

SABRAO Journal of Breeding and Genetics  
 57 (3) 1009-1020, 2025  
<http://doi.org/10.54910/sabrao2025.57.3.13>  
<http://sabraojournal.org/>  
 pISSN 1029-7073; eISSN 2224-8978



## BLACK PEPPER (*PIPER NIGRUM* L.) F1 HYBRIDS ASSESSMENT AGAINST THE STEM BORER (*LOPHOBARIS PIPERIS*) UNDER LABORATORY CONDITIONS

R. ROHIMATUN<sup>1</sup>, S. SISWANTO<sup>1</sup>, N. BERMAWIE<sup>1\*</sup>, S. WAHYUNI<sup>1</sup>,  
 N.L.W. MEILAWATI<sup>1</sup>, M. SUSILOWATI<sup>2</sup>, D.W. UTAMI<sup>2</sup>, R. HERYANTO<sup>1</sup>, S.F. SYAHID<sup>1</sup>,  
 and D. DADANG<sup>3</sup>

<sup>1</sup>Research Center for Estate Crops, National Research and Innovation Agency, Bogor, Indonesia

<sup>2</sup>Research Center for Horticultural, National Research and Innovation Agency, Bogor, Indonesia

<sup>3</sup>Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia

\*Corresponding author's emails: nurl018@brin.go.id, nurliani.bermawie@gmail.com

Email addresses of co-authors: rohimatun@brin.go.id, sisw017@brin.go.id, sriw020@brin.go.id,  
 nurl017@brin.go.id, mari054@brin.go.id, dwin011@brin.go.id, rubi.heryanto@brin.go.id,  
 dadangtea@apps.ipb.ac.id

### SUMMARY

Yield losses from stem borer (*Lophobaris piperis* Marsh) attacks have reached up to 72%. Currently, information on the preference and intensity of stem borer attacks on the black pepper (*Piper nigrum* L.) is limited. This study represents the first effort to develop stem borer-resistant black pepper cultivars through inter-varietal hybridization. An assessment of stem borer preferences among 29 F1 hybrids and their five parental cultivars commenced under laboratory conditions. The F1 hybrids displayed a range of resistance levels, resulting from crosses between highly preferred and less preferred black pepper varieties. Among the 29 F1 hybrids, three were highly resistant, 12 were resistant, 10 were moderately resistant, two were moderately susceptible, one was susceptible, and one was highly susceptible. These findings provide a promising material for developing resistant black pepper cultivars that support integrated pest management strategies and promote environmental sustainability.

**Keywords:** Black pepper (*Piper nigrum* L.), stem borer (*Lophobaris piperis*), damage intensity, inter-varietal hybridization, preference, resistance, susceptible

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: September 13, 2024; Accepted: November 14, 2024.

© Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2025

**Citation:** Rohimatun R, Siswanto S, Bermawie N, Wahyuni S, Meilawati NLM, Susilowati M, Utami DW, Heryanto R, Syahid SF, Dadang D (2025). Black pepper (*Piper nigrum* L.) F1 hybrids assessment against the stem borer (*Lophobaris piperis*) under laboratory conditions. *SABRAO J. Breed. Genet.* 57(3): 1009-1020. <http://doi.org/10.54910/sabrao2025.57.3.13>.

**Key findings:** The F1 hybrids production with various resistant categories came through hybridization among the preferred and less preferred black peppers (*P. nigrum* L.) cultivars. Three and 12 F1 hybrids attained categories as highly resistant and resistant, respectively, which can undergo studies for developing resistant cultivars in black pepper.

## INTRODUCTION

Black pepper (*Piper nigrum* L.), known as the king of spices, is the world's most prominent spice consumed. Indonesia, Brazil, India, Malaysia, Sri Lanka, and Vietnam are the major black pepper-producing countries, contributing approximately 92.80% of the world's production (Entebang *et al.*, 2021). One of the chief obstacles to pepper production is the stem borer (*Lophobaris piperis* Marsh), belonging to the order Coleoptera and family Curculionidae. Information regarding the preference and intensity of the *L. piperis* attack on the black pepper cultivars is limited. *L. piperis* has become the most damaging insect pest globally, not just in Indonesia. In Indonesia, stem borer attacks black pepper in the main growing areas (Amorita *et al.*, 2021).

The stem borer attacks from the nursery to the field stages gradually caused plant death (Lestari *et al.*, 2019). The stem borer larvae enter the stem and burrow/eat the inside of the stem or branches until the pupal and pre-adult phases. The stem tissue eaten by the larvae disrupts nutrient absorption and the distribution of photosynthetic results; over time, the stem will turn black, and the plant will dry and die. One stem borer larva attacking pepper plants can result in 72% yield losses (Lestari *et al.*, 2019). The pest can spread to other black pepper plants by transporting tiny egg seedlings, and controlling this insect pest is quite challenging for the farming community.

The development of host plant resistance through breeding efforts fits an integrated pest management (IPM) (Khew *et al.*, 2022). However, a black pepper-resistant cultivar for the stem borer is still nonexistent. Improving cultivars with genetic traits that are tolerant to stem borer is one of the strategies for managing this pest to reduce yield losses (Dhillon *et al.*, 2021; Horgan *et al.*, 2021). Resistant cultivars complement other pest

control methods (e.g., biological control, cultural practices), creating a multi-layered approach to control stem borer, strengthening the IPM system, and making IPM more effective and sustainable.

Achievements in breeding for stem borer resistance have been slow, mainly due to a less understanding of the inheritance of resistant genes and traits. Therefore, the genetic control of stem borer resistance remains for discovering. However, it is likely a polygenic trait influenced by multiple genes with minor effects (Muturi *et al.*, 2021). Breeding for polygenic traits makes breeding efforts more challenging to identify which genes or combinations have cumulative effects for effective resistance.

Black pepper-resistant cultivars assembly has progressed conventionally through intraspecific crosses and between high-yielding cultivars, which vary in the resistance level against the stem borer. This effort will be the first attempt at crop improvement of black pepper resistance to *L. piperis*. So far, no reports exist regarding breeding black pepper against the stem borer through intraspecific crosses.

