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DOI : https://doi.org/10.22146/jpti.96906

Reference : Mak-683

To appear in : *Jurnal Perlindungan Tanaman Indonesia*

Received date : 18 June 2024

Revised date : 7 August 2024

Accepted date : 27 September 2024

Research Article

Feeding Behavior of Brown Planthopper (*Nilaparvata lugens*) on Pigmented Rice Monitored by Electrical Penetration Graph (EPG)

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ABSTRACT

Rice is one of the essential daily commodity for most of Asian. However, the brown plant hopper (BPH; *Nilaparvata lugens*) infestation had been threatening the increasing demand of rice production. Evaluating resistance level of rice cultivars against BPH will help in managing BPH infestation. The feeding behavior monitoring of brown plant hopper (BPH; *Nilaparvata lugens*) by using an electrical penetration graph (EPG) was conducted to evaluate the resistance level of several rice cultivars against BPH, including pigmented rice (black rice cv. Sembada hitam; red rice cv. Sembada merah); and the commonly consumed white rice (cv. Ciherang). The EPG instrument allowed the monitoring of BPH feeding behaviors by quantifying three unique waveforms, namely, N3, N4, and N5, which represent BPH feeding activities in areas near phloem tissues, in phloem tissues, and in xylem tissues, respectively. EPG monitoring of BPH feeding activities in black rice revealed the absence of the N3 and N4 waveforms. Red rice showed the N3 waveform but not the N4 waveform. White rice showed all three waveforms occurring with high numbers and long total durations. The absence of the N4 waveform in the two pigmented rice cultivars indicated the failure of BPH to access phloem tissues. Overall, the results revealed that the resistance of rice against BPH based on feeding activity could be ordered as follows: black rice > red rice > white rice. This report provides essential information on the resistance mechanism of pigmented rice cultivars against BPH.

Keywords: Brown plant hopper; *Nilaparvata lugens*; Electrical Penetration Graph; resistant cultivar; pigmented rice

INTRODUCTION

Rice (*Oryza sativa*), an important food crop in Indonesia and most Asian countries, has gained increased research interest on account of global requirements for food diversification (Khush, 1997; Muthayya *et al*., 2014). Intensification of rice production to meet increasing demands is often challenged by several limiting factors, one of which is brown plant hopper (BPH; *Nilaparvata lugens*) infestation (Sogawa, 2015). Significant crop losses due to BPH infestation have been reported in many rice-producing countries which initially emerged due to green revolution technologies (Bottrell & Schoenly, 2012; Hu *et al*., 2014). The outbreak, migration, and ability of BPH to overcome the resistance of rice have become major concerns worldwide (Azzam *et al*., 2009; Hu *et al*., 2014; Wu *et al*., 1997).

Brown plant hopper is a piercing and sucking insect belonging to family Delphacidae (order, Hemiptera). The mouthpart of BPH is modified into a stylet for feeding activity, which enables the insects to obtain essential nutrition by feeding on plant tissues, especially the phloem. Phloem feeding through stylets may lead to hopperburn, which could significantly decrease rice production up to 100%. BPH is also known to transmit *Rice ragged stunt virus* (RRSV) and *Rice grassy stunt virus* (RGSV), both of which further contribute to rice production failure (Huang *et al*., 2015; Zheng *et al*., 2014). Brown plant hopper control conventionally relies on chemical pesticides, which are relatively easy to acquire and apply, and produces rapid results. However, the excessive use of chemical pesticides may lead to environment pollution and BPH resurgence (Heinrichs & Mochida, 1984; Khoa *et al*., 2018; Reissig *et al*., 1982). Considering the potential negative impacts of pesticides, the development of new resistant rice varieties through breeding programs has been explored as an environment-friendly control alternative (Cohen *et al*., 1997). Unfortunately, coevolution events leading to the emergence of new BPH biotypes that could breakdown the resistance of existing rice cultivars remains a major concern (Jing *et al*., 2014; Kobayashi *et al*., 2014; Sharma *et al*., 2015; Zhao *et al*., 2016). Today, the emergence of new BPH biotypes and release of new resistant rice cultivars is as never-ending battle.

An efficient but also precise measurement technique to evaluate the resistance of new rice cultivar against BPH is required. The electrical penetration graph (EPG) represents one of the techniques that can be adapted to evaluate the resistance trait of rice cultivars against BPH (Ghaffar *et al*., 2011; Soffan & Aldawood, 2015; Van Helden & Tjallingii, 2000). In principle, EPG enable the monitoring of BPH feeding behavior through visualizing (as a waveforms) electrical resistance change and electromotive force during different feeding activities and location in several tissues of rice cultivars (Seo *et al*., 2009; Tjallingii, 1978; Wan *et al*., 2019).

