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The N-terminal subunit of vitellogenin in planthopper eggs and saliva acts as a reliable elicitor that induces defenses in rice

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Summary

- Vitellogenins (Vgs) are critical for the development and fecundity of insects. As such, these essential proteins can be used by plants to reliably sense the presence of insects.
- We addressed this with a combination of molecular and chemical analyses, genetic transformation, bioactivity tests, and insect performance assays.
- The small N-terminal subunit of Vgs of the planthopper *Nilaparvata lugens* (NlVgN) was found to trigger strong defense responses in rice when it enters the plants during feeding or oviposition by the insect. The defenses induced by NlVgN not only decreased the hatching rate of *N. lugens* eggs, but also induced volatile emissions in plants, which rendered them attractive to a common egg parasitoid. VgN of other planthoppers triggered the same defenses in rice. We further show that VgN deposited during planthopper feeding compared with during oviposition induces a somewhat different response, probably to target the appropriate developmental stage of the insect.
- We also confirm that NlVgN is essential for planthopper growth, development, and fecundity. This study demonstrates that VgN in planthopper eggs and saliva acts as a reliable and unavoidable elicitor of plant defenses. Its importance for insect performance precludes evolutionary adaptions to prevent detection by rice plants.

Introduction

When attacked by herbivores, plants perceive elicitors derived from their attackers via specific receptors and then activate early signaling events, such as the increase in concentrations of cytosolic calcium ion (Ca^{2+}), the activation of mitogen-activated protein kinases (MAPKs) and the production of reactive oxygen species (Erb & Reymond, 2019; Reymond, 2021). These early signaling events lead to the activation of signaling pathways mediated by defense-related phytohormones, which mainly consist of jasmonic acid (JA), salicylic acid (SA), and ethylene (ET). The activated signaling pathways mediate the production of defensive compounds and thus enhance the resistance of plants to herbivores (Schuman & Baldwin, 2016; Erb & Reymond, 2019). On the contrary, herbivores may also secret effectors that suppress plant defenses, thereby enhancing their performance (Erb & Reymond, 2019; Snoeck *et al.*, 2022).

Elicitors are also referred to as herbivore- or egg-associated molecular patterns (HAMPs/EAMP) (Felton & Tumlinson, 2008; Arimura, 2021; Reymond, 2021). Thus far, several elicitors, such as β-glucosidases, fatty acid-amino acid conjugates (FACs), inceptins, caeliferins, bruchins, benzyl cyanide, and indole, have been identified in oral secretions, oviposition fluids and feces of herbivores (Ray *et al.*, 2016; Chen & Mao, 2020). These elicitors are mostly herbivore species-specific and can induce targeted defense responses in plants (Arimura, 2021). So far, elicitors are mainly known for chewing herbivores (Chen & Mao, 2020), but a few have also been identified for piercing-sucking herbivores, such as phosphatidylcholines isolated from female white-backed planthoppers (WBPH, *Sogatella furcifera* (Horváth)), a bacterial chaperonin GroEL from the saliva of potato aphid (*Macrosiphum euphorbiae* (Thomas)), and a mucin-like protein from the saliva of the brown planthopper (BPH, *Nilaparvata lugens* (Stål)) (Chaudhary *et al.*, 2014; Yang *et al.*, 2014; Shangguan *et al.*, 2018). These cases almost exclusively involve elicitors in the insects' oral secretions, but it is known that egg deposition may also activate plant defense responses (Hilker & Fatouros, 2015). Certain insect-derived compounds have been implicated in such oviposition-related responses, but to date, only phosphatidylcholines (Stahl *et al.*, 2020) and an annexin-like protein (Hundacker *et al.*, 2022) have been identified as specific egg-derived elicitors.