Therefore, the novel study aimed to identify resistant genotypes of stem borer on F1 hybrids derived from intraspecific crosses of black pepper and their parental genotypes under laboratory conditions. Confirming the resistance level requires further research at the greenhouse and field levels. The expected result can be a reference for controlling stem borer with black pepper-resistant cultivars that comply with IPM and environmental issues.

## MATERIALS AND METHODS

### Plant preparation

Thirty-four genotypes consisting of 29 F1 hybrids with their parents incurred testing. The

29 hybrids obtained came from crossing five black pepper parental genotypes comprising two commercial cultivars (Natar-2 and Petaling-2), one local cultivar (Belantung), and an improved genotype N2LD (as a female parent) with Ciinten (as a male parent), being a high-yielding and good quality pepper cultivar (Bermawie *et al.*, 2019). The stems of 1.5-year-old seedlings, cut to  $15 \pm 2$  cm long, had 0.5–1.0 cm diameter with 2–3 nodes (Table 1). The criteria for pepper stems used are healthy, smooth appearance, and no spots. The cut area remained covered with wet cotton and parafilm to reduce evaporation.

### Collection and mass rearing of *L. piperis*

The collection of stem borer (*L. piperis*) adults and larvae came from the pepper plantation, then keeping and rearing them in a closed jar with holes drilled on the lid for air circulation in a laboratory environment ( $T 30 \pm 2$  °C; RH 55 – 60%). Adding fresh stems, fruits, and leaves of peppers served as the feed to the male and female adult insects contained in the jars in preparation for egg-laying. Several pairs of adults, placed in a jar to copulate, received monitoring of their condition every two days. In the copulation jar, adding pepper stems as a nesting site ensued. Every seven days, taking the stems and placing them in another jar transpired. Afterward, putting fresh pepper stalks continued in the jar containing some pairs of adults for the next egg-laying process. The eggs hatch into first-instar larvae, which bury themselves in the stems. Moving paired adults for periodic copulation, scientists retained the stems containing eggs for insect growth. Repeating this procedure continued until having sufficient adult female insects for testing.

### Bioassay

The conduct of the preference test in the laboratory used the choice method. Female adult insects were specimens for the test. The test groups, arranged randomly, had three replicates.

### Preference of stem borer to parental cultivars and F1 hybrids

Black pepper stems acquired regular labeling and arranging in a plastic container. In the container, two rows of pepper stems, arranged randomly, had 17 stems per row, with a total of 34 stems representing each of the genotypes tested. Between two rows occurs a distance of  $\pm 5$  cm for placing the imago. In the distance, dividing the pepper stems into five points had equal distance. Each point received 10 adult females to select stems as food. The container, as sealed with a perforated plastic lid for covering with glue, prevented the insect's escape. Preferences observed every 24 hours after releasing the adults into the container up to the 8<sup>th</sup> day. The observed parameters included the percentage of visits by the stem borer one day post-exposure and on the first day of visitation (day 0) over 8 days. The preference calculation of the stem borer used the following formula:

$$D (\%) = \frac{a}{b} \times 100\%$$

Where D = *L. piperis* preference, a = the total number of visiting *L. piperis* on each black pepper genotype, and b = the total number of visiting *L. piperis* on the total genotypes tested.

**Table 1.** Black pepper cultivars and F1 hybrids evaluated against *L. piperis*.

Crosses	F1s tested	F1 hybrids & parental genotypes
Natar 2 × Ciinten	12	N2Ci-P, N2Ci 1-2, N2Ci 2-3, N2Ci 2-7, N2Ci 3-1, N2Ci 3-4, N2Ci 4-3, N2Ci 5-1, N2Ci 5-3, N2 Ci 6-1, N2 Ci 7-2, N2Ci 7-4
Petaling 2 × Ciinten	9	P2Ci-P, P2Ci 1-2, P2Ci 1-5, _2Ci 1-7, P2Ci 2-5, P2Ci 2-6, P2Ci 2-7, P3Ci 4-3, P2Ci 5-1
N2LD × Ciinten	5	N2LDCi 1-5, N2LDCi 2-5, N2LDCi 2-6, N2LDCi 2-7, N2LDCi 4-3
Belantung × Ciinten	3	BLTCi 1-1, BLT Ci 1-3, BLT Ci 1-5
Parental cultivars	5	Natar 2, Petaling 2, Ciinten, N2LD, Belantung

### Attractiveness index (AI), damage intensity, and resistant category

AI calculation relied on the last observation using the following formula:

$$AI = \frac{2E}{(E + S)}$$

Where AI = attractiveness index, E = the number of insects attracted to the evaluated genotype, and S = the number of insects attracted to the most attractive genotype. The AI values range from zero to two, while values closer to zero indicate lower attractiveness (see Table 2 for grouping based on attractiveness).

The damage intensity computation of black pepper stem emerged on the last day of observation, assessed based on percentage of total stem length damage levels as follows: Category 0 = no damage; Category 1 = 1%–20% of total stem length damaged; Category 3 = 21%–40% of total stem length damaged; Category 5 = 41%–60% of total stem length damaged; Category 7 = 61%–80% of total stem length damaged; and Category 9 = >80% of total stem length damaged (Lestari *et al.*, 2019). Next, the damage category on the black pepper stem underwent conversion into damage intensity (DI) using the following formula.

$$DI = \sum_{i=1}^4 \frac{n_i \times v_i}{Z \times N} \times 100 \%$$

Where DI = damage intensity to black pepper stems due to stem borer attack;  $n_i$  = the number of black pepper stems attacked by

stem borer based on  $i^{\text{th}}$  category;  $v_i$  = the value of each category based on the  $i^{\text{th}}$  attack symptom; Z = the highest attack category; and N = the total number of black pepper stems observed (Lestari *et al.*, 2019).

In black pepper genotypes, determining the resistance levels depended on Suhartawan's method (Widiastuti *et al.*, 2014). If the average attack intensity on susceptible cultivars was x%, each percent of damage equally reduces black pepper production. The resistance categories were as follows: 0% attack indicates high resistance (HR), >0–1/4x is resistant (R), >1/4x–1/2x is moderately resistant (MR), >1/2x–3/4x is moderately susceptible (MS), >3/4x–x is susceptible (S), and >x is highly susceptible (HS).