In the present work, we evaluate resistance of two pigmented rice and white rice cultivars against BPH by using an EPG system. Pigmented rice is known to be resistant against several plant diseases, including Bacterial Leaf Blight (Joko *et al*., 2019), however their potential resistance against insect pest including BPH is not known. The results of this study will support the development of resistant rice cultivars against insect pests including for BPH.

MATERIALS AND METHODS

Plant Materials

Two pigmented rice cultivars, black rice (cv. Sembada hitam), and red rice (cv. Sembada merah), and white rice (cv. Ciherang) were used in this work. The seed of all three rice cultivars were provided by Assessment Institutes for Agricultural Technology of Yogyakarta. Prior to EPG monitoring, the rice seeds were dipped in water for 24 h and then germinated for 20 days. All plants were maintained at 28 °C with 80 % relative humidity and a photoperiod of 16 h light/8 h dark.

Brown Plant Hopper Rearing

Brown plant hopper individuals were collected from a rice field in Juwiring, Klaten, Central Java, and reared on rice seedling, cultivar IR 64 in Applied Entomology Laboratory, Department of Plant Protection, Universitas Gadjah Mada. The rice seedling was maintained in cylindrical plastic containers (6 cm diameter, 10 cm height) for BPH feed, whereby water level was maintained at 1 cm height to keep the rice growth. Replacement of the heavily damage rice seedlings by BPH was done as necessary, by flip down them to new rice seedlings in a new container allowing the BPH to migrate naturally, whereby minimizing potential interference related to insect/plant handling. BPH at the 3–4 instar was used for EPG recording.

Brown Plant Hopper Feeding Monitoring by EPG

Brown plant hopper feeding behaviors were monitored by using EPG technique (Tjallingii, 1978). The experiments adopted a completely randomized design with six or seven replications. Twenty-day-old rice seedlings and BPH at the 3–4 instar was used for the EPG experiments. All EPG experiment components were placed in a Faraday cage during feeding monitoring to avoid environment electrical noise event. BPH was placed on the stems of the rice plants about 2-4 cm above the rooting system (Seo *et al*., 2009). An EPG electrical circuit was set up for one plant and one BPH. Gold wire (20 µm diameter; 3 cm length) was attached to the dorsal part of BPH abdomen, with conductive water-based silver glue. Immobilization of the BPH was required prior to insect mounting which was assisted by light air vacuum on the ventral part of the BPH. The mounted insects were connected to a 3 cm-long copper wire (0.2 mm diameter) which in turn, was connected to the input of an amplifier with a 1 GΩ input resistance and 50× gain. The rice plants were connected to the plant voltage output of the EPG device (Giga-4, Wageningen University) by insertion of a copper electrode (thickness, 2 mm; length, 10 cm) into the water-based medium. The electrical circuit was completed when the BPH inserted its stylet into the plant tissue. EPG waveforms were automatically generated and recorded on a computer, and recordings were performed for 4 h. Acquisition and analysis of the EPG waveforms obtained were carried out using STYLET version 3.0 (Tjallingii, Wageningen University, Wageningen, The Netherlands).

Statistical Analysis

All data were analyzed using SAS version 9.2 (2008, SAS Institute, Cary, NC, USA). The normality of the data distribution was tested in PROC UNIVARIATE by the Shapiro–Wilk method, followed by square root transformation prior to analysis of variance. Feeding activity in the xylem, phloem, and areas near phloem tissues are represented by the EPG waveforms N5, N4, and N3, respectively. Numbers; total, maximum, and minimum durations; and the first time a specific waveform occurred were calculated over 4 h of feeding monitoring. Parametric one-way ANOVA was performed in PROC GLM, and multiple comparisons were carried out using the least significant difference test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Resistance characters of rice cultivars monitored by EPG system usually was indicated by three EPG waveforms; N3, N4 and N5, representing stylet feeding activities in areas near phloem tissues, phloem tissues, and xylem tissues respectively (Figure 1). The determination of these waveforms is based on their characteristic shapes, amplitudes, and frequencies. Three other waveforms generated during EPG monitoring of BPH in rice include NP (non-probing), N1, and N2; N1 and N2 are respectively related to the penetration initiation and salivation of stylets. N4 can also be subdivided into N4a and N4b; here, N4a represents intracellular activity in the phloem region, while N4b is related to phloem sap ingestion (Seo *et al.*, 2009). Representative overview of waveform in regards of BPH feeding on three different cultivars were visualized in Figure 2 for white rice (cv. Ciherang), red rice (cv. Sembada merah) and black rice (cv. Sembada hitam) respectively.