Vitellogenins (Vgs) are the major yolk protein precursors that are vital for the egg development in most oviparous vertebrate and invertebrate animals (Tufail & Takeda, 2008). Insect Vgs are mostly synthesized by the fat body in a sex-, stage-, and tissue-specific manner (Raikhel & Dhadialla, 1992). After synthesis in

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the fat body, Vgs are typically cleaved into two subunits, a small N-terminal subunit (<65 kDa; VgN) and a large C-terminal subunit (>150 kDa) at a consensus cleavage site, R/K-X-R/K-R, by subtilisin-like endoproteases (Tufail & Takeda, 2008). Insect Vgs were previously considered as female-adult-specific proteins and only produced in fat body cells. However, recent studies have revealed that Vgs are also found in sexually immature individuals and male adults (Piulachs *et al.*, 2003; Huo *et al.*, 2018). Moreover, *Vg* genes have been found abundantly expressed in hemocytes and ovaries, in addition to fat bodies (Chen *et al.*, 2012; Huo *et al.*, 2018). In some species, albeit at low levels, they are also expressed in salivary glands, midguts, and non-neuronal glial cells (Münch *et al.*, 2015; Shen *et al.*, 2019). Consistent with their distributions in insect bodies, multiple non-nutritional functions have been attributed to Vgs, in addition to their nutritional functions (Amdam *et al.*, 2012; Salmela *et al.*, 2015). Some insect Vgs, for instance, are also involved in the vertical transmission of plant viruses by binding to viral proteins (Wei *et al.*, 2017; Huo *et al.*, 2018). The key role that Vgs play in important physiological processes in insects, as well as their specific chemical features, make them susceptible to recognition by other organisms.

The brown planthopper (Hemiptera: Delphacidae), a monophagous piercing-sucking herbivore, is one of the most important insect pests of rice (*Oryza sativa* L.) in Asia (Dyck & Thomas, 1979). It damages plants by feeding on phloem sap (causing minor tissue damages via its stylet), laying egg clusters in tissues (causing more tissue damages via its ovipositor), but harm is also caused by viruses transmitted by BPH (Sōgawa, 1982; Hattori & Sōgawa, 2002). Newly emerged BPH female adults do not lay eggs until they pass their pre-oviposition periods, which generally takes *c.* 3 d at 25–28°C (Mochida & Okada, 1979). The mature Vg of BPH, NlVg, contains two Vg-N domains at N-terminus, a middle-region domain of unknown function (DUF1943) and a von Willebrand factor type D (vWD) domain at C-terminus (Tufail *et al.*, 2010). The predicted molecular weight of NlVg is 227.94 kDa, and the mature protein is typically cleaved into a small N-terminal subunit (48.33 kDa, NlVgN) and a large C-terminal subunit (179.24 kDa) at the RSRR sequence motif of the N-terminus (Cheng & Hou, 2005; Tufail *et al.*, 2010). In addition to high transcript levels in the fat body of BPH female adults (Tufail *et al.*, 2010), *NlVg* was observed to be abundantly expressed in salivary glands of adult females (Noda *et al.*, 2008; Ji *et al.*, 2013), but also expressed in various other parts of eggs, nymphs, and female and male adults (Wang *et al.*, 2015; Shen *et al.*, 2019). In BPH, there are also two NlVg-like genes, *NlVg-like1* and *NlVg-like2*, both of which are not clustered with the conventional insect *Vg*s, including *NlVg* (Shen *et al.*, 2019). Although each of the three genes influences BPH growth, development, and fecundity, their specific functions appear to differ (Shen *et al.*, 2019).

NlVgN exists not only in BPH hemolymph but also in gelling saliva of fifth-instar nymphs, as well as in their eggs and oviposition fluids (Tufail *et al.*, 2010; Xie, 2012; Huang *et al.*, 2016). During feeding, the saliva enters rice tissues and coagulates to form a salivary sheath around the stylets, whereas during oviposition, fluids are deposited to glue the eggs to the damaged plant tissue. This implies that significant amounts of NlVgN will enter rice tissues when BPH infests plants. It has been reported that defense responses in rice induced by BPH gravid female infestation are distinctly different from those induced by nymphal infestation. For example, infestation by gravid BPH females enhances levels of JA and jasmonoyl-isoleucine (JA-Ile) but decreases ET levels in rice, whereas infestation by BPH nymphs does not change the levels of these phytohormones (Li, 2015; Ji *et al.*, 2017; Ye *et al.*, 2017; Xu *et al.*, 2021). These distinct responses prompted us to hypothesize that NlVgN in BPH saliva and eggs plays a role in regulating the interaction of adult BPH with rice.