### Data analysis

The preference data for the stem borer (*L. piperis*) as well as the AI and intensity of black pepper stem damage bore the analysis of variance (ANOVA) in the IBM SPSS Statistics 22 program. After getting significant differences, the means' comparison used Tukey's Multiple Range Test ( $\alpha \leq 0.05$ ).

## RESULTS

### Preference of stem borer to parental cultivars and F1 hybrids

The preferences of *L. piperis* for black pepper genotype stems varied, with some choosing stems on the first, and others on the second day. However, these differences were nonsignificant. The percentage of *L. piperis* visiting black pepper parental cultivars on the first day showed nonsignificant differences.

**Table 2.** Attractiveness index (AI) value.

AI value	Attractiveness
> 1	The F1 hybrids and parental cultivars of black pepper tested and found more attractive than the susceptible parental cultivar
1	The F1 hybrids' parental cultivars of black pepper tested were as attractive as the susceptible parental cultivar
< 1	The susceptible parental cultivar was more attractive than other F1 hybrids and parental cultivars

Source: De-Oliveira *et al.* (2020).

Among the 29 black pepper F1 hybrids, eight hybrids (N2Ci P, N2Ci 7-2, N2Ci 2-3, N2Ci 5-1, P2Ci 2-6, P2Ci 5-1, BLTCi 1-5, and N2LDCi 4-3) gave no significant variations. The hybrid genotype N2Ci 6-1 had the fewest visits on the first day, with only one of three replicates visited, while other genotypes, such as N2Ci 1-2, had visits later and indicated a lower preference (Figure 1).

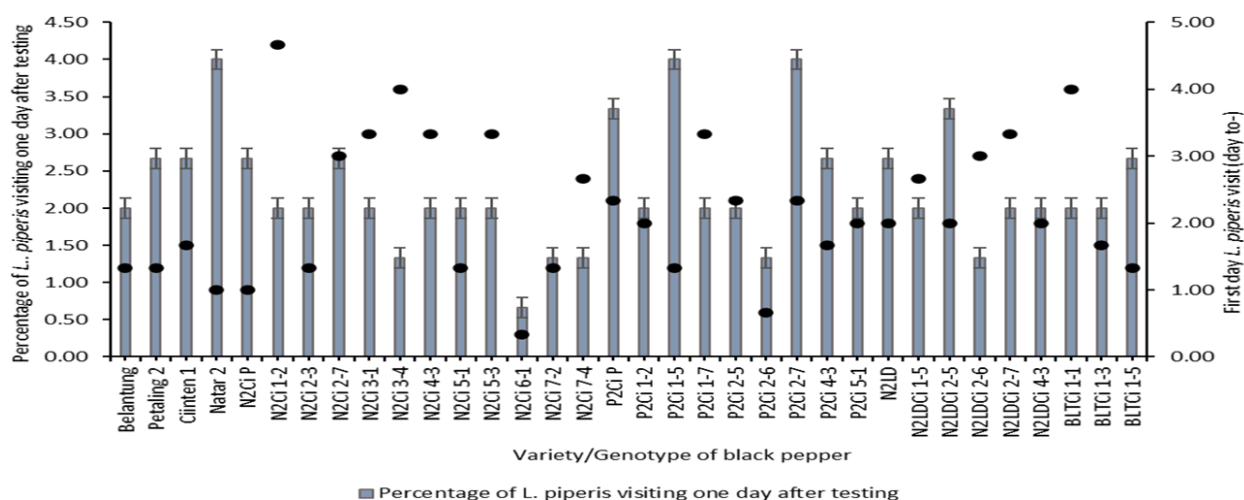
The visitation frequency of stem borer on black pepper stems over eight days showed considerable differences ( $F = 1.20$ ;  $p = 0.008$ ). Among the black pepper parental cultivars, the cultivar Natar-2 had the highest frequency (6.33 visits), followed by Ciinten-1 (4.67), Petaling-2 (4.00), Belantung (3.67), and N2LD (3.33) (Figure 2). Among the 29 F1 hybrids, the hybrid P2Ci P had the most frequent visits (4.67 times), followed by P2Ci 1-5 (4.33), N2Ci 5-1, and BLTCi 1-5 (3.67). Several other F1 hybrids, like N2Ci 3-4 (0.67 visits) and N2Ci 6-1, had, at most, three visits (Figure 2).

The stem borer exhibited the highest preference for parental cultivar Natar-2 (9.01%), while the other parent cultivars, namely Belantung, Petaling-2, N2LD, and Ciinten displayed at par preferences of 4.36%, 4.07%, 4.07%, and 4.94%, respectively. Among F1 hybrids, the hybrid P2Ci P had the

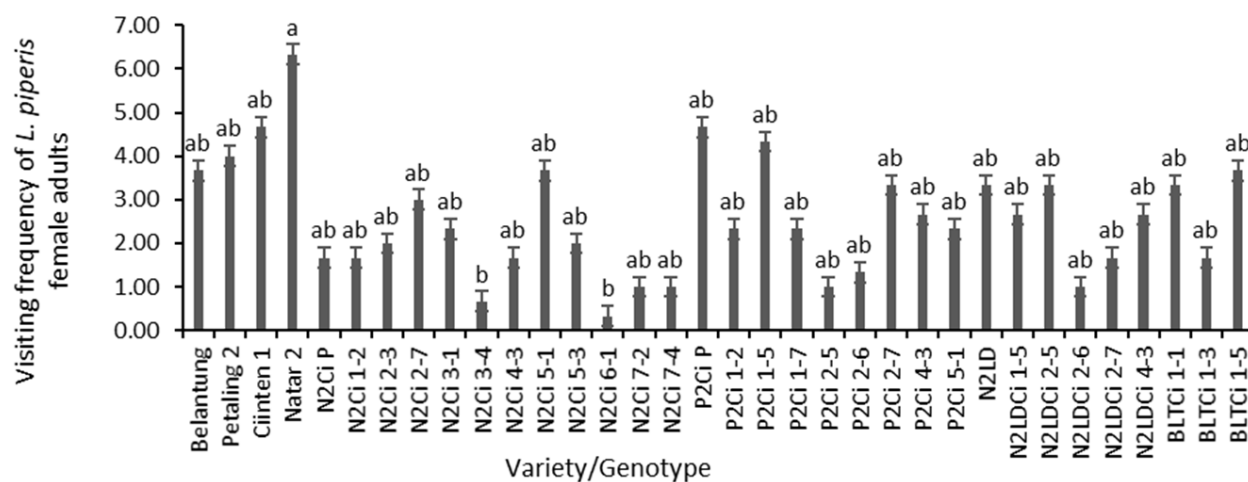
premier selection (7.56%), though this was not significantly different from the preference for several other black pepper hybrids, ranging from 1.74% to 5.81%. In contrast, the hybrid N2Ci 6-1 (0.29%) was the least preferred, followed by the hybrid N2Ci 3-4 (0.58%) and some other F1 hybrids, which also showed lower preferences (Figure 3).

