INTRODUCTION

Tea is one of the perennial crops widely planted in Indonesia and other countries because it is highly beneficial. Furthermore, tea is also well-known in society since commonly used as an ingredient for drinks, medicines, and cosmetics. The demand for tea increases as the human population grows. However, according to Statistics Indonesia (2017), tea production in Indonesia decreased from 2004 to 2016. The productivity of dry tea is affected by several factors, including biotic and abiotic stress. For example, *Helopeltis* is the most common pest that causes yield losses in the Pagilaran tea plantation. According to Bandopadhyay et al. (2015) about 75% of the total tea production could decrease. Roy, Muraleedharan, Mukhopadhyay, & Handique (2015) explained that *Helopeltis* seriously damages and reduces the quantity and quality of tea plants.

*Helopeltis* attacks tea plantations by sucking buds, shoots, and leaves and cause various symptoms, including curled, dried, and black tea leaves. This damage significantly affects yield losses (Roy, Mukhopadhyay, & Gurusubramanian, 2010). This pest can be controlled using chemical agents, such as pesticides, but they pose a high risk and harm tea products, ecosystems, and humans. Nevertheless, this pest can be effectively controlled using resistant plants. Thus, the distribution of *Helopeltis* and its damage can be reduced without leaving any residues and causing damage to the environment and products (Suganthi, Senthilkumar, Arvinth, Rajkumar, & Chandrashekara, 2014). Afifah, Murti, & Wahyudhi (2021) explained that plant breeding programs have been facilitated to produce superior clones and varieties as genetic resources and thus obtain high yields and superior traits, including resistance against pests and diseases.

Genotypes produced through plant breeding programs are evaluated and screened to select the most desirable plants in pests and diseases (Arief et al., 2015). During the evaluation, information about the morphological characteristics and biochemical defence mechanisms of tea plants against *H. bradyi*. 

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**ABSTRACT**

*Helopeltis bradyi* is a significant pest that causes yield losses and reduces the quality of tea plantations by piercing and sucking the sap of tea leaves. This pest can be appropriately controlled by using resistant clones. The PGL series (clones) owned by Pagilaran plantation has high yield and quality. However, information their resistance level against *H. bradyi* is limited. This research was conducted at Pagilaran tea plantation at an altitude of 990 masl. Six PGL clone series (PGL 4, PGL 6, PGL 9, PGL 10, PGL 11, and PGL 15) and control clones (TRI 2025 and Gambung 7) were used as plant materials. Their morphological and biochemical characteristics were determined to evaluate their resistance level against *H. bradyi*. The results revealed that the score symptoms (under 10%) of PGL 4, PGL 9, PGL 10, PGL 11, and PGL 15 clones were the lowest, so these clones were categorized to be highly resistant. In addition, the total phenol content and trichome density of PGL 4, PGL 10, and PGL 15 clones were the highest. Among them, the PGL 4 clone had the thickest epidermis. These characteristics likely contributed to the morphological and biochemical defence mechanisms of tea plants against *H. bradyi*. 

**INTRODUCTION**

Tea is one of the perennial crops widely planted in Indonesia and other countries because it is highly beneficial. Furthermore, tea is also well-known in society since commonly used as an ingredient for drinks, medicines, and cosmetics. The demand for tea increases as the human population grows. However, according to Statistics Indonesia (2017), tea production in Indonesia decreased from 2004 to 2016. The productivity of dry tea is affected by several factors, including biotic and abiotic stress. For example, *Helopeltis* is the most common pest that causes yield losses in the Pagilaran tea plantation. According to Bandopadhyay et al. (2015) about 75% of the total tea production could decrease. Roy, Muraleedharan, Mukhopadhyay, & Handique (2015) explained that *Helopeltis* seriously damages and reduces the quantity and quality of tea plants.