The representative 1-hour EPG waveform of Ciherang (Figure 2A) showed the presence of the N3, N4, and N5 waveforms, which are commonly observed during the normal feeding of BPH on rice. The presence of these three waveforms reflects the ability of BPH to access and utilize phloem tissues, which is essential for BPH development (Ghaffar *et al*., 2011; Yu *et al*., 2024). The absence of the N4 waveform in Sembada hitam and Sembada merah (Figures 2B and 2C) indicates the disturbance of phloem tissue access by BPH. Quantitative measurement of the N3 and N4 waveforms of Sembada hitam and Ciherang revealed significant differences in total number and duration (Table 1). Sembada merah, numerically has fewer number and total duration of N4, and significantly has fewer maximum duration of phloem ingestion (N4) as compared to Ciherang (Table 1).

Interestingly, although feeding activities in areas near phloem tissues (N3) were not significantly different between Sembada merah and Ciherang, feeding activities in phloem (N4) revealed a shorter period of access to phloem ingestion/acquisition in Sembada merah than that in Ciherang. Thus, Sembada merah has more resistance traits than Ciherang. BPH feeding activity indicated by the N3 and N4 waveforms in Sembada hitam was completely absent, which means this cultivar has resistance characteristics that completely prohibit BPH from utilizing its phloem tissues (Table 1). Xylem feeding in Sembada hitam by BPH was significantly reduced compared with that in other cultivars, indicating the relative resistant character of Sembada hitam has relation with fewer activities of BPH in xylem (represented by N3 waveform) (Table 1).

Obvious differences in BPH feeding behaviors among the three different rice cultivars could clearly be observed by EPG monitoring (Figure 2). Representative 1-hour BPH feeding patterns (Figure 2A) revealed that BPH stylets had greater access to the phloem tissues of Ciherang than to the corresponding tissues of the other rice cultivars. The ability to access phloem tissue is a crucial indicator of host plant suitability for phloem-feeding insects (PFIs). Studies using electrical penetration graph (EPG) technology have demonstrated that the frequency and duration of phloem feeding phases can differentiate between susceptible and resistant plants (Liu *et al*., 2012; Wu *et al*., 2022). BPH feeding on Sembada merah (Figure 2B) revealed the N3 and N5 waveforms but not the N4 waveform. Finally, BPH feeding on Sembada hitam (Figure 2C) demonstrated the absence of N3 and N4 waveforms. This phenomenon clearly shows that BPH has no access to nutrients in the phloem tissues of Sembada hitam, which could lead to insufficient nutrient uptake for survival and reproduction.

The absence of the N4 wave as an indicator of varietal resistance is supported by the results of Ghaffar *et al*. (2011) which showed that in susceptible varieties, BPH ingested phloem sap for a long period of time without interruption. BPH can only ingest phloem sap in susceptible genotypes. In addition, Quais *et al*. (2020) also showed that BPH spent significantly less time in the phloem and sustained phloem sap consumption was lower in salinity-resistant rice plants. Results of these research about feeding behavior of BPH on pigmented rice support Hattori's (2001) suggestion that resistance to BPH is determined by phloem-related characters. Phloem-based resistance may have a basis in phloem chemistry where silicic, oxalic and phenolic acids, sterols and apigenin-C-glycosides have been implicated in resistance to BPH.

CONCLUSION

Pigmented rice particularly var. Sembada hitam appeared to have potential resistant character as compared to white rice cv. Ciherang which was showed from strong evidence of the inhibition of phloem access (N4 waveform) during BPH feeding and the absence of N3 waveform (probing activities prior phloem access). This result is essential as a baseline to develop new rice cultivar resistant to BPH, therefore securing rice production in the future.

ACKNOWLEDGMENTS

We thank the Assessment Institutes for Agricultural Technology of Yogyakarta for supporting our experiments and providing the necessary materials for testing. We also thank Yuni Apriliana S.P., M.Sc, who provided excellent assistance during the EPG experiments.