In this study, we tested the above hypothesis by exploring the role of *NlVg* (GenBank: AB353856) in BPH-induced defense responses in rice. Combining molecular tools, chemical analyses, and bioassays, we revealed that NlVgN can indeed enter rice plants via the BPH saliva or from the surface of BPH eggs. We show that, together with the damage caused by BPH feeding or oviposition, the small N-terminal subunit of NlVg allows rice plants to specifically recognize planthopper attacks. As this protein is essential for planthopper survival, it is seemingly impossible for the planthoppers to avoid this recognition.

Materials and Methods

Plant growth

Rice genotypes used in this study were Taichun Native 1 (TN1), Xiushui 11 (wild-type), and transgenic lines expressing *NlVgN* (oe-1 and oe-3; details described later) or lines with knocked out *OsJAR1* (JASMONATE RESISTANT 1; the JA pathway in these lines is impaired) (jar1-3 and jar1-4) (Fu, 2021); TN1 and Xiushui 11 are two rice varieties susceptible to BPH. Pregerminated seeds were cultured in plastic bottles (diameter 8 cm; height 10 cm) in a glasshouse (27 ± 1°C, photoperiod, 14 h : 10 h, light : dark). Ten-d-old seedlings were transferred to 20-l hydroponic boxes with a rice nutrient solution (Yoshida *et al.*, 1976), and 30-d-old plants were transferred to individual 500 ml hydroponic plastic pots. Five days later, plants were used for experiments. In all experiments, unless otherwise specified, we used the rice variety TN1.

Insects

A colony of BPH was originally provided by the Chinese National Rice Research Institute (Hangzhou, China) and maintained on TN1 plants in a climate chamber at 27 ± 1°C and 80% relative humidity under a photoperiod, 16 h : 8 h, light : dark. Colonies of WBPH and the small BPH (SBPH, *Laodelphax striatellus* (Fallén)) were originally collected from rice fields in Hangzhou, China, and maintained on TN1 plants in the climate chamber. A colony of *Anagrus nilaparvatae* (Pang et Wang), the egg parasitoid of rice planthopper, was established with individuals collected from rice fields in Hangzhou, China, and maintained on BPH eggs on Xiushui 11 rice plants in a similar climate chamber.

Cloning of *NlVgN* and sequence analysis

The cDNA fragment encoding NlVgN was obtained by reverse transcription (RT)-PCR using a pair of primers listed in Supporting Information Table S1. Sequence analysis was performed on DNAMAN (www.lynnon.com/) and CLUSTAL OMEGA (https://www.ebi.ac.uk/Tools/msa/clustalo/). Details provided in Methods S1.

RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated using the SV Total RNA Isolation System (Promega Corp.) by following the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed with the CFX96™ Real-Time system (Bio-Rad) using the SYBR Premix EX Taq Kit (Takara Bio Inc., Kusatsu, Japan). A relative quantitative method ($2^{-\Delta\Delta C_t}$) described previously (Pfaffl, 2001) was applied to evaluate the variation in expression levels of target genes among samples. Details provided in Methods S1.

Expression of NlVgN in *Escherichia coli*

The cDNA fragment encoding NlVgN was obtained by RT-PCR using the method as stated above. The PCR product was cloned into the pET-28a vector (Novagen Inc., Madison, WI, USA) and sequenced. The recombinant vector *NlVgN*:pET-28a (Fig. S1a) and empty vector pET-28a (as a control) were transformed into *E. coli* BL21 (DE3) strain. The protein fused with His-tag was expressed after induction with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 16°C for 12 h and purified by using Ni-NTA resin columns (Qiagen) according to the manufacturer's instructions. All products purified from recombinant vectors or empty vectors were mixed with 5 × protein loading buffer, separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) in a 12% (w/v) acrylamide gel, followed by staining with 0.025% Coomassie Blue R-250 in water. Because the products purified from the recombinant vector still contain many nontarget bands (Fig. S1b), the collected products were purified again using the same method described above. After purifying twice, we obtained products without significant nontarget bands (Fig. S1c), which were then used for the experiments. The products from twice-purified empty vectors were used as controls. The methods for the preparation of polyclonal antibodies of NlVgN and the western blot analysis are described in Methods S1. The predicted mass of the mature recombinant protein NlVgN containing six N-terminal His-tags is 52.46 kDa.