### Attractive index (AI), damage intensity, and resistant category

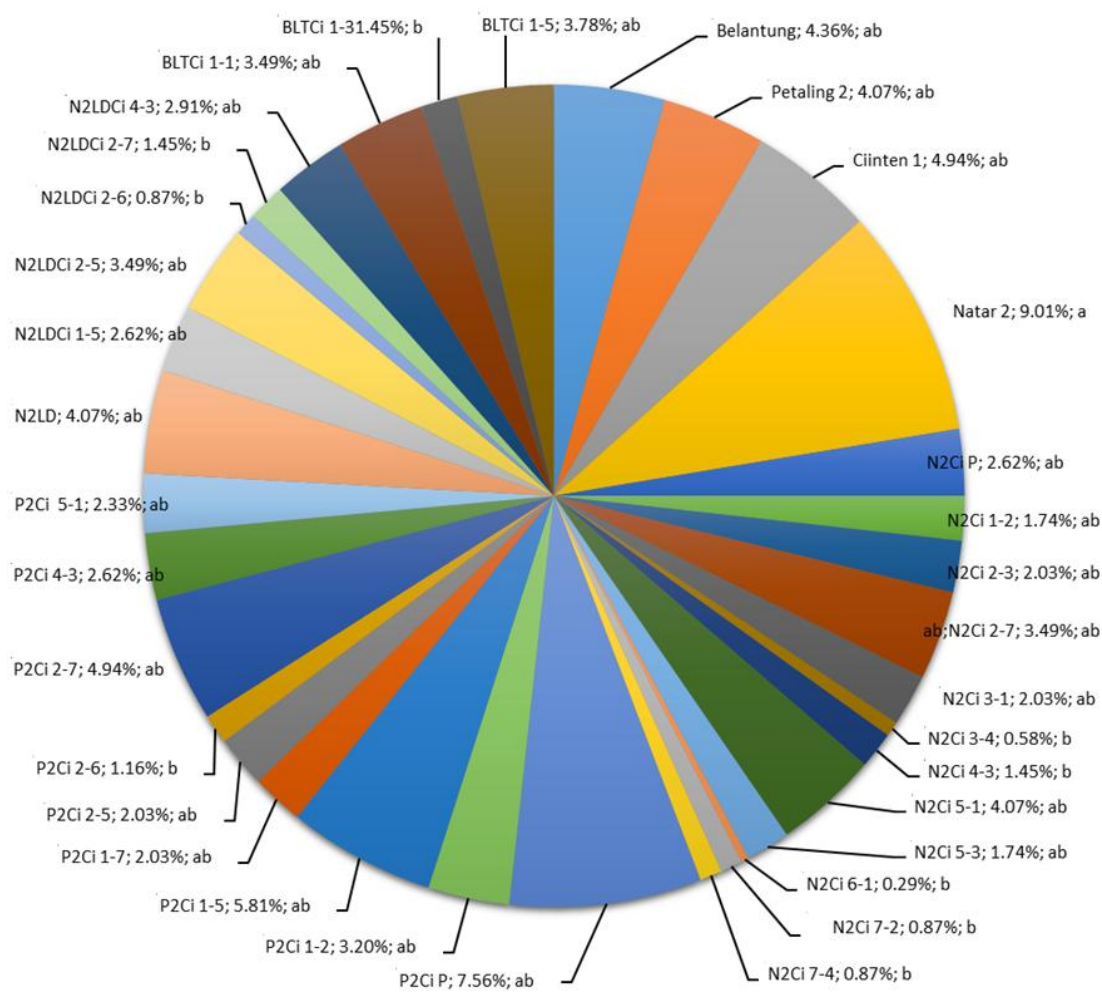
The attractive index (AI) values calculation depended on the number of stem borers selecting black pepper stems compared with the most frequently chosen cultivar, Natar-2. All the F1 hybrids had AI values below 1, indicating the susceptible parent (Natar-2) was more attractive than the other parental genotypes. The four other parental cultivars had AI values close to 1, ranging from 0.61 to 0.74. The F1 hybrids with the same values included the hybrids N2Ci 5-1 (0.65), P2Ci P (0.74), P2Ci 1-5 (0.76), and P2Ci 2-7 (0.68). The F1 black pepper hybrids with the lowest AI values were N2Ci 6-1 (0.05), N2Ci 3-4 (0.10), N2LDCi 2-6 (0.14), and P2Ci 2-6 (0.18) (Table 3).



**Figure 1.** First day of *L. piperis* visit (day to-) (●) ( $p = 0.414$ ) and percentage of visiting *L. piperis* one day after testing (■) ( $p = 0.104$ ).



**Figure 2.** Frequency of visiting *L. piperis* (time) on each black pepper parental cultivar and F1 hybrid.



**Figure 3.** Preference of *L. piperis* to black pepper parental cultivars and F1 hybrids.

**Table 3.** AI, damage intensity, and resistance category of black pepper parental cultivars and F1 hybrids to stem borer.