*Helopeltis* attacks tea plantations by sucking buds, shoots, and leaves and cause various symptoms, including curled, dried, and black tea leaves. This damage significantly affects yield losses (Roy, Mukhopadhyay, & Gurusubramanian, 2010). This pest can be controlled using chemical agents, such as pesticides, but they pose a high risk and harm tea products, ecosystems, and humans. Nevertheless, this pest can be effectively controlled using resistant plants. Thus, the distribution of *Helopeltis* and its damage can be reduced without leaving any residues and causing damage to the environment and products (Suganthi, Senthilkumar, Arvinth, Rajkumar, & Chandrashekara, 2014). Afifah, Murti, & Wahyudhi (2021) explained that plant breeding programs have been facilitated to produce superior clones and varieties as genetic resources and thus obtain high yields and superior traits, including resistance against pests and diseases.

Genotypes produced through plant breeding programs are evaluated and screened to select the most desirable plants in pests and diseases (Arief et al., 2015). During the evaluation, information about the morphological characteristics and biochemical defence mechanisms of tea plants against *H. bradyi*.
biochemical properties of resistant genotypes should be obtained. Characteristics that contribute to defence mechanisms should be identified and examined to produce superior plants. Furthermore, these characteristics can be differentiated between resistant and susceptible genotypes. Therefore, this study aimed to evaluate the morphological and biochemical characteristics associated with the resistance level of tea clones against *Helopeltis*.

War et al. (2012) stated that resistant plants, directly and indirectly, defences to confront the pests. Moreover, indirect defence in plants could affect the survival and reproductive rate of the herbivory mediated by plant morphology (such as trichomes, thicker epidermis) and production of toxic chemicals (such as phenol, terpenoid, alkaloid, quinone, and anthocyanin). However, indirect defence in plants was mediated by producing some volatiles that attracts biological agents (enemy of the pest).

Xing et al. (2017) studied the efficiency of trichome-based plant defence against insect behaviour. They explained that trichomes are among the plant morphological characteristics that act as physical barriers to protecting sites from insects and trap insects. Trichomes are also among the main factors correlated with the resistance against herbivory. Moreover, trichomes participate in the natural defence by disrupting the feeding site of insects, preventing insects from reaching the surface of plants, and releasing toxic exudates that kill insects (Glas et al., 2012). Trichomes and epidermis are morphological defence systems that help prevent pests from sucking through a plant surface.

Haldhar et al. (2018) demonstrated that resistant plants might produce numerous biochemical compounds that counter-attack pests. Plant phenolics are also biochemical defensive factors produced by plants against pests. Nisha, Prabu, & Mandal (2018) explained that phenol and flavonoids are biochemicals in defensive systems against insects. Phenolics negatively affect the mortality of insects. Gantner, Najda, & Piesik (2019) showed that phenolic compounds develop the resistance of hazel cultivars against aphids. They are an essential group of compounds that protect plants from insect attack, enhance plant resistance, and alleviate oxidative stress.

In the Pagilaran plantation, new tea clones with high productivity and quality have been developed. However, the Pagilaran plantation has several problems with pests and diseases affecting the productivity and quality of dry tea. One of the pests widely known in Pagilaran is *H. bardyi*. Arnanto (2016) observed that PGL clone series are highly resistant to *Empoasca* and likely resistant to *H. bardyi*. However, information about the resistance level of the PGL clone series to *H. bardyi* is limited. As such, host plant resistance should be investigated to support the breeding program, determine the reaction of clones to pests, and identify the resistance factor between clones and *Helopeltis* (Das, Kalita, & Hazarika, 2017). Therefore, the morphological and biochemical characteristics of the PGL clone series against *Helopeltis* should be evaluated.

**MATERIALS AND METHODS**

**Experimental Procedure**

This study was carried out in the Karangnongko Block, Afdeling of Pagilaran, a tea plantation owned by Pagilaran Plantation, Batang District, Central Java, Indonesia, with an altitude of 990 masl from April to August 2018. In this period, a high infection of *H. bardyi* was widely distributed in the Pagilaran Plantation. Therefore, data were collected from the Pagilaran Plantation, including the score of *H. bardyi* attack, the number of shoots, shoot weight, trichome density, epidermal thickness, and total phenol content. This study was arranged with a completely randomized block design with four blocks as replications. Six PGL clone series (PGL 4, PGL 6, PGL 9, PGL 10, PGL 11, and PGL 15), TRI 2025, and Gambung 7 (as a susceptible clone) were used as plant materials.