Plant treatment

Wounding treatment (W) Leaf sheaths of individual plant shoots (4 cm length) were punctured 80 times using a #3 insect pin (diameter 450 µm; Beijing Bao Yuan Industrial Technology Co. Ltd., Beijing, China). Unmanipulated plants were used as controls (Con).

NlVgN and biomaterial extract treatment For NlVgN treatment, plants were individually wounded as stated above, and then were individually treated with 40 µl of the recombinant protein His-NlVgN (26.8 ng µl^{-1}) or the purified products of the empty vector (EV), or kept unmanipulated (Con). For ovary, egg, or eggshell extract treatments, plants were wounded as stated above, and then were individually treated with 40 µl of the extract of either ovaries (Ovary), eggs (Egg) or eggshells (Eggshell), or with PBS (containing 1 mM PMSF), or kept unmanipulated (Con). The method for preparing these extracts is described in Methods S1.

Brown planthopper treatment Individual plant shoots were confined in glass cylinders (diameter 4 cm, height 8 cm, with 48 small holes, and diameter 0.8 mm) and were infested with either (1) 20 newly emerged BPH female adults that were injected with double-stranded RNA (dsRNA) of the N-terminal sequence of *NlVg* (*dsNlVg*) as fifth-instar nymphs (dsNlVg-BPH), (2) 15 newly emerged BPH female adults that were injected with dsRNA of green fluorescent protein (*dsGFP*) as fifth-instar nymphs (dsGFP-BPH), or (3) 15 unmanipulated newly emerged BPH female adults (C-BPH). The method for dsRNA injection (RNA interference (RNAi)) is described in Methods S1. The different numbers of BPH used for plant treatments dsNlVg-BPH, dsGFP-BPH, or C-BPH, were based on observed differences in their food intake in preliminary tests, ensuring that each plant received a similar amount BPH-inflicted damage. To ensure there were no eggs laid on the plants by these BPH females, they were replaced, every 48 h, with a new set of newly emerged females with the same treatment. Plants with empty glass cylinders (Noninfested) and kept unmanipulated (Con) were used as controls. When plants expressing *NlVgN* and wild-type (variety Xiushui 11) plants were used, each cylinder received 10 gravid C-BPH females for BPH treatments.

Generation of transgenic plants

The full-length coding sequence of *NlVgN* was PCR-amplified using a pair of primers listed in Table S1 and was digested by *Kpn*I and *Xba*I; the product was then cloned into the binary vector pCAMBIA1301, yielding an overexpression transformation vector *NlVgN*:pCAMBIA1301, which is driven by the cauliflower mosaic virus 35S promoter (Fig. S1d). The vector was inserted into the Xiushui 11 plants via *Agrobacterium tumefaciens*-mediated transformation. Rice transformation, screening of the transgenic plants, and identification of the number of insertions were performed following the same method as described previously (Zhou *et al.*, 2009). Two *NlVgN*-expressing lines at T_2 generation, oe-1 and oe-3, each with one insertion (Fig. S2), were used for experiments.

Intracellular calcium ion variation determination

Fluo-3 AM (acetoxy-methyl ester of Fluo-3) was used to determine the intracellular calcium ion variation as described previously (Ye *et al.*, 2017). The shoots of TN1 plants were individually confined in the glass cylinders into which 15 newly emerged female adults of dsNlVg-BPH or dsGFP-BPH were released as explained above. Infested parts of leaf sheaths (*c.* 3 cm) were individually harvested 1 and 3 h after infestation and were immediately incubated in 1 ml of 5 μM Fluo-3 AM working solution at 37°C for 30 min. The samples were mounted on a Zeiss LSM 780 confocal laser scanning microscope and were observed at 488 nm excitation wavelength. Images generated by the ZEN 2010 software were analyzed by using the IMAGEJ software (https://imagej.nih.gov/ij). The fluorescence intensity at BPH feeding sites was individually measured at least 16 times.