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APPENDIX

Table 1. Brown plant hopper (BPH) feeding activities represented by electrical penetration graph (EPG) waveforms of three rice cultivars, including white rice (cv. Ciherang), red rice (cv. Sembada merah), and black rice (cv. Sembada hitam)*

BPH Feeding activities on		Rice cultivars		
Rice Tissue location	Parameter (Unit)	Ciherang	Sembada merah	Sembada hitam
Phloem (N4)	Number (n)	1.2 ± 0.5 a	0.4 ± 0.2 ab	0 ± 0 b
	Duration (min)	41.8 ± 26 a	8 ± 4.6 ab	0 ± 0 b
	First (min)	72.7 ± 24.9 a	65.4 ± 36.8 ab	0 ± 0 b
	Maximum (min)	53.1 ± 27.9 a	4.4 ± 4.4 b	0 ± 0 b
	Minimum (min)	30.7 ± 26.4 a	4.4 ± 4.4 ab	0 ± 0 b
Areas near phloem (N3)	Number (n)	2.7 ± 0.6 a	3 ± 1.1 a	0 ± 0 b
	Duration (min)	4.4 ± 0.7 a	2.9 ± 0.8 a	0 ± 0 b
	First (min)	137.1 ± 29.7 a	72.9 ± 22.1 b	0 ± 0 c
	Maximum (min)	6 ± 0.8 a	4 ± 1.2 a	0 ± 0 b
	Minimum (min)	3.1 ± 0.9 a	1.9 ± 0.5 a	0 ± 0 b
Xylem (N5)	Number (n)	1.8 ± 0.3 a	3.7 ± 0.9 a	0.6 ± 0.3 b
	Duration (min)	17.7 ± 7.4 a	11.7 ± 2.9 a	28.3 ± 18.4 a
	First (min)	85.1 ± 33.1 a	85.3 ± 36.9 a	52.9 ± 32 a
	Maximum (min)	22.3 ± 7 a	17.3 ± 3.5 a	28.3 ± 18.4 a
	Minimum (min)	13.4 ± 8.3 a	7.9 ± 3.6 a	28.3 ± 18.4 a

*Means followed by the same letter in the same row are not significantly different (least significant difference test, α = 0.05).

