from the field rating conducted in September 2019 (unpublished data). These three accessions rated susceptibility based on larval survival and a Bt-introgressed APN 0909 also showed developmental lag based on the final larval weight (0.0388 g).

In Set B, APN 0913 gained a resistant rating, with APN 0397 and APN 0938 intermediate, along with IPB VAR13. In this set, Bt showed resistance at 24.91% MLS. APN 0397 is Bt-introgressed, while APNs 0913 and 0938, as well as, IPB VAR13, scored all negative to Bt. IPB VAR6 remained susceptible to FAW. The highest average number of FAW egg masses appeared in susceptible varieties APN 0445, APN 0296, and APN 0432; in contrast, the lowest number in the check IPB VAR6.

Dual resistance to ACB and FAW

Most of the accessions found susceptible to ACB showed susceptible to FAW also. More accessions are susceptible to FAW than ACB, indicating that FAW-resistant variety search is trickier. In summary, four accessions have dual resistance to ACB and FAW: APN 0120 variants SB, SG, and SI; and APN 0397. Among these four, APN 0120 SI is the only one without Bt. CIMMYT researchers have been successful in breeding maize with multiple borer resistance to southwestern corn borer (*Diatraea grandiosella*), sugarcane borer (*D. saccharalis*), European corn borer (*O. nubilalis*), and fall armyworm in 1984 (Benson, 1986; Smith *et al.,* 1989), with resistance mechanism through lower nitrogen content, thus lower nutritional value, and high fiber and phenolics content (Bergvinson *et al.,* 1994). This is one of the possible resistance mechanisms exhibited by variant SI.

The Asian corn borer and fall armyworm belong to the insect order Lepidoptera. ACB is highly susceptible to corn hybrids expressing *cry1Ab* and other *cry* toxins (Alcantara *et al.,* 2011). On the other hand, FAW is more susceptible to *cry1F* than other *cry1* genes (Luo *et al.,* 1999; Siebert *et al.,* 2008). Nevertheless, the presence of *cry1Ab* may still provide partial resistance against FAW. Prasanna *et al.* (2018) listed some maize germplasm or inbred lines developed with potential and at least partial resistance to FAW damage. Among those listed include corn-borer-tolerant inbreds: B49, B52, B64, B68, and B96. Hence, the potential of ACB-resistant varieties against FAW may need further study by corn breeders and researchers. Moreover, Singh *et al.* (2022) listed several traditional maize landraces with potential FAW resistance as well, which suggests that the local maize may serve as a prospective source of resistance factors for FAW resistance breeding.

CONCLUSIONS

The Philippine traditional maize remains susceptible to ACB and even more to FAW. Despite that, this study explored the very few varieties exhibiting potential resistance to ACB or FAW and more so to both pests. Screening for resistance among local traditional maize needs continuity, and efforts can focus first on already identified ACB-resistant varieties. As planting of Bt hybrids widely spread in the country, the introgression of transgenic pollen from Bt hybrids to local traditional landraces proved inevitable. This gene flow has provided the latter an opportunity to acquire resistance to the target pests. Even though the use of transgenic crops as food is still controversial, it serves as an advantage to local maize farmers in this "tug-of-war" between the host plant and the insect pests.

RECOMMENDATIONS

The potential of ACB-resistant varieties and the Bt-introgressed accessions require exploring to develop FAW-resistant cultivars from local traditional maize that may be deployed to local maize farmers to boost the utilization of open-pollinated varieties. Resistance mechanisms of resistant accessions, especially non-Bt varieties, need understanding, including phytochemical and morphological properties. Bt hybrids with Cry1F may be used, as well as, one of the resistant checks, as FAW is more susceptible to this toxin than Cry1Ab. The mechanisms of resistance exhibited by APN 120 (SI), and other varieties exhibiting resistance without the presence of Bt, need further study also.

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