



Increased probing activities of green peach aphid (GPA), *Myzus persicae*, on chitosan-treated caisim (*Brassica juncea*) monitored by electrical penetration graph (EPG)

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Abstract

Green peach aphid (GPA), *Myzus persicae*, is a major pest of most horticulture crops. Chitosan-based pest management emerges as an alternative to pesticides due to its biocompatibility and biodegradability properties. Population growth and electrical penetration graph (EPG)-based feeding behavior studies were conducted to assess the effect of chitosan application on caisim against GPA at three concentrations (0.1%, 0.5%, and 1%) including two controls of water and acetic acid. Evident GPA population growth reduction was observed in the chitosan-treated caisim. The effect of chitosan was further monitored by 10 h of EPG recording, which revealed a significant increase in probing activities due to frequent stylet withdrawal that generated high levels of short probing activities. Additionally, inter- and intracellular stylet punctures (waveform C and potential drop-Pd, respectively) displayed a significant increase. However, once the stylet reached the phloem tissue, GPA under chitosan treatment and water control can access the phloem tissue equally, either in terms of the number and duration. Therefore, we suggest that the reduced population growth due to chitosan treatment was related to extra energy consumption during frequent stylet withdrawal and intracellular puncture. This finding indicates the role of chitosan as a plant defense elicitor. However, further investigation regarding this topic is required.

Keywords Chitosan · Plant elicitor · Caisim · Population · Feeding behavior · EPG

Introduction

Green peach aphid (GPA), *Myzus persicae*, is considered to be one of the most important horticultural pests in the world, with the infestation often aggregating in high densities in more than 300 host plants in over 40–66 plant families including caisim (*Brassica juncea*) plant (Cao et al. 2017; Capinera 2008; Davis et al. 2006; Shim et al. 1977). Significant loss in Brassicaceae family due to GPA may reach 97–100% (Patel et al. 2004). The pest status of GPA gradually increased due to their ability to efficiently transmit

virus diseases to more than 100 plants (Kennedy et al. 1962). Additionally, they developed resistance to major insecticides (Li et al. 2016; Rubiano-Rodriguez et al. 2014; Silva et al. 2012; Tang et al. 2017). The conventional methods used to manage GPA include utilization of rice–straw mulch (Silva-Filho et al. 2014), predators (De Backer et al. 2015; Down et al. 2003), entomopathogens (Vu et al. 2007; Mohammed et al. 2018), resistant cultivars (Davis et al. 2007; Pascal et al. 2002), with majority by using insecticides (Wang et al. 2008; Yu et al. 2010). Overuse of chemical pesticides to manage this pest has caused many environmental problems, and may lead to resurgence (Harrington et al. 1989). For these reasons, an alternative measures should be developed, one of which involves the use of the natural compound chitosan (Katiyar et al. 2014; Orzali et al. 2017).

Chitosan is derived from chitin, a natural amino polysaccharide, which is extracted from the exoskeleton of crustaceans, insect, fungal cell walls, present in abundant numbers and known has nontoxic and biodegradable properties (Badawy and Rabea 2011; Jia et al. 2016; Katiyar et al. 2014). Chitosan had been reported has potential in

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plant defense through priming thus protecting from pests and diseases (El Hadrami et al. 2010). Similarly, several reports considered chitosan as a plant defense elicitor that reduces plant diseases caused by viruses, fungi, bacteria, and nematodes by inducing biochemical changes in plants (Farag et al. 2017; Fitza et al. 2013; Jia et al. 2016; Mwaheb et al. 2017). However, chitosan application on insects had mostly focused on their effect as insecticides, such as those for *Aphis gossypii*, *Callosobruchus maculatus* (Sahab et al. 2015), and *Spodoptera littoralis* (Badawy et al. 2005), with additional function able to prolonged mosquitocidal activity of *Siparuna guianensis* essential oil (Ferreira et al. 2019). However, little is known about the possible properties of chitosan as a plant defense elicitor when chitosan-treated plants are challenged with insect pest.

