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Efficacy Study of Selected Maize (*Zea mays* L.) Genotypes Against the Asian Corn Borer, *Ostrinia furnacalis* (Guenée)

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Laboratory and screen house studies were conducted to assess the efficacy of selected twenty-six maize varieties against Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée). Hierarchical tree based on resistance parameters clearly revealed that the twenty-six maize varieties are best classified into three groups, viz. resistant, intermediate, and susceptible. In laboratory test, the varieties RCS2, RCS4, RCS5, 'IPB Var13', *Bt2*, *Bt3*, and *Bt4* were consistently identified as highly resistant. Further examinations in stalk feeding assay showed that the resistant varieties had sharp decline in larval survival (LS) on the 2nd to the 5th day of larval feeding in stalk tissue. On the other hand, the susceptible varieties showed high LS across the 5-day feeding duration. This research highlighted that resistant maize varieties were mainly attacked below the ear nodes and internodes, with the most frequent observation on the 8th node below the ear node but had zero attack on the crucial ear node. Meanwhile, both the intermediate and the susceptible varieties were attacked on the ear node, with the feeding attacks distributed throughout the whole plant. This research provided further evidence that ACB resistance is available in Philippine native maize varieties. These experiments corroborate with previous results that transgenic maize expressing the *Cry1Ab* protein is lethal to ACB larvae. High resistance observed from the non-*Bt* 'IPB Var13' suggests that natural resistance is present. This research have presented that *Bt* varieties (*Bt2, Bt3, Bt4*) together with RCS2, RCS4 and 'IPB Var13' were the consistently resistant varieties in both laboratory and screen house assays. The plant resistance indices used in this study can effectively screen different varieties of maize in the Philippines. Further experimentations would be needed to determine the mechanism involved in the resistance and susceptibility of maize varieties infested with ACB.

Keywords: Asian corn borer, bioassay, *Bt* maize, *Cry1Ab* protein, herbicide-tolerant maize, larval damage, recycled commercial seeds.

Abbreviations: ACB— Asian corn borer, DAI—days after infestation, DAP—days after planting, LDA—laboratory leaf disc assay, LS—larval survival

INTRODUCTION

Maize is believed to have been introduced to the Philippines through the Manila-Acapulco (Mexico) galleon trade during the Spanish colonial period. From then on, there were substantial domestic increases in maize volume of production (PSA 2017). The increase is primarily attributed to the higher demand for maize for human food, animal feed formulations, and industrial purposes. The yield-enhancing outcomes of the now accessible agricultural inputs contributes to the increased maize production that affects the pest population present in the field which used to reduce maize production and incur severe losses for farmers.

Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée) was first recorded as a pest of maize in Southeast Asia in 1905 (Banks 1906). This pest is a serious destructive and recurrent pest to maize in the Philippines (Dalmacio et al. 2007). Corn borer foliar damage results in reduction of total leaf area and depression of photosynthetic capacity of the plant (Tefera et al. 2016). Yield loss due to ACB incurred by maize farmers in the past decades ranges from 20–80% (Sanchez 1971) and can shift to 80-100% under heavy infestation (Klaus-Quemada 2005). With this, it is imperative to have ACB resistant maize germplasm available for breeding because maize resistance is a critical component of ACB control and integrated management (He et al. 2003; Stout 2014).

Resistance might be found in the cultivars, landraces, wild progenitors, related species and genera (Balconi et al. 2012). Therefore, identification of sources of resistance to ACB among maize varieties in breeding material and gene bank collections is of great importance.

Nowadays, Philippine maize farmers have wider range of choices of maize varieties as planting material such as the landraces, improved open-pollinated varieties (OPVs), conventional hybrids, transgenic *Bt* or glyphosate -resistant hybrids (or oftentimes stacked). There are even unauthorized or recycled commercial seeds (RCS) locally termed as "*ukay-ukay*" or "*sige-sige*" which are factual across local niches in the country, where farmers exploit the simply inherited transgenic traits. Initial local study of Caasi-Lit et al. (1989) found that there is genetic variation to be exploited in breeding for resistance to ACB from maize hybrids and open-pollinated varieties. On December 4 of 2002, the Department of Agriculture authorized the propagation of *Bt* maize varieties incorporated with the MON810 event (Trade name: YieldGuard® maize) as an option to control ACB. YieldGuard® maize expresses the *Cry1Ab* protein and is lethal to feeding ACB larvae (He et al. 2003; Alcantara et al. 2011). Recently, ACB resistance is suggested to be available in the Philippine native maize varieties (Salazar et al. 2016; Caasi-Lit et al. 2018). Thus far there are no local studies and information available which deals with comparing the reaction of different maize varieties towards ACB feeding. Hence, this study aims to (1) screen the different maize varieties for resistance to ACB in laboratory and screen house experimentation; (2) evaluate which parts of maize plants are susceptible to ACB, and (3) compare the reaction of the different maize varieties against the ACB both in laboratory and screen house experimentation.

MATERIALS AND METHODS

Plant Materials and Management

Twenty-six varieties of maize were used that is composed of the selected six accessions of Philippine native maize (coded as "APN"), five recycled commercial hybrid seeds (coded as "RCS"), two farmer's cultivar (coded as "FC"), four transgenic herbicide-tolerant (coded as "HT"), four *Bt* maize (coded as "*Bt*"), two public-bred non-transgenic OPVs (viz. 'IPB Var6' and 'IPB Var13'), a non-transgenic F_1 Hybrid (P30B80), and two special type maize varieties as susceptible checks viz. cv. 'Lagkitan' and 'Supersweet' which are glutinous and sweet maize, respectively. The varieties were planted at the Institute of Plant Breeding (UPLB, Los Baños, Laguna, Philippines) in the screen house following Randomized Complete Block Design (RCBD) with three replications (Davis and Williams 1997). Planting was done during the wet season of 2017 from July to October. One row per plot of 5 meters length was assigned for each variety and the distance between rows were spaced at 50 cm apart. Plants within a row were spaced at 20 cm. Thus, 25 hills were accommodated per plot. Seeding rate was two seeds per hill and was thinned-to-one fifteen days after sowing. Complete fertilizer (14%N-14%P_2O_5-14%K_2O) was applied as basal fertilizer at a rate of 120-60-60 and nitrogen side-dressing was implemented using granular urea (46% Nitrogen) at a rate of 3 bags per hectare on the thirty day-old maize plants. No insecticide application was implemented. However, supplemental overhead irrigation was carried out when rainfall was insufficient throughout the duration of the study.