Parental cultivars & F1 hybrids	AI $\pm$ SE	Damage intensity (%) $\pm$ SE	Resistance category
Belantung	0.61 $\pm$ 0.35 <sup>a</sup>	13.07 $\pm$ 6.29 <sup>ab</sup>	Moderate Susceptible
Petaling-2	0.65 $\pm$ 0.22 <sup>a</sup>	10.17 $\pm$ 2.62 <sup>ab</sup>	Moderate Resistant
Ciinten-1	0.74 $\pm$ 0.17 <sup>a</sup>	12.34 $\pm$ 1.45 <sup>ab</sup>	Moderate Susceptible
Natar-2	1.00 $\pm$ 0.00 <sup>a</sup>	22.51 $\pm$ 4.76 <sup>ab</sup>	Susceptible
N2Ci-P	0.45 $\pm$ 0.22 <sup>a</sup>	6.54 $\pm$ 3.77 <sup>ab</sup>	Moderate Resistant
N2Ci 1-2	0.37 $\pm$ 0.22 <sup>a</sup>	0.73 $\pm$ 0.73 <sup>b</sup>	Resistant
N2Ci 2-3	0.41 $\pm$ 0.20 <sup>a</sup>	4.36 $\pm$ 2.51 <sup>ab</sup>	Resistant
N2Ci 2-7	0.56 $\pm$ 0.22 <sup>a</sup>	8.71 $\pm$ 3.33 <sup>ab</sup>	Moderate Resistant
N2Ci 3-1	0.40 $\pm$ 0.15 <sup>a</sup>	6.54 $\pm$ 3.77 <sup>ab</sup>	Moderate Resistant
N2Ci 3-4	0.10 $\pm$ 0.05 <sup>a</sup>	0.73 $\pm$ 0.73 <sup>b</sup>	Resistant
N2Ci 4-3	0.31 $\pm$ 0.10 <sup>a</sup>	4.36 $\pm$ 2.51 <sup>ab</sup>	Resistant
N2Ci 5-1	0.65 $\pm$ 0.13 <sup>a</sup>	7.26 $\pm$ 3.63 <sup>ab</sup>	Moderate Resistant
N2Ci 5-3	0.36 $\pm$ 0.16 <sup>a</sup>	3.63 $\pm$ 1.92 <sup>ab</sup>	Resistant
N2Ci 6-1	0.05 $\pm$ 0.05 <sup>a</sup>	0.73 $\pm$ 0.73 <sup>b</sup>	Resistant
N2Ci 7-2	0.22 $\pm$ 0.15 <sup>a</sup>	0.73 $\pm$ 0.73 <sup>b</sup>	Resistant
N2Ci 7-4	0.22 $\pm$ 0.15 <sup>a</sup>	1.45 $\pm$ 1.45 <sup>b</sup>	Resistant
P2Ci P	0.74 $\pm$ 0.24 <sup>a</sup>	31.23 $\pm$ 21.87 <sup>a</sup>	Highly Susceptible
P2Ci 1-2	0.48 $\pm$ 0.10 <sup>a</sup>	18.88 $\pm$ 12.60 <sup>ab</sup>	Susceptible
P2Ci 1-5	0.76 $\pm$ 0.05 <sup>a</sup>	14.53 $\pm$ 4.04 <sup>ab</sup>	Moderate Susceptible
P2Ci 1-7	0.41 $\pm$ 0.20 <sup>a</sup>	1.45 $\pm$ 1.45 <sup>b</sup>	Resistant
P2Ci 2-5	0.36 $\pm$ 0.18 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	Highly Resistant
P2Ci 2-6	0.18 $\pm$ 0.12 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	Highly Resistant
P2Ci 2-7	0.68 $\pm$ 0.27 <sup>a</sup>	12.35 $\pm$ 5.67 <sup>ab</sup>	Moderate Susceptible
P2Ci 4-3	0.48 $\pm$ 0.23 <sup>a</sup>	6.54 $\pm$ 2.51 <sup>ab</sup>	Moderate Resistant
P2Ci 5-1	0.31 $\pm$ 0.19 <sup>a</sup>	1.45 $\pm$ 1.45 <sup>b</sup>	Resistant
N2LD	0.63 $\pm$ 0.18 <sup>a</sup>	6.54 $\pm$ 4.53 <sup>ab</sup>	Moderate Resistant
N2LDCi 1-5	0.39 $\pm$ 0.18 <sup>a</sup>	6.54 $\pm$ 4.36 <sup>ab</sup>	Moderate Resistant
N2LDCi 2-5	0.57 $\pm$ 0.05 <sup>a</sup>	5.81 $\pm$ 3.16 <sup>ab</sup>	Moderate Resistant
N2LDCi 2-6	0.14 $\pm$ 0.08 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	Highly Resistant
N2LDCi 2-7	0.27 $\pm$ 0.07 <sup>a</sup>	3.63 $\pm$ 1.45 <sup>ab</sup>	Resistant
N2LDCi 4-3	0.51 $\pm$ 0.08 <sup>a</sup>	2.18 $\pm$ 2.18 <sup>ab</sup>	Resistant
BLTCi 1-1	0.58 $\pm$ 0.25 <sup>a</sup>	8.71 $\pm$ 3.33 <sup>ab</sup>	Moderate Resistant
BLTCi 1-3	0.31 $\pm$ 0.10 <sup>a</sup>	2.18 $\pm$ 1.26 <sup>ab</sup>	Resistant
BLTCi 1-5	0.60 $\pm$ 0.05 <sup>a</sup>	9.44 $\pm$ 1.92 <sup>ab</sup>	Moderate Resistant
F	1.591	1.831	
P	0.054	0.018	

Means followed by the same letter in each column do not differ statistically by the Tukey's test ( $\alpha \leq 0.05$ ).