Potential mechanism of chitosan as a plant elicitor against the GPA was evaluated by monitoring their feeding behavior using Electrical Penetration Graph (EPG). Basically EPG monitor the association of the target insect (including GPA) to their plant substrate, which is essential for their fitness (Backus et al. 2020). Some detail parameters observed during EPG monitoring will determine specific probing behavior due to external factors, such as the use of resistant cultivars, or exogenous material application such as naringenin, and silicon (Goussain et al. 2005; Civolani et al. 2010). Therefore, through the feeding behavior data, complemented with plant population growth study, we reported here the possible effect of chitosan as on plant defense elicitor against GPA, which further can be optimized in future as one of the potential strategies to manage GPA population.

Materials and methods

Plant, chitosan, and insect material

Caisim plants (*Brassica juncea* var. Tosakan) were grown in a mixture of soil and organic fertilizer (2:1) contained in a plastic polybag (20 cm × 20 cm). All plants were maintained in a glass house until 17–25 days to ready them for chitosan treatment. Chitosan (Shrimp Shell, Black Tiger) was dissolved in 1.5% acetic acid (AA) at the final concentrations of 0.1%, 0.5%, and 1%. Control plants were prepared using water and 1.5% AA to determine the possible effect of AA solvent. Chitosan treatment was conducted thrice by drenching the plants with 10 ml chitosan for 12 days.

Green peach aphid (GPA), *Myzus persicae*, were collected from a caisim field in Wukirsari, Yogyakarta (7°39'59.8"S 110°25'54.4"E). A virginoparous apterous adult female aphid was used to initiate GPA culture synchronization in the laboratory. The replacement of new caisim seedlings was routinely conducted when previous caisim seedlings suffered from GPA infestation. Three to five days old nymphal GPA

were utilized for the population growth study and EPG monitoring of the feeding behavior.

Green peach aphid (GPA) population growth study

Each single plant was introduced with six GPA apterous nymphs. The plants were further covered with a clear plastic polymerized vinyl chloride tubes (height: 30 cm; diameter: 10 cm) and ventilated using a muslin cloth (15 × 5 cm). The perforated plastic tube prevented GPA from escaping while allowing them to feed on any part of the plant freely. Artificial infestation was terminated after 14 days by cutting the plant from the base. Different stages of GPA, including adults (alatae and apterae) and nymphs (alatae and apterae), were collected and preserved in 75% ethanol for counting.

Green peach aphid (GPA) feeding monitoring by EPG

GPA feeding behavior of five chitosan-treated caisim (including control) was monitored using a four-channel EPG monitor (type Giga 4, EPG Systems, Netherlands). A Faraday cage, which covered the plant, was used to cancel electrical noise during EPG monitoring. The abaxial side of the second leaf from the apex was twisted up to ease the GPA access to the plant (Prado and Tjallingii 2007; Soffan and Aldawood 2015). GPA were immobilized by gentle air vacuum which sucked on the ventral side (abdomen), followed by attachment of a thin gold wire (diameter: 20 µm; length: 3 cm) on the dorsal side using conductive water-based silver glue. A 3 cm-long copper wire (diameter: 0.2 mm) was used to connect the other end of the gold wire and the input of the EPG amplifier at a 1 GΩ input resistance and 50× gain. Each plant electrode (a 2 mm-thick, 10 cm-long copper rod) was connected to an adjustable EPG plant voltage output and was further connected to the plant by insertion through the soil medium (Prado and Tjallingii 2007). Finally, 10 h EPG recording was initiated with four aphids at a time, with one aphid considered per plant.

EPG recording was mediated by Stylet + software installed on a computer, which generated several unique waveforms, for further analysis. The EPG recording was achieved by using AD conversion at 100 Hz (Di158U converter, Dataq, USA). In signal analysis, five EPG waveforms were distinguished. The nonprobe (Np) waveform occurs due to stylet withdrawal; waveform C represents the intercellular stylet pathway in epidermis and mesophyll; waveform E denotes the total phloem phase, representing the feeding activity in phloem tissue; waveform G indicates xylem ingestion or active drinking of water from xylem elements; potential drop (Pd) indicates intracellular cell puncture

Table 1 Population growth study of Green peach aphid (GPA) under three chitosan treatments, including two controls for specific population parameters (par.)*