Asian Corn Borer Rearing

The initial population of ACB egg masses was collected from maize fields in Laguna and mass reared in the laboratory in a meridic diet developed by Caasi-Lit et al. (2016) and allowed to become adults to generate the next cycle of larva batches as test insect larvae which are the 4 - 5 day-old second instars.

Excised Plant Tissue Experiment

A. No - Choice Antibiosis Study of Excised Whorl Leaf Discs

Sample leaves from the whorl of maize varieties at V6 stage which occurred at 30 days after planting (DAP) was used for the bioassay. Laboratory leaf disc assay (LDA) was executed as follows: for each variety, sampled whorls were unfurled and leaves were excised to form discs to fill thirty Petri dishes arranged following the Complete Randomized Design (CRD) for three replications. The set up was placed in a constant temperature-humidity room at 26.7 ± 2°C and 60 ± 5% RH in 16L:8D. Filter paper was moistened with 300 μL of distilled water to maintain a suitable moisture level. One ACB larva (4 - 5 day old second instar) was placed on an excised leaf disc in each Petri dish. Each petri dish were then covered with a lid and sealed with parafilm and supervised for five days while leaf disc was replaced daily. The number of surviving larvae was counted for the five-day monitoring period. Larval survival (LS) expressed in percentage was computed as the number of larvae that survived over the total number of introduced larvae.

B. No - Choice Antibiosis Study of Excised Stalk Internode

Stalk feeding assay (SFA) were executed as follows: ten random maize plants were harvested at 45 DAP and internodes from top to bottom were picked-out measuring 1 inch and were placed on designated bioassay cups. The Bioassay cups containing the excised stalk were infested with one 4 - 5 day old second instar ACB larva.

The cups were then covered. Daily monitoring was done but final assessment was implemented at five days after infestation (DAI). The number of surviving larvae was counted. The tunnel length, width, and tunnel area produced by the feeding larva were measured and recorded.

Screen House Experimentation

The "Barbeque" infestation technique (Caasi-Lit et al. 2016) developed at the Entomology Laboratory – Institute of Plant Breeding was used for artificial infestation. Briefly, the infestation medium was prepared separately where leaves and stalks of the 'IPB Var6' maize plants were collected at 25 DAP and were cleaned and cut into standardized sizes of 1.5 inches for leaves and one inch for stalks. Fifty 4 - 5 day-old second instar of ACB feeding initially on a rearing media were transferred onto the infestation medium using a pointed and soft Camel-hair brush, placed in plastic cups sealed with tissue paper, and covered. Plastic cups holding the infestation media were incubated under ambient conditions in the laboratory for 3 - 4 days prior to the day of infestation coinciding with the targeted stage of the standing maize plants in the screen house which was 25 - 30 days old. One infestation media was placed in the inner whorl of the randomly tagged 10 plants per plot and were allowed to settle for five days. The infestation was conducted early morning at the V6 whorl stage which occurred within the 25 - 30 DAP.

At 7 and 14 days after infestation (DAI), the test plots were visually rated for ACB leaf-feeding damage using a 9-class rating scale developed by Guthrie et al. (1960). Damage rating was done on the whorl and leaves including the partially and fully expanded leaves. With this scale, resistant accessions receive the lowest numeric ratings. At 60 DAP, plant heights of the artificially infested ten plants were measured from the soil surface to the first branch of the tassel. The sample plants were then harvested by cutting the stalk close to the soil surface leaving no more than 20 cm remnant stalk. The harvested sample plants were then evaluated as to the (i) number and location of node/s and internodes with ACB damage and (ii) tunnel length produced by the feeding larva. ACB damage on stalk was evaluated by split dissection procedure and the length and width of the tunnel damage were measured. Percent stalk tunnel damage was calculated as the total tunnel length within the whole stalk over the plant height.

Statistical Analysis

One-way ANOVA was conducted to detect significant difference in the performance or reaction of maize varieties against the ACB in laboratory bioassay and screen house artificial infestation using R statistical computing language and environment v.3.6.2 (R CoreTeam, 2019), together with the 'agricolae' (De Mendiburu 2020) R package, consistent with the design of the experiment. Means detected with significant difference were compared according to Tukey's Honest Significant Difference (HSD; Tukey 1949) post hoc means comparison. Multivariate cluster analysis of varieties was also executed utilizing the Euclidean distance measure in average linkage or UPGMA agglomerative method. Larval survivability and stalk feeding tunnel length data in laboratory assay were used in clustering, whereas, 7 and 14 DAI leaf damage rating and stem tunnel length data in screen cage experiment were used in clustering.

RESULTS AND DISCUSSION

Performance and Reaction of Maize Varieties to ACB on Leaf Discs and Stalk Feeding Bioassays

ANOVA revealed that variety means are declared highly significantly different ($p \leq 0.01$) in terms of their effect to ACB percent LS in LDA, SFA, and the resulting tunnel damage measurements. Figure 1 illustrates LS in LDA and SFA. All the four *Bt* varieties which expresses the ACB larvae toxic *Cry1Ab* protein resulted to zero ACB LS in LDA. Notably, RCS2, RCS4 and 'IPB Var13' also resulted to zero ACB LS. They are identified as highly resistant varieties where LS ≤ 17%. HSD mean comparison test showed that APN36, RCS3, RCS5, APN120, and RCS1 are not significantly different from the highly resistant varieties although their LS ranged from 3.3 – 13.3%. Moderately resistant varieties are the HT4, FV1 and FV2 where LS ≥ 17% but < 33%. Resistant varieties are HT1, HT3 and APN32 where LS ≥ 33% but < 50%. P30B80 is identified as susceptible where LS ≥ 50% but < 67%. Moderately susceptible varieties are the 'Lagkitan' (check), APN24, 'Supersweet' maize (check) and 'IPB Var6' where LS ≥ 67% but < 83%. Finally, APN40, HT2 and APN33 are identified as highly susceptible where LS ≥ 83%.