JA, JA-Ile, ethylene, and H_2O_2 analysis

Jasmonic acid and JA-Ile in rice tissues were extracted following the method described in Lu *et al.* (2015) and analyzed by HPLC–MS/MS. Ethylene accumulation from individual plants was measured by GC using the same method as described previously (Lu *et al.*, 2006). H_2O_2 in rice tissues was extracted and determined following the method in Lou & Baldwin (2006). We also analyzed H_2O_2 by in situ detection with 3,3′-diaminobenzidine (DAB) as described previously (Asano *et al.*, 2012). Details provided in Methods S1.

Volatile collection and isolation

TN1 plants of the same age and size were randomly assigned to NlVgN, EV, and control treatments. Twelve hours after treatment, the volatiles emitted from individual plants were collected (for 8 h), isolated, and identified using the method described previously (Lou *et al.*, 2005). The compounds were expressed as a percentage of peak areas relative to the internal standard (IS, diethyl sebacate) per 8 h of trapping for one plant. Collections were replicated four times for each treatment.

Olfactometer bioassays

Behavioral responses of newly emerged *A. nilaparvatae* females (0–24 h post ecdysis, experienced) to rice volatiles were performed in a Y-tube olfactometer using the same method as described previously (Lou *et al.*, 2005). The attraction of the parasitoid females exposed to the following pair of odor sources was recorded: TN1 plants treated with wounding plus purified recombination protein NlVgN for 12 h vs TN1 plants treated with wounding plus purified elution products of the empty vector for 12 h. For each treatment, eight plants were used, and the odor sources were replaced by a new set of 8 plants after testing 20 wasps. In total, six sets of plants and 120 female parasitoids were used.

BPH feeding and performance assays

We also investigated the effect of the knockdown of *NlVgN* in BPH on the feeding, fecundity, growth, and survival of BPH, as well as the effect of treating plants with the recombinant protein NlVgN on the survival of BPH and the hatching rate of BPH eggs. In addition, we studied the effects of plants expressing *NlVgN* or *OsJAR1*-knocked out plants that were treated with the recombinant protein NlVgN on the hatching rate of BPH eggs (details described in Methods S1).

Data analysis

Two-treatment data were analyzed using Student's *t*-tests or chi-squared test (olfactometer bioassays). Data from three or more treatment groups were compared using one-way ANOVA; if the ANOVA was significant ($P < 0.05$), Tukey's honest significant difference (HSD) *post hoc* test was used to detect significant differences between treatments. When necessary, data were log-transformed to meet requirements for the homogeneity of variance. All statistical analyses were performed using IBM SPSS Statistics 20.

Results

NlVgN enters rice tissues during BPH feeding and oviposition

Brown planthopper causes damage to rice plants by sucking phloem sap or laying its eggs in rice leaf sheaths (Fig. 1a–d). As stated above, NlVgN is present in BPH-gelling saliva and eggs. We therefore examined whether NlVgN enters rice tissues during BPH feeding as well as oviposition. Western blot analysis with anti-NlVgN showed a band of *c.* 50 kDa in total proteins from rice leaf sheaths that had been infested by newly emerged BPH females (feeding only) for 24 h, whereas no NlVgN band was detected in proteins from noninfested plants (Fig. 1e), implying that NlVg indeed enters rice tissues when BPH feeds on plants. Additionally, a NlVgN band was detected in the PBS buffer solution used to extract pieces of rice leaf sheaths on which BPH females had oviposited but from which eggs had been carefully removed. The band was also found in similar extracts from intact BPH eggs, whereas no band was detected in PBS solutions used to extract pieces of rice leaf sheaths infested with newly emerged BPH females (no eggs) or uninfested leaf sheaths (Fig. 1f). These results confirm that NlVgN is also present on the surface of BPH eggs and/or in oviposition fluids, and it can come in contact with damaged rice tissues during oviposition. Taken together, these results suggest that NlVgN reaches rice plants during BPH feeding and oviposition.