Regarding damage intensity, parental cultivar Natar-2 had the topmost value (22.51%) among the parental genotypes, classifying it as susceptible (S). In contrast, the parental genotypes Belantung (13.07%) and Ciinten (12.34%) provided lower damage and received a moderately susceptible (MS) category. Parental cultivars Petaling-2 (10.17%) and N2LD (6.54%) belonged to the moderately resistant (MR) classification (Table 3). Among F1 hybrids, the P2Ci P was the only highly susceptible (HS) genotype, with 31.23% damage, while the F1 hybrid P2Ci 1-2

(18.88%) reached the category of susceptible (S). The F1 hybrids P2Ci 1-5 (14.53%) and P2Ci 2-7 (12.35%) attained the moderately susceptible (MS) category. Nine black pepper F1 hybrids were moderately resistant (MR), including N2Ci P and N2LDCi 1-5, with damages ranging from 6.54% to 9.44%. Thirteen F1 hybrids were visible as resistant (R), with three hybrids (P2Ci 2-5, P2Ci 2-6, and N2LDCi 2-6) found highly resistant (HR). These F1 hybrids showed hybrid vigor compared with their parents.



## DISCUSSION

Insects undergo several stages in host selection. First, they recognize the host's habitat, then locate it using visual (size, shape) and chemical cues, followed by host recognition through the tasting. If suitable, the insect feeds and may damage the plant; if not, it moves on to other plants. Host acceptance depends on whether the plant's favorable chemical compounds allow the insect to continue feeding and potentially harm the plant. Host suitability, determined by the plant's nutritional content, influences the insect's survival (Horgan *et al.*, 2021). Insects detect chemical compounds in low concentrations, using familiar ones as attractants. Unknown compounds may lead to rejection, and insect resistance to certain chemicals can help control pests, as these compounds can deter feeding, inhibit growth, and even cause death (Cheema *et al.*, 2021; Horgan *et al.*, 2021).

The interaction between herbivorous insects and plants is highly complex, with host plant selection influenced by factors like plant morphology and chemical compounds (Maron *et al.*, 2019). Physical characteristics, such as stem shape, diameter, internode length, color, and glandular secretions, significantly affect insect behavior (Follett, 2017).

Surface conditions and plant tissue strength may limit an insect's ability to land and feed; however, these traits correlate to antixenosis mechanisms (De-Bastos-Pazini *et al.*, 2022). The biophysical traits influence the insect response, which is not the primary cause of non-preference, as insects can easily distinguish morphologically identical cultivars with varying resistance (Sharma *et al.*, 2024; Soujanya *et al.*, 2023). *L. piperis* adults typically feed on black pepper plants' flowers, leaves, shoots, and twigs. In preference tests, some black pepper F1 hybrids, like N2Ci 6-1 and P2Ci 2-6, bore visits quickly; however, they attracted fewer female stem borers as compared with other F1 hybrids.

After one day of testing, *L. piperis* visited the parental cultivar Natar-2 most frequently, while parental genotype Petaling-2 was the least preferred. Among F1 hybrids, the

P2Ci P was the most visited genotype (4.67%), though visits occurred after more than two days. In contrast, the F1 hybrid N2Ci 6-1, with a frequency of 0.33%, gained no visits until after the fourth day (Figure 1). The variation in visitation time and preferences was likely due to the stem borer's ability to identify the plants suitable for consumption and reproduction, which may be due to differences in plant morphology and chemical compounds found in the plants. Plants, in turn, deploy various defense mechanisms, including morphological, biochemical, and molecular strategies, to counter pest attacks (War *et al.*, 2020).

*L. piperis* tends to visit black pepper stems with larger diameters and longer internodes more frequently than those with smaller diameters and shorter internodes (Soetopo *et al.*, 1993). In sorghum, the extent of stem borer damage had a significant correlation with plant height (Muturi *et al.*, 2021), suggesting food resource availability influences the development of stem borer pests (Doležal *et al.*, 2021). The black pepper parental genotype Natar-2 was morphologically distinct from the two other parental cultivars, i.e., Petaling 2 and Ciinten, as shown by RAPD and SSR markers. However, the stem borer showed nonsignificant differences in preference between Petaling-2 and Ciinten. Parental genotype Natar-2 also has a shorter internode length than the other two cultivars (Meilawati *et al.*, 2020). This suggests that secondary metabolites may play a more significant role in *L. piperis* preferences than morphological characteristics, as these compounds serve as the primary defense against phytophagous insects (Divekar *et al.*, 2022).

The plant's physical-chemical traits and environment play a complex role in selecting host and feed. *L. piperis* prefers laying eggs on the softer nodes of black pepper stems. Without proper control, these herbivores can cause substantial damage and potentially kill the plants. Among parental cultivars, Natar-2 gained the most frequent visits from stem borers, while the four other parental genotypes, viz., Belantung, Petaling 2, N2LD, and Ciinten, were less preferred. Crossbreeding among the Natar-2, Belantung, Petaling-2, and N2LD with Ciinten produced the F1 hybrids that



attract or repel the stem borer. These preference variations could refer to plant morphology and secondary metabolites, which influence the resistance provided to pests (Divekar *et al.*, 2022; War *et al.*, 2020). Variations in the morphology of F1 hybrids were evident, especially in the stem softness, leaf shape, and sizes (data not presented).

The secondary metabolites received no studying in the presented research. However, the existing literature highlights differences in essential oil (EO) and alkaloid piperine levels among the parent cultivars. Hussain *et al.* (2017) reported black pepper extract containing piperine exhibits insecticidal activity, with piperine—responsible for pungency—linked to resistance against stem borer pests. Therefore, one can presume that the stem borer may avoid black pepper genotypes with higher piperine content, which exclusively targets the genotypes belonging to the Piperaceae family.

Black pepper is distinct for its elevated volatile compounds. Petaling-2 has the maximum essential oil (EO) and piperine levels among the parental cultivars, with 4.90% EO and 3.79% piperine. Parental genotype Natar-2 contains 3.13% EO and 2.21% piperine, while Ciinten has 2.62% EO and 3.85% piperine (Bermawie *et al.*, 2019). Natar-2 has a lower piperine content and may attract *L. piperis*, influencing its visitation preferences. These findings indicate that EO and piperine content combinations remarkably affect the *L. piperis* preferences among the parental cultivars.