In SFA, *Bt2* and RCS4 are identified as the highly resistant varieties which resulted to zero and 2.2% ACB LS, respectively (Figure 1). HSD mean comparison test showed that *Bt3*, RCS2, 'IPB Var13', *Bt4* and RCS5 are not significantly different from the highly resistant varieties although their LS ranged from 11.1 – 15.6%. *Bt1* and RCS3 are identified as moderately resistant and resistant varieties, respectively. APN40, APN36, FV2, APN120, HT2, APN24, 'Supersweet' maize (check) and RCS1 are identified as susceptible. Moderately susceptible varieties are the HT4, 'IPB Var6', FV1 and HT3. Finally, HT1, 'Lagkitan' (check), APN33, P30B80 and APN32 are identified as highly susceptible varieties. LS in SFA has

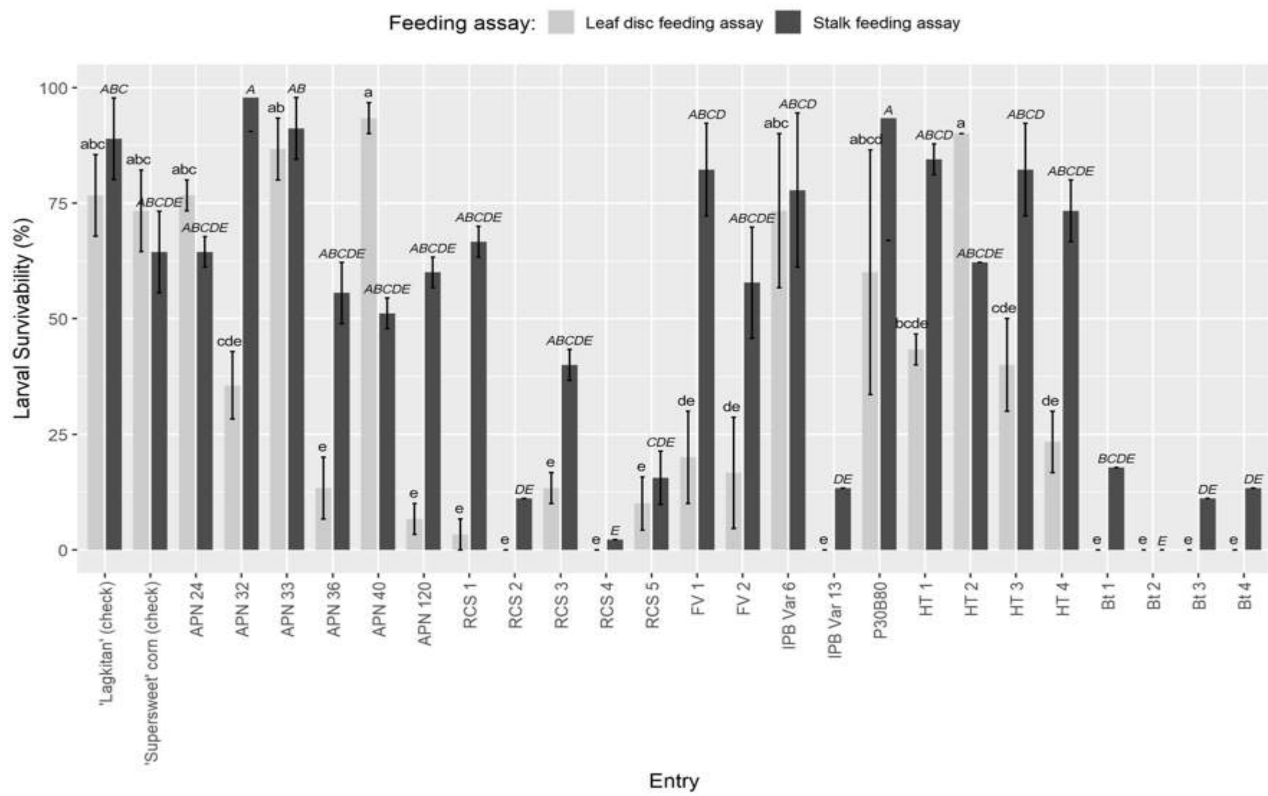

Fig. 1. Mean larval survival (%) of Asian corn borer, *Ostrinia furnacalis* (Guenée) larvae feeding on excised leaf disc and stalk internode tissue of the different maize varieties in laboratory experimentation. Any two means having a common letter are not significantly different at 1% level of significance. Lowercase and uppercase alphabet notation compares larval survival in leaf disc and stalk feeding assay, respectively.

high positive correlation with tunnel length (r = 0.90) and tunnel area (r = 0.83) produced by the feeding larva. These correlations indicate that resistant varieties which caused low LS has short tunnel length and tunnel area damaged by ACB larvae (Figure 2).

From LDA to SFA, the highly resistant *Bt2* consistently accomplished a zero ACB LS. The varieties RCS2, RCS4, RCS5, 'IPB Var13', *Bt3*, and *Bt4* were consistently identified as highly resistant although there were small increased in LS from LDA to SFA. The highly resistant *Bt1* and RCS3 in LDA downgraded as moderately resistant and resistant varieties, respectively, in SFA. In addition, highly resistant APN36, APN120, RCS1 and FV2 in LDA downgraded as susceptible varieties in SFA. Transgenic maize expressing the *Cry1Ab* protein has been identified that it is lethal to feeding ACB larvae (He et al. 2003; Alcantara et al. 2011). The same results were obtained with *Bt* corn varieties and ACB in this research.

The moderately resistant FV1 and HT4 in LDA downgraded as moderately susceptible in SFA. The 'IPB Var6' was consistently identified as moderately susceptible although there was small increase in LS from LDA to SFA. The moderately susceptible 'Lagkitan' (check) in LDA downgraded as highly susceptible variety in SFA. The resistant HT3 in LDA downgraded as moderately susceptible varieties in SFA. The resistant APN32 and HT1 in LDA downgraded as highly susceptible varieties in SFA. The susceptible and highly susceptible P30B80 and APN33 in LDA are downgraded both as highly susceptible in SFA. On the other hand, both 'Supersweet' maize (check) and APN24 which are moderately susceptible in LDA are upgraded as susceptible in SFA, and both APN40 and HT2 which are highly susceptible in LDA are upgraded as susceptible in SFA. The changes in the resistance can be accounted for the categorization bracket and by the increased (downgrading) and decreased (upgrading) LS in SFA in relation to LS in LDA.