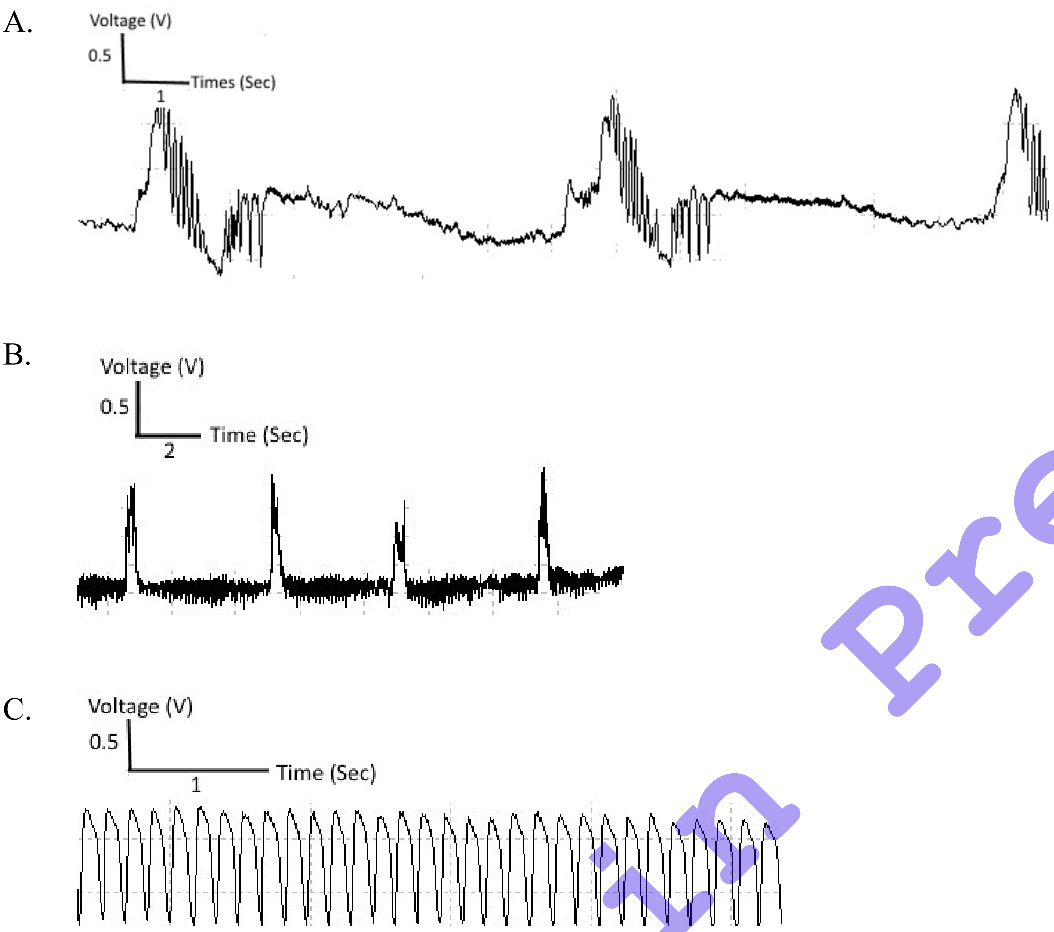

Figure 1. Feeding behaviors of brown plant hopper (BPH) as monitored by an electrical penetration graph (EPG): (A) Typical N3 waveform indicating feeding activity in areas near phloem tissues; (B) Typical N4 waveform indicating feeding activity in phloem tissues; (C) Typical N5 waveform indicating feeding activity in xylem tissues

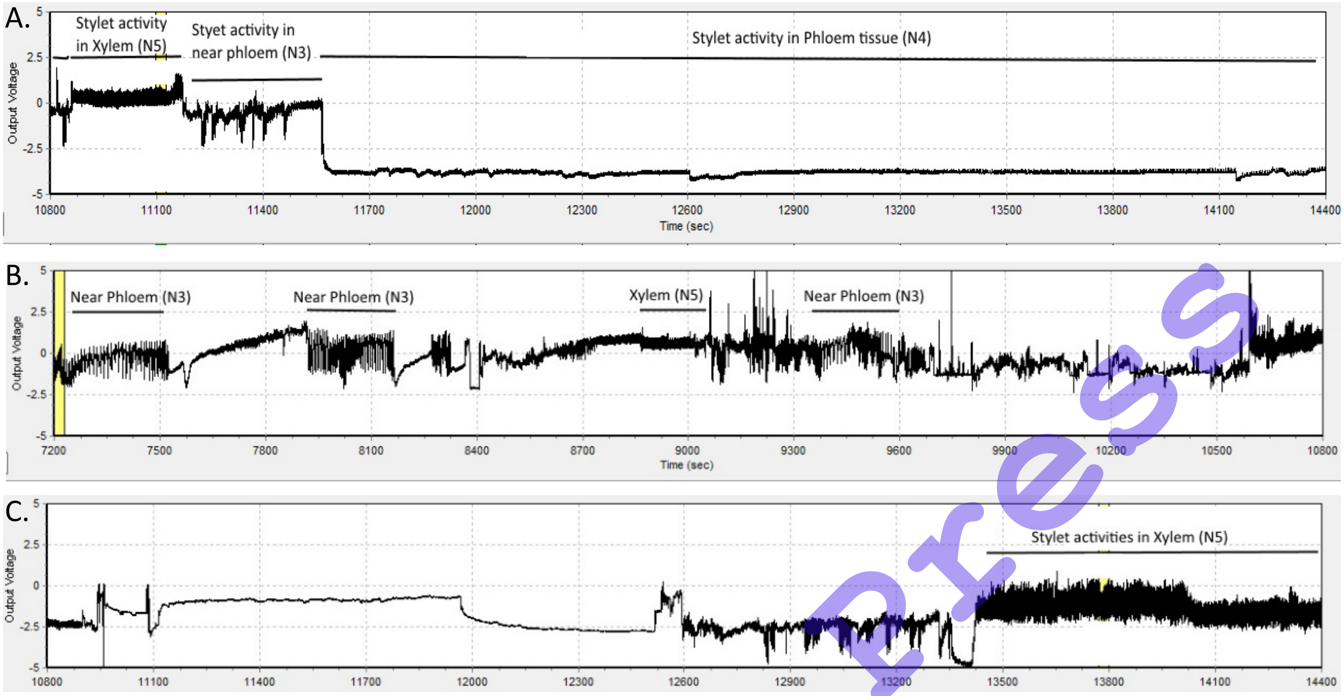

Figure 2. Representative 1-hour electrical penetration graph (EPG) waveforms of brown plant hopper (BPH) in (A) white rice (cv. Ciherang), (B) red rice (cv. Sembada merah), and (C) black rice (cv. Sembada hitam); in (A), N3 and N4 are present; in (B), N3 is present but N4 is absent; and, in (C), N3 and N4 are both absent