The F1 hybrid P2Ci-P reached the most frequent visits, derived from the crossing between Petaling-2 and Ciinten. However, these findings contradict the observed preferences of *L. piperis*, which tends to favor neither parent. In contrast, the F1 hybrid obtained from crosses between the parents favored by stem borer, such as Natar 2 × Ciinten, produced descendants like N2Ci 3-4 and N2Ci 6-1, less preferred by *L. piperis*. The cross Natar 2 × Ciinten resulted in the F1 hybrid displaying significant variation in preference and visitation frequency by *L. piperis*. However, F1 hybrids from other crosses, such as Petaling 2 × Ciinten, N2LD × Ciinten, and Belantung × Ciinten, revealed

nonsignificant differences. Likewise, no prominent variations emerged among the parental genotypes and F1 hybrids for AI, while damage intensity differed significantly among the F1 hybrids. These variations in damage intensity were visible in the F1 hybrids obtained from the crosses, such as Natar 2 × Ciinten, Petaling 2 × Ciinten, and N2LD × Ciinten, except in Belantung × Ciinten.

The variability in damage intensity and resistance in the F1 hybrids is likely due to the heterozygous nature of the parental genotypes and the polygenic inheritance nature of resistance traits. Despite black pepper being a self-pollinating, vegetatively propagated crop, inherent heterozygosity can lead to segregation among the F1 hybrids. The segregation in F1 progenies was also evident in the morphological characters, especially leaf shapes and sizes (data not presented). The polygenic nature of resistance to stem borer indicates that each contributing gene can have an additive effect, either enhancing or reducing resistance. Different combinations of these additive alleles from each parent can result in various resistance levels, which explain why the F1 hybrids obtained from this study do not show uniform resistance. Instead, they displayed a range of resistance, from high to low, depending on which alleles they inherited.

Among the 12 F1 hybrids from the Natar-2 × Ciinten cross, eight had the resistant (R) category, indicating hybrid vigor and increased resistance of their F1 hybrids. Four F1 hybrids of Natar-2 × Ciinten were moderately resistant (MR), despite the genotypes Natar-2 being susceptible (S) and Ciinten being moderately susceptible (MS). In contrast, the cross Petaling 2 × Ciinten produced more variable results, ranging from highly susceptible (HS) to highly resistant (HR). Four F1 hybrids showed hybrid vigor in resistance to stem borer (R and HR). These F1 hybrids inherited superior genes from both parents. However, two F1 progenies decreased to HS and S, possibly due to receiving a high proportion of identical recessive alleles from their parents. At the same time, three other hybrids maintained resistance levels like their parental cultivars (MS to MR).

The analysis of AI and damage intensity revealed notable patterns. Parental genotype Natar-2 had the highest AI (1); however, it was not like the highest damage intensity, as instead observed in P2CiP. The lowest AI values resulted in N2Ci 6-1 (0.05), N2Ci 3-4 (0.10), N2LDCi 2-6 (0.14), and P2Ci 2-6 (0.18), and these genotypes did not show the lowest damage intensity. Interestingly, the F1 hybrids P2Ci2-5, P2Ci2-6, and N2LDCi 2-6 exhibited 0% damage, suggesting factors like sensory preferences may deter insect attacks, as insects adaptively respond to plant compounds (Divekar *et al.*, 2022).

Notably, a high AI only sometimes corresponds to higher damage intensity, as frequent insect visits did not necessarily lead to stem damage. Morphological and chemical factors likely influence the stem borer host and food selection, intertwining food quantity (stem size) and quality (nutrition). Parental cultivars showed consistently high AI (0.61–1.00) but demonstrated variable damage intensity (10.17%–11.51%). Among the F1 hybrids, the P2Ci 1-5 had the highest AI (0.76), followed by P2Ci 2-7 (0.68), and both F1 hybrids exhibited relatively low damage intensity (14.53%, and 12.35%, respectively), classifying them as moderately susceptible.

During evaluation, the F1 hybrid P2Ci P appeared the most frequently visited by female stem borers, with an AI of 0.74 and the maximum damage intensity (31.33%), classifying it as highly susceptible. The F1 hybrid also surpasses its parental cultivars, Petaling-2 (10.17%; MR) and Ciinten-1 (12.34%; MS). A report on the reduction in the performance of F1 hybrids compared with their parental genotypes has also come out in black pepper hybridization (Bhat *et al.*, 2023). Conversely, the F1 hybrid N2Ci 6-1 obtained a visit only once in three replications, showing very low AI and damage intensity (0.73%), classifying it as resistant (R). Three F1 hybrids, P2Ci 2-5, P2Ci 2-6, and N2LDCi 2, had visits from *L. piperis*; however, they observed nonsignificant damage, categorizing them as

highly resistant (HR). These findings highlight the complex relationship between AI and actual damage. Morphological, environmental, and chemical factors may be influencing the stem borer behavior, and the same pattern was also apparent in maize stem borer (Soujanya *et al.*, 2023).

Among the black pepper parental cultivars, Natar 2 was the most favored by *L. piperis*. It had consistent visits from the first day of testing and showed the highest damage intensity, classifying it as the most susceptible genotype. The other four black pepper parental cultivars, Belantung, Petaling-2, N2LD, and Ciinten, sustained the MS, MR, and MS categories, respectively.

## CONCLUSIONS

Crossing black pepper (*P. nigrum*) cultivars produced F1 hybrid offsprings with resistance levels to *L. piperis*, ranging from highly susceptible to highly resistant. Among the 29 hybrids tested, three received highly resistant (HR) categories, 12 as resistant (R), 10 as moderately resistant (MR), two as moderately susceptible (MS), one as susceptible (S), and one as highly susceptible (HS). Further research is necessary to evaluate these resistance levels under semi-field and field conditions, which will provide more accurate data and insights into IPM strategies, supporting sustainable black pepper production by reducing reliance on chemical pest control.

## ACKNOWLEDGMENTS

The authors acknowledge the National Research and Innovation Agency, Bogor, the Republic of Indonesia, for funding the research through the In-House Program for Superior Seeds, No. 9/III.11/HK/2023. They also thank the Indonesian Institute for Testing Instrument Standard of Spices, Medicinal, and Aromatic Crops, for facilitating the collection of *L. piperis*.