Average linkage hierarchical tree were constructed among the varieties constructed from the euclidean distance matrix computed from LS in both LDA and SFA together with tunnel length and tunnel area measured from SFA (Figure 3). The tree revealed that the twenty-six varieties are best classified into three groups. RCS2, RCS4, RCS5, 'IPB Var13', *Bt1*, *Bt2*, *Bt3* and *Bt4* form the first

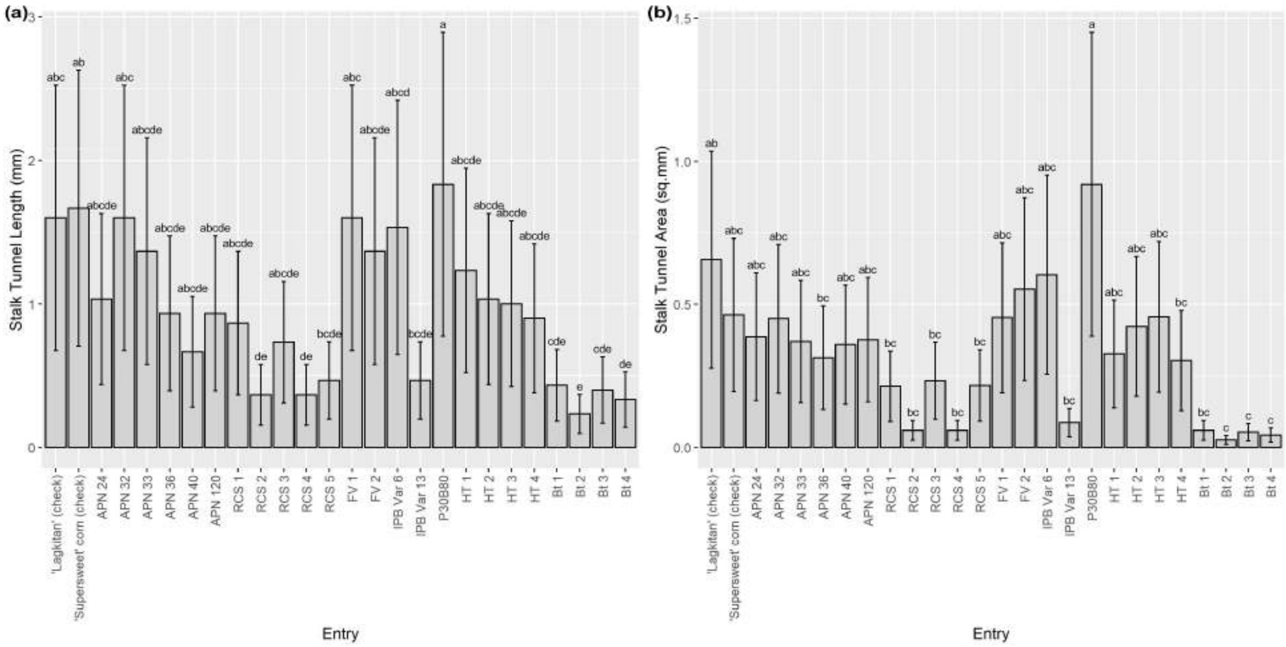

Fig. 2. Mean stalk tunnel length (a) and tunnel area (b) bored by the feeding larva on excised stalk internode tissue of the different maize varieties in stalk feeding assay laboratory experimentation. Any two means having a common letter are not significantly different at 1% level of significance.

group which are the resistant varieties. 'Lagkitan' (check), 'Supersweet' maize (check), APN24, APN33, APN40, 'IPB Var6', P30B80 and HT2 form the second group which are the susceptible varieties. Finally, APN32, APN36, APN120, RCS1, RCS3, FV1, FV2, HT1, HT3 and HT4 form the third group which are the intermediate varieties. Further examinations showed that the resistant cluster showed low LS rate at 46.67% ± 21.38 a day after. This further declined sharply during the 2nd to the 5th day at 7.50 ± 8.50%, 1.67 ± 2.52%, 0.83 ± 2.36%, and 0.83 ± 2.36%, respectively (Figure 4a, d). The susceptible cluster shows high LS across the 5 day larval challenge duration (Figure 4b, d). Larval survival throughout the 5 day duration was high and declined slowly at 97.92 ± 3.96%, 94.58 ± 5.33%,