Parameters	Chitosan Treatment			Control	
	0.1%	0.5%	1%	Water	AA**
a. Nymph (Instar 1)	9 ± 1.09 c	11.6 ± 1.2 bc	11.5 ± 0.9 bc	12.5 ± 1.04 ab	15.7 ± 1.63 a
b. Nymph Alatae	3.7 ± 0.72 c	3.2 ± 0.5c	4.3 ± 0.67bc	5.9 ± 0.71 ab	7.3 ± 0.76 a
c. Nymph Apterous	16.9 ± 0.96ab	12.0 ± 0.9c	14.8 ± 0.9bc	17.8 ± 1.7ab	19.2 ± 1.69a
d. Adult Alatae	1.9 ± 0.31b	1.9 ± 0.4c	1.5 ± 0.5b	4.8 ± 0.25a	4.5 ± 0.37a
e. Adult Apterous	12.3 ± 0.65bc	11.4 ± 0.9bc	10.2 ± 0.6c	16.1 ± 1.09a	13.7 ± 1ab
f. Total Population	43.8 ± 1.6b	40.1 ± 1.8b	42.3 ± 0.9b	57.1 ± 1.72a	60.4 ± 2.19a

*Means followed by the same letter in the same row are not significantly different at LSD, $\alpha=0.05$

**AA: Acetic acid solvent

during intercellular stylet pathway (Tjallingii 1990). Next, to waveform analysis, the data were processed to calculate 19 selected EPG parameters (par.) (Tables 2 and 3) using an Excel macro workbook (Sarria et al. 2009).

Statistical analysis

The experimental design was a complete randomized design. The study of feeding behavior and population growth utilized 6 and 10 replications of caisim plants, respectively, for the five chitosan treatments (including control). All the data were analyzed using SAS program ver. 9.2 (SAS I 2008). Nonparametric one-way analysis of variance and Kruskal–Wallis test were performed by using PROC NPAR1WAY, followed by multiple comparison using least significant difference (LSD) test ($\alpha:0.05$).

Results

We reported two methods; population growth studies and EPG-monitored feeding behavior to study the effect of chitosan on GPA, when the compound is used to drench the caisim plant. Our results in population growth studies (Table 1), particularly in total population (par.f, Table 1) showed chitosan affected the reduction of the GPA population during 14 days of observation (43.8, 40.1 and 42.3 for chitosan treatment 0.1%, 0.5%, 1% respectively) compared with the water control (57.1). However, the usage of AA as solvent alone had no effect on the reduced population (60.4). The reduced total population of chitosan-treated plants was followed by the reduced number of adult-stage GPA (either alatae or apterous) compared with the water control plants. The nymph phase (either for instar 1, alatae, or apterous) showed no significant reduction after chitosan treatment, although such result was evident numerically.

Table 2 Probing activities of Green peach aphid (GPA) on nonspecific tissues in different chitosan-treated caisim monitored by 10 h EPG recording (mean ± standard error [SE])

No	EPG parameters	Chitosan			Control	
		0.1%	0.5%	1%	Water	AA
General probing activities						
1	Number of probes/nonprobing	104.3 ± 17.9a	78.8 ± 3.4ab	91 ± 14.3a	44.8 ± 7.8b	74.5 ± 24.7ab
2	Number of short probes	83.3 ± 16.6a	59 ± 4.7a	65 ± 9.9a	22.8 ± 5.1b	52.3 ± 20.2ab
3	Total duration of probing	421.2 ± 32.8b	486 ± 22.5ab	467 ± 26b	540 ± 15.2a	499.4 ± 37.2ab
4	Total duration of nonprobing	178.8 ± 32.8a	114 ± 22.5ab	133 ± 26a	60 ± 15.2b	100.6 ± 37.2ab
5	Duration of 1st probe	7.8 ± 2a	13.7 ± 5.3a	23.1 ± 7.5a	14.8 ± 14.4a	42 ± 23a
6	Duration of 2nd probe	0.5 ± 0.1a	19 ± 13.2a	4.2 ± 2.2a	37 ± 27.9a	35 ± 34.2a
Intercellular probing						
7	Number of C	105 ± 17.4a	82.5 ± 4ab	96 ± 14.5a	48.5 ± 7.3b	77.3 ± 24.8ab
8	Total duration of C	379 ± 30.5a	391.4 ± 46.6a	381.1 ± 26.9a	429.4 ± 35.2a	393.3 ± 30.1a
9	Duration C' (C/number of C)	3.8 ± 0.6b	4.7 ± 0.5ab	4.2 ± 0.6b	9.5 ± 1.8a	8.8 ± 4.4ab
Intracellular probing						
10	Number of Pd	195 ± 25.3a	264.3 ± 50.6a	323.8 ± 9.4a	294.5 ± 56.1a	233 ± 53.3a
11	Pd/C; (Pd')	55.6 ± 14.1ab	55.6 ± 8.8ab	81.2 ± 9.6a	32.5 ± 7b	48.2 ± 16.8ab