NlVgN induces the production of cytosolic Ca^{2+} and H_2O_2 in rice

Infestation by both BPH nymphs and gravid females induces increases in the concentration of cytosolic Ca^{2+} and H_2O_2 in rice plants (Zhou *et al.*, 2009; Ye *et al.*, 2017). To explore the role of NlVgN plays in activating these two pathways, we silenced the *NlVg* gene in BPH using RNAi as described previously (Liu *et al.*, 2010) and then investigated the effect of *NlVg* silencing on the levels of cytosolic Ca^{2+} and H_2O_2 in rice. Injecting fifth-instar nymphs with *dsNlVg* reduced the transcript level of *NlVg* in BPH female adults by 97.90, 90.64, and 73.06%, respectively, at 1, 3, and 5 d after emergence (2–6 d post-injection), compared with the levels in BPH injected with *dsGFP* (Fig. S3a; Table S2). The protein level of NlVgN also decreased drastically in BPH injected with *dsNlVg* (dsNlVg-BPH) (Fig. S3b). This dsRNA

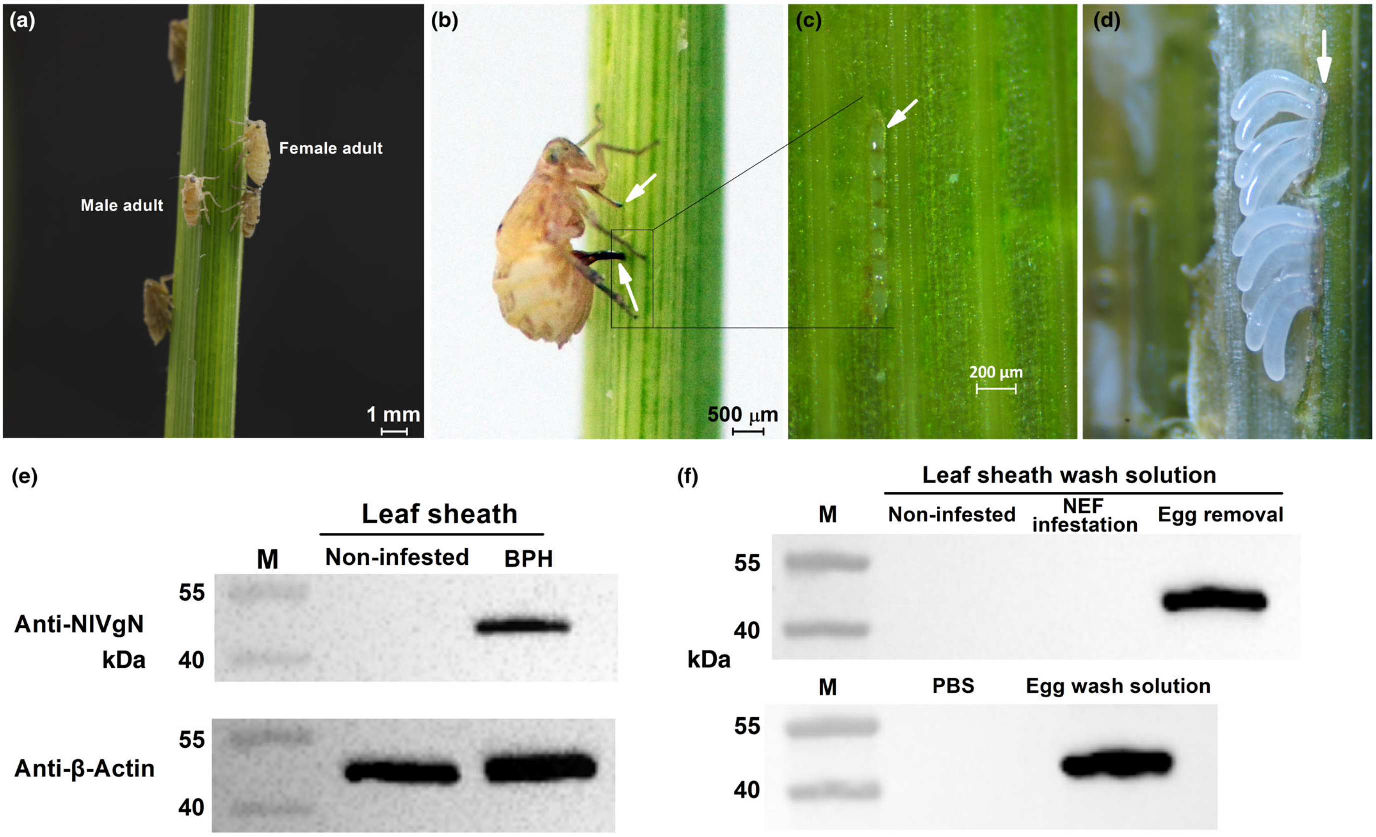

Fig. 1 NlVgN enters rice tissues during brown planthopper (BPH) feeding and egg deposition. (a) A rice plant exposed to BPH infestation, showing male and female adults. (b) A gravid BPH female feeding (see upper arrow) and ovipositing (see lower arrow) on a rice plant. (c) BPH eggs in the rice leaf sheath with the arrow pointing out the egg caps. (d) BPH eggs are visible after the leaf sheath was removed. The upper approximately transparent part of the egg, indicated by the arrow, is the egg cap. (e) Western blot showing that NlVgN is present in rice plants that were infested for 24 h by 50 newly emerged BPH female adults, but not in noninfested plants. (f) Western blot showing that NlVgN is present in the PBS buffer in which pieces of leaf sheaths that were infested by BPH gravid females but BPH eggs were removed (Egg removal) and intact BPH eggs (Egg wash solution) were immersed for 8–10 min and 2–3 min, respectively, but not in the buffer in which pieces of leaf sheaths that were kept noninfested or infested by BPH Newly Emerged Females (NEF infested) were immersed for 8–10 min or in the buffer alone. M, molecular weight markers (kDa).