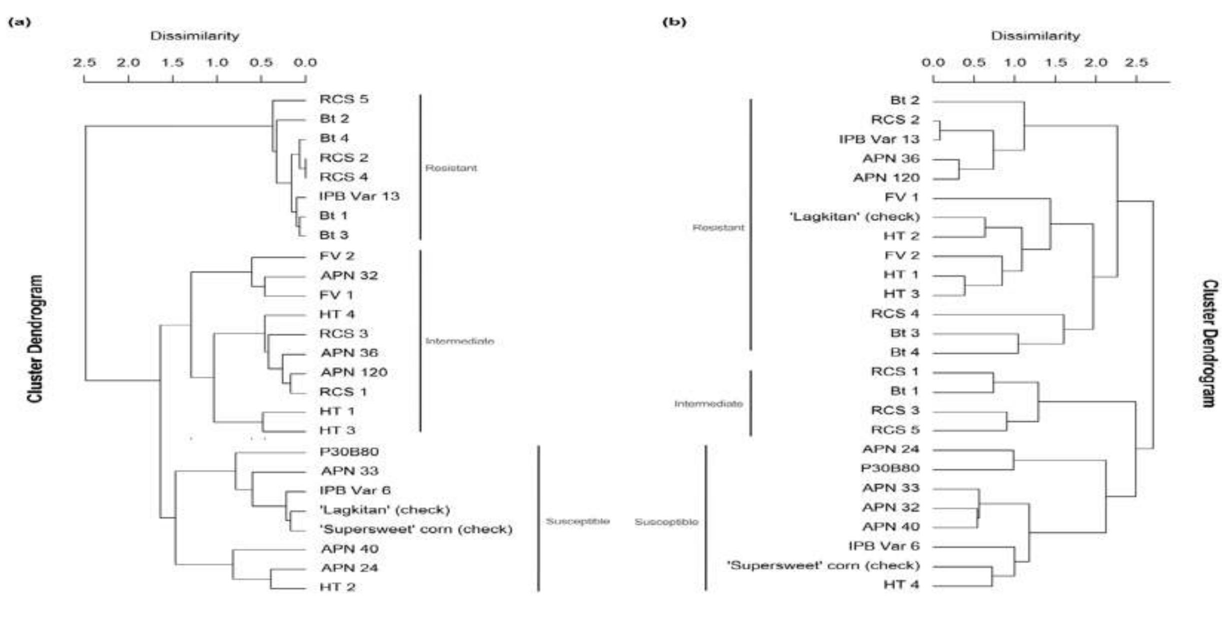

Fig. 3. Dendrogram of maize varieties utilizing the Euclidean distance measure in average linkage agglomerative clustering method. Varieties under laboratory bioassay clustered (a) using the leaf disc larval survivability and stalk feeding tunnel length, and under screen cage clustered (b) using the 7 and 14 days after infestation (DAI) leaf damage rating, and stem tunnel length.

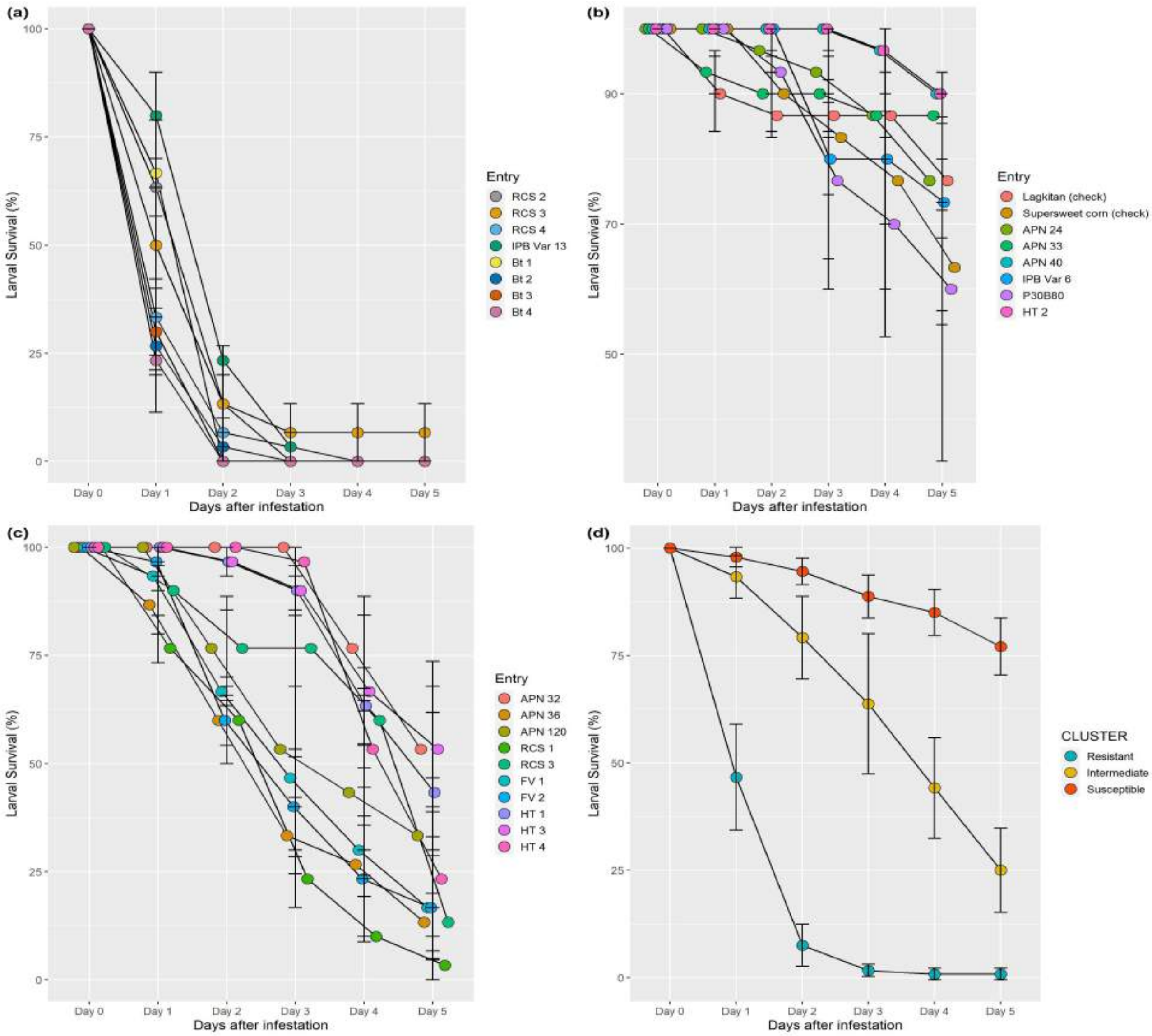

Fig. 4. Mean larval survival (%) of Asian corn borer, *Ostrinia furnacalis* (Guenée) larvae feeding on excised stalk internode tissue of resistant (a), susceptible (b), intermediate clustered maize varieties (c), and pooled value in stalk feeding assay laboratory experimentation.

88.75 ± 8.72%, 85.00 ± 9.26%, and 77.08 ± 11.47% for 1 to 5 DAI, respectively. The intermediate cluster showed a 93.33 ± 8.54% larval survive on 1 DAI, and gradually declined to 79.17 ± 16.69%, 63.75 ± 28.25%, 44.17 ± 20.30%, 25.00 ± 17.00% on the 2nd to the 5th DAI, respectively (Figure 4c, d).

Performance and Reaction of Maize Varieties on Screen House Experiment

ANOVA revealed that variety means for damage rating and tunnel length damage by the feeding larva are declared highly significantly different ($p \leq 0.01$) in the screen house experiment under artificial infestation of ACB larva.