*Means followed by the same letter in the same row are not significantly different at LSD, $\alpha=0.05$

**AA: Acetic acid solvent

Table 3 Probing activities of Green peach aphid (GPA) on specific tissues (xylem and phloem) in different chitosan-treated caisim plants monitored by 10 h EPG recording (mean \pm standard error [SE])

No	EPG parameters	Chitosan			Control	
		0.1%	0.5%	1%	Water	AA
Xylem probing						
12	Number of G	0.7 \pm 0.3ab	0 \pm 0b	0.3 \pm 0.3b	1.8 \pm 0.5a	1 \pm 0.6ab
13	Total Duration of G	16.4 \pm 12.1ab	0 \pm 0b	3.2 \pm 3.2b	58.8 \pm 14.7a	81.3 \pm 57ab
Phloem probing						
14	Number of phloem phases	0.3 \pm 0.3c	4.3 \pm 0.8ab	5 \pm 0.6a	2.3 \pm 0.8ab	2 \pm 1.4bc
15	Total duration of E	25.8 \pm 25.8a	94.7 \pm 41.4a	82.7 \pm 28a	51.9 \pm 45.1a	24.8 \pm 23.7a
16	Total duration of nonphloematic phase	522.5a	505.4 \pm 41.4a	517.3 \pm 28a	548.1 \pm 45.1a	550.4 \pm 46.4a
17	Duration of 1st E	77.5a	38.6 \pm 19.3a	24.1 \pm 12.3a	49.5 \pm 45.9a	16.9 \pm 15.3a
18	Time to 1st probe from start of EPG	7.7 \pm 0.7a	11.5 \pm 4.3a	7.4 \pm 2.9a	7.7 \pm 5.4a	3.9 \pm 3.7a
19	Time from start of EPG to 1st E	513 \pm 87a	258.7 \pm 37.7b	220.8 \pm 77.6b	411.2 \pm 29.2ab	438.2 \pm 139.9ab

*Means followed by the same letter in the same row are not significantly different at LSD, $\alpha=0.05$

**AA: Acetic acid solvent

Effect of chitosan on GPA was further observed using EPG monitoring for 10 h. EPG was used to observe different parameters (par.), which were categorized into two major groups of probing activities: those that showed feeding behavior on nonspecific (Table 2) and specific tissues (phloem and xylem) (Table 3). Probing activities on nonspecific tissues comprised general probing (par. 1–6, Table 2), intercellular probing (par. 7–9, Table 2), and intracellular probing (par. 11–12, Table 2). The overview of probing activities resulting from chitosan treatment was distinguished by comparing the table columns of the highest concentration (1% chitosan) treatment and water control. A significant increase was observed in the probing activities under the 1% chitosan treatment (par. 1, Table 2). This result was due to the increased number of short probing activities (par. 2, Table 2) caused by the increase in stylet withdrawal and displayed as nonprobing activities (par. 3 and 4, Table 2).

The differences in general probing categories were inconspicuous when observing the 1st and 2nd probing (par. 5 and 6, Table 2 respectively), which means that chitosan had no effect at the beginning of probing activities. However, although chitosan-treated plants and water control showed no significance difference in the 1st and 2nd probing (par. 5 and 6, Table 2 respectively), a numerically decreased probing duration was noted in most of chitosan-treated plants, whereas an increase was displayed in the water control plants.

Another feeding alteration was observed in the inter- and intracellular stylet activities represented by waveform C and Pd, respectively (Table 2, par. 7–11). The increased intercellular probing (C) number (par. 7) and the duration for every single C (par. 9) were due to the significant increase in the number of Pd' (par. 11).