**Percentage of *H. bardyi* Symptom**

Tea shoot per clones (with a pick formula of shoot + 1, shoot + 2, and shoot + 3) were harvested and then scored for *H. bardyi* attack. The percentage of damages was calculated with the following formula:

\[
\text{Percentage of damage} = \frac{\sum_{n} x}{N} \times 100\% \quad \text{1)
}
\]

Where: \( n \) is the total number of shoots damaged, and \( N \) is the total number of shoots observed. The result of this calculation was used to categorize the resistance level against *H. bardyi* following the classifications of Das, Kalita, & Hazarika (2017): 0%, immune; 1%–10%, highly resistant; 11%–20%, resistant; 21%–35%, medium resistant; 36%–50%, susceptible; and 51%–100%, highly susceptible.
Measurement of Trichome Density

Trichome density was measured with a qualitative method based on Pachrudin, Witjaksono, & Wijonarko (2007) by observing the second leaves of tea shoots under a microscope with 40× magnification. Each sample was observed four times and then averaged. Trichomes on the bottom of the tea clones leaves were evaluated because they do not exist on the surface of the leaves. Trichome density was scored based on this value (Pachrudin, Witjaksono, & Wijonarko, 2007):

1 : Trichome infrequently distributed in the leaf bones.
2 : Trichome frequently distributed in the leaf bones.
3 : Trichome infrequently distributed in all parts of the leaves.
4 : Trichome frequently distributed in all parts of the leaves.
5 : Trichome infrequently distributed in all parts of the leaves and stems.
6 : Trichome distributed in all part of the leaf and stem frequently.

Measurement of Epidermal Thickness

Epidermal thickness was measured via anatomical observation and the paraffin method (single staining). Tissues were fixed with liquid formalin, acetic acid glacial, and 70% alcohol for 24 h. The leaves were cleaned and dehydrated by removing the fixative with 70%, 80%, 95%, and 100% alcohol for 30 minutes. Then, they were cleansed (de-alcoholic) with a mixture of alcohol and xylol at ratios of 3:1, 1:1, and 1:3 for about 30 minutes. Afterwards, a mix of xylol and paraffin with a ratio of 1:9 was added at 57°C for 24 h. In infiltration, the mixture of xylol and paraffin was removed and replaced with pure paraffin at 57°C for 24 h. The tissue blocks were sliced using a rotary microtome with a thickness of 6–12 μm. The sample was placed in an object-glass with a mixture of alcohol and xylol at ratios of 3:1, 1:1, and 1:3 for about 30 minutes. The object-glass was then placed on a hot plate at 57°C. Subsequently, 1% safranin, 70% alcohol, and fast green were used to stain the sample. Lastly, the sample was covered and added with Canada balsam. The preparation was observed under a binocular microscope (Olympus CX 21) with a magnification of 40× to determine its epidermal thickness.

Total Phenol Content

The total phenol contents of the infected and non-infected leaves of tea clones were analyzed using a gallic acid equivalence method. Then, 0.125 g of gallic acid was placed in a volumetric flask until 25 ml was reached, and 2.5 ml of ethanol and distilled water were added to prepare a standard solution of 5 mg/ml. The standard solution of gallic acid was diluted into 0, 250, 500, 750, and 1000 ppm. Each concentration was placed in different tubes, added with 0.5 ml of Folin–Ciocalteau, 1.5 ml of Na₂CO₃, and 7.9 ml of distilled water. The standard solution was obtained after about 7 minutes. The standard solution was kept in the dark for about 2 h to wait for the reaction. To measure the total phenol of each sample, 1 g of tea leaves was cleaned with distilled water, ground with mortar, placed in 2 ml tubes, added with 80% ethanol, centrifuged at 12,000 rpm for about 12 minutes. This extraction diluted into 0, 250, 500, 750, and 1000 ppm. Each dilution was placed in a tube, added with 0.5 ml of Folin–Ciocalteau, 1.5 ml of Na₂CO₃, and 7.9 ml of distilled water. The solution was kept in the dark for about 2 h to wait for the reaction. The absorbances of each sample and standard were measured with a UV–vis spectrophotometer at a wavelength of 650 nm. The result of the measurement was expressed as gallic acid equivalent with weight per sample (Shah, Saied, Mahmood, & Malik, 2014).