The varieties APN36, APN120, RCS1, RCS2, RCS3, RCS4, RCS5, 'IPB Var13' together with the four *Bt* varieties (*Bt1*,*Bt2*, *Bt3*, *Bt4*) were consistently identified as highly resistant in 7 and 14 DAI damage rating (Figure 5). The highly resistant FV1, HT1, HT3 and HT4 in 7 DAI downgraded as resistant in 14 DAI. The resistant FV2 in 7 DAI rating downgraded as intermediate in 14 DAI rating. The 'Lagkitan' (check), APN33 and HT2 were consistently identified as resistant in 7 and 14 DAI. On the other hand, the intermediate APN24 in 7 DAI rating was upgraded as highly resistant in 14 DAI. The APN40 was consistently identified as intermediate in 7 and 14 DAI damage rating. The intermediate 'Supersweet' maize (check), APN32 and P30B80 in 7 DAI rating were upgraded as resistant in 14

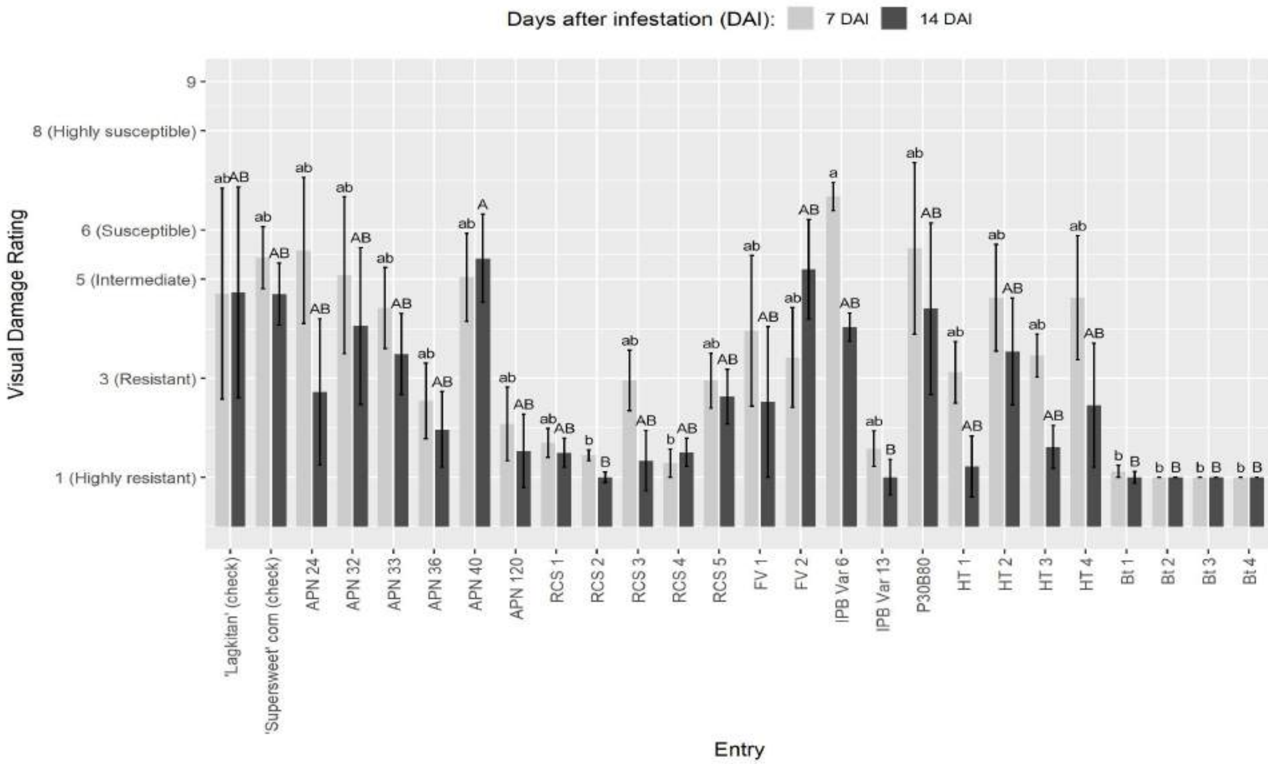

Fig. 5. Mean visual ACB leaf damage rating to the different maize varieties in screen house experimentation. Varietal test plots rated at 7 and 14 days after infestation (DAI) using the 9-class rating scale (1 = low to 9 = high amount of leaf damage) and categorization developed by Guthrie et al. (1960). Damage rating was done on the whorl and leaves including the partially and fully expanded leaves. Any two means having a common letter are not significantly different at 1% level of significance. Lowercase and uppercase alphabet notation compares damage rating at 7 and 14 DAI, respectively.

DAI. The susceptible 'IPB Var6' was upgraded as resistant in 14 DAI. The difference in the resistance can be attributed for by the used Guthrie et al. (1960) 9-class rating scale and categorization bracket, such that, with reference to the damage rating at 7 DAI, downgraded and upgraded varietal resistance category reflects extended and arrested foliar feeding of ACB larva at 14 DAI, respectively.

Seven DAI damage rating has high positive correlation with 14 DAI (r = 0.81), tunnel length (r = 0.84) and percent stalk tunnel damage (r = 0.86). Fourteen DAI damage rating has high positive correlation with tunnel length (r = 0.92) and percent stalk tunnel damage (r = 0.92). Finally, tunnel length has high positive correlation with percent stalk tunnel damage (r = 0.98). These correlations indicate that resistant varieties which displayed low visual damage rating had shorter tunnel length and smaller percent stalk tunnel damage damaged by ACB larvae. Susceptible varieties demonstrated more leaf damage and stalk tunneling. This study demonstrated that the amount of tunneling was very low for resistant varieties (Figure 6), which indicated a poor survival following infestation in screen house experiment. High leaf damage rating and long stem tunneling in susceptible varieties suggest that the potential loss of grain yield is high (Tefera et al. 2016). The high positive correlation between visual damage rating and tunnel length is worth mentioning because the simpler and easy to collect data would be helpful for further screening for ACB resistant maize.

The observed highly significantly different ($p \leq 0.01$) variety means for tunneling in the screen house experiment under artificial infestation of ACB larva share similarity with the results of Munyiri et al. (2013) for the *Chilo partellus* stem borer to maize.