Although the nonspecific probing events (Table 2) showed evident differences due to chitosan treatment, when considering specific tissue-probing activities (Table 3), feeding activities in xylem showed strong evidence of reduced number and duration of xylem feeding in most of the chitosan-treated plants (par. 12 and 13) compared with the water control. However, in the phloem tissue, most of the EPG parameters related to phloem feeding activities showed no remarkable difference between the chitosan-treated plants and water control (par. 14–19, Table 3).

Discussion

The potential use of chitosan as a plant elicitor that induces resistance against plant disease had been observed in numerous reports. However, little is known about whether a similar mechanism occurs in plants challenged by insect pests. Although several studies had proven the potential use of chitosan to control insect pests, most of them acted as insecticide and not as a plant defense elicitor (Badawy et al. 2005; Ferreira et al. 2019; Sahab et al. 2015). Initial experiment by conducting population growth study had confirmed the reduction of total GPA population followed by the reduced number of adult-stage (either alatae or apterous) on chitosan treated plant. Further 10 h EPG monitoring revealed the chitosan application had induced the increase number of GPA probing. The increased probing activities could be due to the need for probing re-adjustment and potentially due to the unfavorable surrounding stylet environment, as responded by the several probing attempts observed through stylet withdrawal. This unfavorable surrounding stylet environment could be a biochemical response of the plant induced by chitosan treatment. However, further investigations are

needed to confirm such assumption. It was noted that the chitosan probably had no effect at the beginning (1st probe) of probing activities, rather in the next probing (2nd probe), thus, there was indication that chitosan treatment induced physiological alterations in the GPA.-affected plants, which responded immediately by reducing the probing duration in the second probing. The performance in early probing is important to indicate the immediate response of insect feeding, given that insect stylet operation is sensitive for any small biochemical changes (Will and Vilcinskis 2015).

The increased intracellular stylet puncture as noted in par.11, Table 2 (which is potential drop -Pd- waveform) was potentially attributed to the induced resistance properties of chitosan, as confirmed by previous findings obtained using citrus psyllid (Shi et al. 2019). Other reports also highlighted that the increased number of probing activities was correlated with the induced resistance of host plant due to silicon treatment, silencing proteins such structural sheath protein, and acoustic stimuli (Goussain et al. 2005; Huang et al. 2018; Lee et al. 2012; Will and Vilcinskis 2015).

Finally we noted the reduced number and duration of xylem feeding in most of the chitosan-treated plants, but no obvious difference in the phloem tissues, here we hypothesized that GPA., once it reached the phloem tissue, finally utilized the tissue in an equal manner as the water control plant, indicating the absence of chitosan effect on the phloem tissues; a similar result was reported regarding the effect of silicon on the plant (Goussain et al. 2005). Thus, although GPA feeding behavior was normal when accessing the phloem tissue, the probing in the stylet pathway to the phloem tissue increased, as reported with silicon and dihydrojasmonone treatments (Goussain et al. 2005; Paprocka et al. 2018), representing the use of extra energy due to the increased number of stylet withdrawal and inter- or intracellular stylet puncture. Given that GPA is a small insect that efficiently uses and conserves energy for many biological functions (Barribeau et al. 2010), the energy consumed caused by increased probing might have affected the population growth, however, further investigation is required to confirm such finding.

Conclusion

Our experiment reported the effect of chitosan treatment on the reduced population of GPA after 14 days. Further investigation on the feeding behavior using EPG showed that chitosan treatment induced stylet withdrawal frequently, which resulted in a high number of probing activities. These events were followed by an increased number of inter- and intracellular puncture (waveform C and Pd, respectively). However, once the stylet reached the phloem tissue, the feeding response was equal between chitosan-treated plants and the

water control. Extra energy utilization due to increased probing activities was one of the possible reasons for the reduced population growth of GPA caused by chitosan, confirming that chitosan potentially acts as a plant defense elicitor. However, further experiments are needed to confirm this result.

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Data Availability All relevant data are within the paper.

Declarations

Conflicts of interest/Competing interests The authors declare that they have no conflicts of interest.

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