RESULTS AND DISCUSSION

*Helopeltis* attacks tea plants by sucking stems, buds, and leaves. This study revealed that a new symptom appeared to be translucent and then changed quickly to light brown approximately 10–15 minutes after *Helopeltis* infection. The sign changed from translucent to necrotic at the initial stage and was surrounded by a dark ring (Fig. 1a). This symptom transformed from dark necrotic to curled, dried, and black (Fig. 1b). This study observed *Helopeltis* symptoms in six PGL clone series and two of the control clones (TRI 2025 and Gambung 7 for susceptibility check) in the study period when the highest infection and distribution of *H. bradyi* occurred in the Pagilaran Plantation.

Table 1 shows the percentage of tea clones damaged by *H. bradyi*. The data revealed that PGL clones significantly differed from the controls (Gambung 7 and TRI 2025). In particular, the lowest percentage of *Helopeltis* damage was found in PGL.
4 clones, followed by PGL 15, PGL 10, PGL 11, PGL 9, and PGL 6. By contrast, the highest percentage of Helopeltis damages was detected in TRI 2025 and Gambung 7. The score value indicated that PGL 4, PGL 15, PGL 10, PGL 11, and PGL 9 were highly resistant clones with <10% of damage. However, PGL 6 was categorized as a resistant clone with 19.83% of damage. Conversely, Gambung 7 and TRI 2025 as controls were highly susceptible and susceptible, respectively. In addition, the percentage of damage of PGL 4 was not different from those of PGL 9, PGL 10, PGL 11, and PGL 15. Therefore, PGL 4, PGL 9, PGL 10, PGL 11, and PGL 15 were more resistant to Helopeltis than TRI 2025 and Gambung 7.

Haldhar, Samadia, Bhargava, & Singh (2017) explained that plants with different genetic backgrounds generally influence herbivores’ host preference and non-reference. They use several strategies to counter-attack herbivores. The resistant plant may synthesize some biochemical compounds and physical barriers, such as trichomes, thorns, cell walls, and chemical compounds, including phenolic compounds recognized as the most active plant defence groups. Therefore, the percentage of Helopeltis damage and the morphological and biochemical characteristics of tea clones resistant to Helopeltis were observed in this study.

**Fig. 1.** Symptoms of H. bradyi attack: (a) New attack and (b) further attack

**Table 1.** Percentage of H. bradyi damages, epidermal thickness, phenol content, and score of trichome density of nine tea clones

| Clones  | Percentage of damage (%) | Category*  | Epidermal thickness (µm) | Phenol (mg/g) | Score of trichome density (**) |
|---------|--------------------------|------------|---------------------------|---------------|--------------------------------|
|         |                          |            | Upper     | Lower    |                  |                          |
| GMB 7   | 67.41 a                  | Highly susceptible | 1.00 d   | 0.82 d   | 0.58 d           | 3.75 b                   |
| TRI 2025| 46.87 b                  | Susceptible  | 1.02 cd  | 0.86 d   | 0.66 c           | 1.75 c                   |
| PGL 4   | 2.40 d                   | Highly resistant | 1.49 a   | 1.56 a   | 0.82 a           | 5.25 a                   |
| PGL 6   | 19.83 c                  | Resistant   | 1.22 b   | 0.84 d   | 0.65 c           | 5.00 a                   |
| PGL 9   | 8.78 d                   | Highly resistant | 1.20 b   | 1.00 c   | 0.77 b           | 3.00 b                   |
| PGL 10  | 7.28 d                   | Highly resistant | 1.20 b   | 1.14 b   | 0.8a b           | 5.25 a                   |
| PGL 11  | 8.75 d                   | Highly resistant | 1.14 bc  | 0.90 cd  | 0.67 c           | 1.75 c                   |
| PGL 15  | 5.62 d                   | Highly resistant | 1.18 b   | 1.02 bc  | 0.81 ab          | 5.75 a                   |
| CV%     | 24.39                    |             | 10.72    | 12.84    | 2.89             | 18.69                    |