Average linkage hierarchical tree were constructed among the varieties constructed from the euclidean distance matrix computed from 7 and 14 DAI leaf damage rating and stem tunnel length data (Figure 3b). The tree revealed that the twenty-six varieties are also best classified into three groups. 'Lagkitan' (check), APN36, APN120, RCS2, RCS4, FV1, FV2, 'IPB Var13', HT1, HT2, HT3, *Bt2*, *Bt3* and *Bt4* form the first group which are the resistant varieties. 'Supersweet' maize (check), APN24, APN32, APN33, APN40, 'IPB Var6', P30B80, and HT4 form the second group which are the susceptible varieties. Finally, RCS1, RCS3, RCS5 and *Bt1* form the third group which are the intermediate varieties.

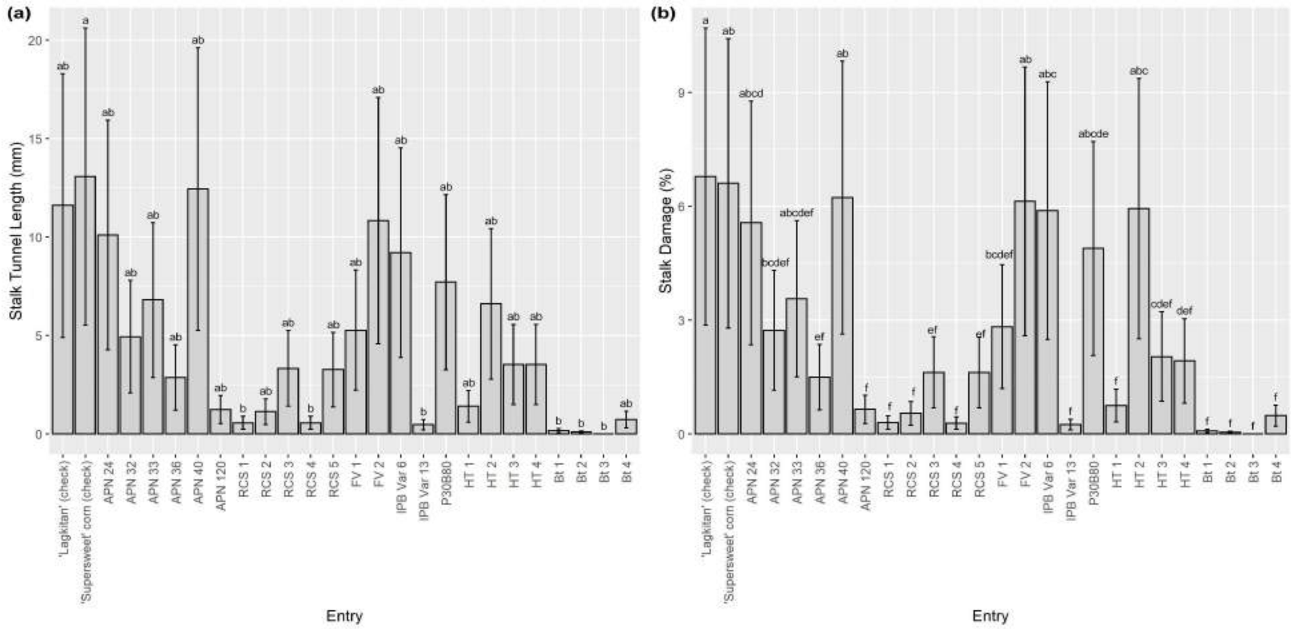

Fig. 6. Tunnel length (a) and percent stalk tunnel damage (b) produced by the feeding ACB larva to the different maize varieties in screen house experimentation. ACB damage on stalk was evaluated by split dissection procedure and the length and width of the tunnel damage were measured. Percent stalk tunnel damage was calculated as the total tunnel length within the whole stalk over the plant height. Any two means having a common letter are not significantly different at 1% level of significance.

The number of ACB larval exit holes is an indicator of the number of borers that have successfully completed the life cycle within a maize stalk. The screen house experiment showed that resistant maize was mainly attacked below the ear nodes and internodes, with the most frequent (0.16) observation on the 8th node below the ear node (Figure 7). Notably, the resistant varieties had zero attacks on the crucial ear node. Meanwhile, both the intermediate and the susceptible clustered varieties were attacked on the ear node, with the feeding attacks distributed across the whole plant from the lowest node to the tassel (12th node). Finally, the susceptible varieties had heavier attacks on the first and second node below the ear node, whereas the intermediate cluster suffered more damage on the second node above the ear.

Overall Performance of Maize Varieties Against ACB in Two Experiments

Simple pairing stability tests of varieties responses to ACB feeding in laboratory and screen house experiments based on separate multivariate cluster analysis revealed that RCS2, RCS4 and 'IPB Var13' together with the three *Bt* varieties (*Bt2*, *Bt3*, *Bt4*) were consistently identified as resistant varieties. The resistant RCS5 and *Bt1* in the laboratory assay downgraded as intermediate in screen house artificial infestation experiment. The RCS1 and RCS3 were the consistently identified as intermediate varieties. The other intermediate varieties viz. APN36, APN120, FV1, FV2, HT1 and HT3 in laboratory experiment were upgraded as resistant in screen house experiment. On the other hand, the intermediate APN32 and HT4 varieties in laboratory were downgraded as susceptible screen house. The 'Supersweet' maize (check), APN24, APN33, APN40, 'IPB Var6' and P30B80 were consistently identified as susceptible in laboratory and screen house experiment. On the other hand, the susceptible 'Lagkitan' (check) and HT2 in laboratory were upgraded as resistant screen house experiment.

Larval survival in laboratory assay and damage ratings in screen house experiment indicated that transgenic maize varieties expressing the *Cry1Ab* protein was highly resistant to ACB (Figure 1 and 5). This is not particularly surprising given the fact that *Cry1Ab*-expressing maize varieties are lethal when ingested by ACB larvae (He et al. 2003; Alcantara et al. 2011). Interestingly, high resistance observed from the non-Bt 'IPB Var13' against ACB larvae suggest that natural resistance is present. This public-bred OPV has the potential to be a parental source in breeding for natural ACB resistance. The resistance can either be attributed to deterrent morphological structures and/or toxic chemical compounds (Stout 2013; Franeta et al. 2018) which demands for further experimental investigations to determine the mechanism involved. We speculated that the high resistance observed from RCS varieties viz.