## REFERENCES

- Amorita C, Daryanto A, Sahara (2021). Competitiveness analysis of Indonesian pepper in international market. *Int. J. Res. Rev.* 8(5): 38–52.
- Bermawie N, Wahyuni S, Heryanto R, Darwati I (2019). Morphological characteristics, yield and quality of black pepper Ciinten variety in three agro ecological conditions. *IOP Conf. Ser.: Earth Environ. Sci.* 292: 012065. doi: 10.1088/1755-1315/292/1/012065.
- Bhat DS, Hegde L, Hegde NK, Vijayakumar N, Shet RM, Rajashekara E (2023). Inter-varietal hybridization in black pepper (*Piper nigrum* L.). *Pharm. Innov. J.* 12(11): 1317–1323.
- Cheema JA, Carraher C, Plank NOV, Travas-Sejdic J, Kralicek A (2021). Insect odorant receptor-based biosensors: Current status and prospects. *Biotechnol. Adv.* 53: 1–13.
- De-Bastos-Pazini J, da Silva Martins JF, da Rosa Dorneles K, Crizel RL, da Silva FF, Chaves FC, Fernando JA, Dallagnol LJ, Seidel EJ, Stout MJ, Grützmacher AD (2022). Morphoanatomical and biochemical factors associated with rice resistance to the South American rice water weevil, *Oryzophagus oryzae* (Coleoptera: Curculionidae). *Sci. Rep.* 12: 1–17.
- De-Oliveira JRF, de Resende JTV, de Lima Filho RB, Roberto SR, da Silva PR, Rech C, Nardi C (2020). Tomato breeding for sustainable crop systems: High levels of Zingiberene providing resistance to multiple arthropods. *Horticulturae* 6(2): 1–34.
- Dhillon MK, Tanwar AK, Kumar S, Hasan F, Sharma S, Jaba J, Sharma HC (2021). Biological and biochemical diversity in different biotypes of spotted stem borer, *Chilo partellus* (Swinhoe) in India. *Sci. Rep.* 11: 1–12.
- Divekar PA, Narayana S, Divekar BA, Kumar R, Gadratagi BG, Ray A, Singh AK, Rani V, Singh V, Singh AK, Kumar A, Singh RP, Meena RS, Behera TK (2022). Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *Int. J. Mol. Sci.* 23(5): 1–24.
- Doležal P, Kleinová L, Davidková M (2021). Adult feeding preference and fecundity in the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). *Insects* 12: 473.
- Entebang H, Wong S-K, Mercer ZJA (2021). Development and performance of the pepper industry in Malaysia: A critical review. *Int. J. Bus. Soc.* 21(3): 1402–1423.
- Follett PA (2017). Insect-plant interactions: Host selection, herbivory, and plant resistance – an introduction. *Entomol. Exp. Appl.* 162(1): 1–3.
- Horgan FG, Romena AM, Bernal CC, Almazan MLP, Ramal AF (2021). Stem borers revisited: Host resistance, tolerance, and vulnerability determine levels of field damage from a complex of Asian rice stemborers. *Crop Prot.* 142: 1–13.
- Hussain A, Rizwan-Ul-Haq M, Al-Ayedh H, Aljabr AM (2017). Toxicity and detoxification mechanism of black pepper and its major constituent in controlling *Rhynchophorus ferrugineus* Olivier (Curculionidae: Coleoptera). *Neotrop. Entomol.* 46(6): 685–693.
- Khew CY, Koh CMM, Chen YS, Sim SL, Mercer ZJA (2022). The current knowledge of black pepper breeding in Malaysia for future crop improvement. *Sci. Hortic.* 300: 1–10.
- Lestari T, Apriyadi R, Husein A (2019). Damage intensity of pepper stem-borer (*Lophobaris piperis*) on different weed control in Bangka Belitung Archipelago Province. *Adv. Engin. Res.* 167: 145–149. doi: <https://doi.org/10.2991/icoma-18.2019.30>.
- Maron JL, Agrawal AA, Schemske DW (2019). Plant-herbivore coevolution and plant speciation. *Ecology* 100(7): 1–11.
- Meilawati NLW, Susilowati M, Bermawie N (2020). Phylogenetic of nine superior black pepper (*Piper nigrum* L.) varieties based on morphological and molecular markers. *IOP Con. Ser.: Earth Environ. Sci.* 418: 01056. doi: 10.1088/1755-1315/418/1/01056.
- Muturi PW, Mgonja M, Rubaihayo P, Mwololo JK (2021). QTL mapping of traits associated with dual resistance to the African stem borer (*Busseola fusca*) and spotted stem borer (*Chilo partellus*) in sorghum (*Sorghum bicolor*). *Int. J. Genomic* 2021: 1–17.
- Sharma KR, Raju SVS, Singh SK, Singh R, Dhanapal R, Kumar R (2024). Differential preference of grain of landrace and commercial rice genotypes to *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) attack. *J. Stored Prod. Res.* 108: 1–9.
- Soetopo D, Trisawa IM, Handayani T (1993). Influence of pepper stem diameter and length on biology of *Lophobaris piperis* Marsh (Coleoptera: Curculionidae). *Bull. Litro.* 8(1): 17–23.
- Soujanya PL, Sekhar JC, Karjagi CG, Ratnavathi CV, Venkateswarlu R, Yathish KR, Suby SB, Sunil N, Rakshit S (2023). Role of

- morphological traits and cell wall components in imparting resistance to pink stem borer, *Sesamia inferens* Walker in maize. *Front. Plant Sci.* 14: 1–12.
- War AR, Buhroo AA, Hussain B, Ahmad T, Nair RM, Sharma HC (2020). Plant defense and insect adaptation with reference to secondary metabolites. In: J.-M. Merillon and K.G. Ramawat (Eds.), *Co-Evolution of Secondary Metabolites*. Springer Cham. pp 1–28.
- Widiastuti RP, Rahardjo BT, Tarno H (2014). Resistance of several commercial sugarcane varieties to stem borer attacks *Chilo auricilius* Dudgeon (Lepidoptera: Pyralidae) in green house. *J. Hortic. Prod. Technol.* 2(2): 38–46.