Remarks: Numbers in columns followed by the same letter are not significantly different based on Duncan multiple range test at α = 5%. (*): Classification based on Das, Kalita, & Hazarika (2017). (**: The score ranged from 1 to 6; 1 = Trichome infrequently distributed in the leaf bones; 2 = Trichome frequently distributed in the leaf bones; 3 = Trichome infrequently distributed in all parts of the leaves; 4 = Trichome frequently distributed in all parts of the leaves; 5 = Trichome infrequently distributed in all parts of the leaves and stems; 6 = Trichome distributed in all part of the leaf and stem frequently (Pachrudin, Witjaksono, & Wijonarko, 2007).
The epidermal thickness (Table 1 and Fig. 2) significantly differed between the PGL clones and the controls (Gambung 7 and TRI 2025) in the upper epidermis. PGL 4 clones had the thickest epidermis in the upper (1.49 μm) and lower (1.56 μm) parts of the leaves. The epidermis on the upper part of the leaves did not significantly vary among PGL 6, PGL 9, PGL 10, PGL 11, and PGL 15. This result indicated that PGL series clones had a physical barrier that inhibited the infection of *Helopeltis* by having a thick epidermis. By contrast, the epidermis in the upper part of the leaves of Gambung 7 and TRI 2025 was the thinnest (1.00 and 1.02 μm, respectively).

The epidermal thickness in the lower part of the leaves (Table 1 and Fig. 2) depicted that the epidermis of PGL 4 was the thickest at around 1.56 μm, followed by PGL 9 and PGL 10 with the epidermal thickness of 1 and 1.14 μm, respectively. Conversely, the epidermal thickness in the lower part of the leaves of PGL 6, PGL 11, and PGL 15 did not significantly differ from that of Gambung 7 and TRI 2025. The epidermis is one of the morphological characteristics of direct defence mechanisms. It acts as a physical barrier and first-line plant defence against herbivores and inhibits and protects plants from herbivores’ feeding sites. It is implicated in the hardening of tea leaves and plant resistance mechanism by reducing the sucking site and tissue palatability; consequently, the damage caused by *Helopeltis* decreases (War et al. 2012). A study by Irieda & Takano (2021) described that multicellular organisms’ epidermis serves as a barrier between pest and their environment, protecting them from a wide range of biotic and abiotic challenges. Therefore, these barriers protect leaf infection from the herbivore. Moreover, in response to the entry trial of herbivore infection, epidermal chloroplasts arise at the higher periclinal wall. In Table 1, PGL 4, PGL 9, PGL 10, and PGL 15 had total phenol contents of 0.82, 0.77, 0.8, and 0.81 mg/g, respectively. These values were significantly different compared to Gambung 7 and TRI 2025. However, PGL 6 and PGL 11 were not significantly different from the control (TRI 2025). Phenol content plays an important role in plant defence mechanisms against herbivory. The resistant clones, namely, PGL 4, PGL 9, PGL 10, and PGL 15, had different biochemical contents that were likely associated with their defence mechanism against *Helopeltis*. Phenol is one of the secondary metabolites contributing to the immune system of...
plants, increasing oxidative stress after pest infection. Gantner, Najda, & Plesik (2019) showed that resistant plants have a high content of phenolic compounds. For example, a resistant hazel cultivar has a high content of phenolic compounds and harms aphids. Therefore, phenolic compounds affect the mortality of insects. Flavonoids, phenolic acids, stilbenes, and lignans are the major categories of polyphenols. These play an important role in plant chemical defenses as well as flavor, color, odor, astringency, oxidative stability, and bitterness (Singh, Kaur, & Kariyat, 2021). For instance, the infestation of winter triticale seedlings with grain aphids (Sitobion avenae F.) produces bioactive chemicals such as phenolic acids, which confer resistance to them (Singh, Kaur, & Kariyat, 2021).