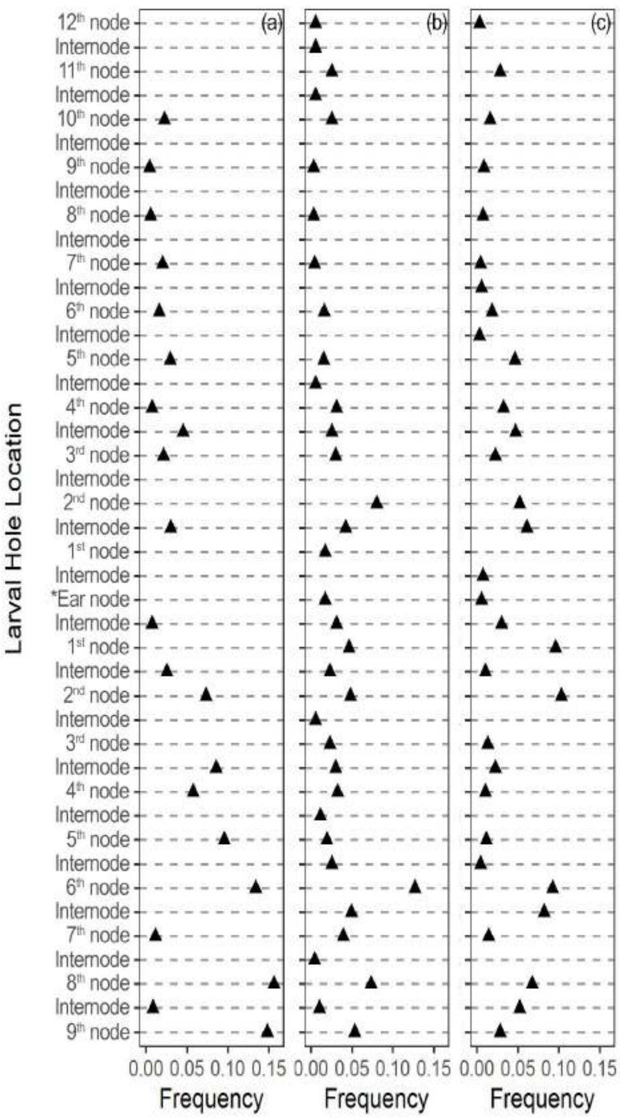

Fig. 7. Pooled frequency and location of node/s and internodes having ACB larval exit holes for resistant (a), intermediate (b), and susceptible (c) clustered varieties in screen house experiment. Varieties were clustered utilizing the Euclidean distance measure of the 7 and 14 days after infestation (DAI) leaf damage rating, and stem tunnel length in average linkage agglomerative clustering method.

RCS2, RCS3, RCS4, and RCS5 to be derived from *Bt*-introgressed maize populations. It's almost two decades (2002-2021) since the propagation of MON810-introgressed maize varieties were authorized in the country. Those RCS varieties may contain and express the *Cry1Ab* toxin from the transgenic maize varieties because farmers commonly use $F_{1:2}$ seeds as planting materials. It is recommended that further research should be undertaken to confirm the resistance mechanism of the RCS. These experiments corroborate with previous results that ACB resistance is available in Philippine native maize varieties (Salazar et al. 2016; Caasi-Lit et al. 2018) as demonstrated by the intermediate resistance of APN36 and APN120. The observed susceptibility of the 'Supersweet' maize was not surprising. In fact it is consistent with the previous observation (Caasi-Lit et al. 2018). Given that our findings are based on a limited number of maize varieties in each class i.e. native, recycled, *Bt*, public-bred OPVs, etc., generalization from the results should be treated with considerable caution. Large amount of varieties must be evaluated in ACB resistance breeding programs.

The no choice LDA and SFA laboratory feeding experiments are extremely useful in the preliminary evaluation of resistance. LDA and SFA are a reliable and rapid method for confirming insect resistance as demonstrated by Caasi-Lit et al. (2018). A number of studies attribute the significantly low ACB larval survival demonstrated by resistant varieties to antibiosis (Kalode and Pant 1966; Mathur and Jain 1972; Lal and Pant 1980). Meanwhile, being able to reflect field condition qualifies the screen house experimentation as more competent (Selvi and Sakthi 2018). In these resistance test environment, various authors resolved that foliar damage rating and stalk tunneling are reliable indicators of resistant or susceptibility of maize to ECB and ACB (Patanakamjorn 1975; Mangoendidjojo 1978; Gethi 1997; Munyiri et al. 2013; Caasi-Lit et al. 2018). Of the two indicators, leaf damage rating system is the best and fastest method since it is practical, rapid, and reliable (Guthrie et al. 1960). Splitting stalks and measuring tunnels is slow and costly, therefore, more maize varieties could be evaluated with the rating system (Patanakamjorn 1975). The nine class rating scale (1 = low to 9 = high amount of leaf damage), similar to the one used by Guthrie et al. (1960), can be used for measuring resistance to a first-generation infestation.

ACB is closely related to the European corn borer (He et al. 1997) and both first-generation neonate larvae of ECB and ACB tend to establish on whorl leaves (Zhou et al. 1995) especially at the vulnerable mid-whorl stage (Guo et al. 2019). The present study has only investigated the varietal resistance against the newly hatched ACB larvae of the first-generation infestation on maize in the early whorl stage of growth i.e. at 25-30 DAP. Different generations of the ACB feed on different parts of the maize plant. The second-generation ACB eggs are deposited on corn plants during the late-whorl stage (V17 stage; He et al. 2003) which damage the stalks and is more critical for yield than the corn borer damage in younger leaves (Velasco et al. 2007). Guthrie et al. (1971) pointed out for the case of the closely related ECB, that first- and second-generation resistance may not be correlated. This is a vital issue for further studies which takes into account the resistance to both the ACB larval generations.

Despite the limitation of low sample size and replicates, the plant resistance indices used in this study do however can effectively screen different varieties of maize in the Philippines. Further experimental investigations would be needed to determine the mechanism/s involved in the identified ACB resistant and susceptible maize varieties.

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