Trichome density is one of the morphological characteristics related to the relationship between host plant resistance and herbivory. The trichome densities of PGL 4, 6, 10, and 15 were higher (with the score 5.25, 5.00, 5.25, and 5.75) than those of the other plant materials and significantly different from susceptible clones (TRI 2025 and Gambung 7). By contrast, PGL 9 and PGL 11 were not significantly different from TRI 2025 and Gambung 7 at 3.00, 1.75, and 1.75. Plant trichomes protect the surface of plants from herbivory infection and act as physical and chemical barriers. They consist of the main class of secondary metabolites, such as terpenoids, phenylpropenies, flavonoids, methyl ketone, acyl sugars, and defensive proteins (Glas et al., 2012). Trichomes are physical barriers to indirect plant defense mechanisms against insects with toxic and other harmful effects (War et al., 2012). This is in line with the study of Fortes, Fernández-Muñoz, & Moriones (2020), leaf glandular trichomes are useful to control the persistent transmitting of begomovirus Tomato Yellow Leaf Curling (TYLCV) which is infected by a vector Bemisia tabaci. Moreover, trichome consisted of acylsucroses that has a negative effect on B.tabaci.Correlation analysis (Table 2) revealed that the density of trichomes was negatively correlated with the percentage of Helopeltis damage (r = −0.368). This result indicated that trichomes did not significantly affect the reduction of Helopeltis damage in tea clones. Helopeltis has long styles measuring 1.5–2.1 mm (Bhau, Mech, Borthakur, Bhuyan, & Bhattacharyya, 2014). Trichomes did not sufficiently prevent a Helopeltis attack in the bottom part of the leaves because tea leaves have trichomes in the bottom part, while Helopeltis attack the upper surface of leaves.

**Table 2.** Correlation of the percentage of damage with trichome density, epidermal thickness, and phenol content

| Variables         | r-value | Significance |
|-------------------|---------|--------------|
| Trichome density  | −0.368  | 0.368        |
| Epiderm thickness | −0.732  | **0.038**    |
| Phenol content    | −0.820  | **0.012**    |

Remarks: *) significant at α = 5%.

In Table 2, the epidermal thickness had a significantly negative correlation with the percentage of damage (r = −0.732). The epidermal thickness contributed to preventing a Helopeltis attack. The thicker the epidermis was, the lower the rate of Helopeltis damage would be. Chen & Chen (2012) described that epidermal thickness affects insect deposition. It contributes to the hardening of leaves and consequently affects the host preference of insects and their preference for attacking young leaves. Lutfi, Hidayat, & Maryana (2018) reported that epidermal thickness could prevent pest attacks. For instance, soybean leaves with thick epidermal layers are less susceptible to the development of B. tabaci. The thin epidermal layer on leaves favours the development of B. tabaci nymphs.

The phenol content had a significantly negative correlation with the percentage of Helopeltis damage with r of −0.82. The higher the phenol content was, the lower the percentage of Helopeltis attack would be. This result indicated that phenol content contributed to the increase in the resistance of tea clones against Helopeltis. Glas et al. (2012) demonstrated that phenolic compounds participate in defensive mechanisms to herbivory and microorganisms. Phenol content is also involved in the cyclic reduction of reactive oxygen species, such as hydroxide radicals, superoxide anion, and singlet oxygen, which cause oxidative stress. Plant phenols are parts of a highly efficient biological defence system against pests and diseases. Nisha, Prabu, & Mandal (2018) described that flavonoid, and phenolic contents provide a wide range of beneficial effects, including protection against abiotic and biotic stress, among plants. They serve as factors that contribute to plant resistance. For instance, the expression of genes that encode this pathway is activated after Exobasidium vexans infection.
CONCLUSION AND SUGGESTION

Eight tea clones could be classified under the following categories: Highly susceptible (Gambung 7), susceptible (TRI 2025), resistant (PGL 6), and highly resistant (PGL 4, PGL 9, PGL 10, PGL 11, and PGL 15). Resistant clones against Helopeltis had a high epidermal thickness and phenol content. These morphological and biochemical characteristics likely contributed to the resistance of these clones against Helopeltis. Resistant clones such as PGL 6, PGL 4, PGL 9, PGL 10, PGL 11, and PGL 15 are recommended for extensification or rehabilitation of tea plantations. Moreover, the epidermis thickness and phenol content can be applied to select the resistant clone to Helopeltis.

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