injection did not cosilence *NlVg-like1* and *NlVg-like 2* (Fig. S3c, d; Table S3), showing that the RNAi is specific. Compared with the levels in plants infested by newly emerged dsGFP-BPHs adult females (feeding only), the level of NlVgN in plants infested by newly emerged dsNlVg-BPHs adult female was also considerably lower (Fig. 2a). Cytosolic Ca^{2+} and H_2O_2 analysis revealed that feeding by dsNlVg-BPHs, compared with feeding by dsGFP-BPHs and C-BPHs, induced a weaker fluorescence intensity around feeding sites at 3 h (Fig. 2b,c; Table S4) and lower levels of H_2O_2 at 3 and 8 h after BPH infestation (Fig. 2d; Table S5). Moreover, application of recombination protein NlVgN increased the H_2O_2 level in rice plants 30 min after treatment (Fig. 2 e,f; Table S5), whereas expressing *NlVgN* in rice that had no obvious effect on the plant growth phenotype (Fig. S2), enhanced constitutive and BPH-induced (especially 8 h after infestation) levels of H_2O_2 in plants (Fig. 2g; Table S5). These findings demonstrate that NlVgN, either secreted by BPH salivary glands or on the surface of BPH eggs, contributes to BPH-induced increases of cytosolic Ca^{2+} and production of H_2O_2 in rice.

Silencing *NlVg* does not affect the production of JA and JA-Ile in rice fed on by BPH but exogenous NlVgN or expressing *NlVgN* in rice does

Jasmonic acid- and ET-mediated signaling pathways play a central role in regulating the resistance of rice to BPH (Zhou *et al.*, 2009; Lu *et al.*, 2014; Xu *et al.*, 2021). Hence, we wondered whether NlVgN plays a role in modulating the biosynthesis of these phytohormones in rice. Consistent with previous results (Ji *et al.*, 2017), BPH feeding did not induce the production of JA and JA-Ile, nor did the knockdown of *NlVg* affect JA and JA-Ile levels after BPH feeding. In each case, hormone production was very low (Fig. S4a,b; Table S2). Although applying EV to fresh wounds resulted in higher JA and JA-Ile levels than in unmanipulated plants, the application of purified recombination protein NlVgN to fresh wounds induced still higher levels of JA (Fig. 3a; Table S5) and JA-Ile (Fig. 3b; Table S5) than in plants with applying EV to fresh wounds. Consistent with these results, applying either the homogenized fresh BPH egg extract in a phosphate buffer (pH 7.4), the homogenized BPH eggshell

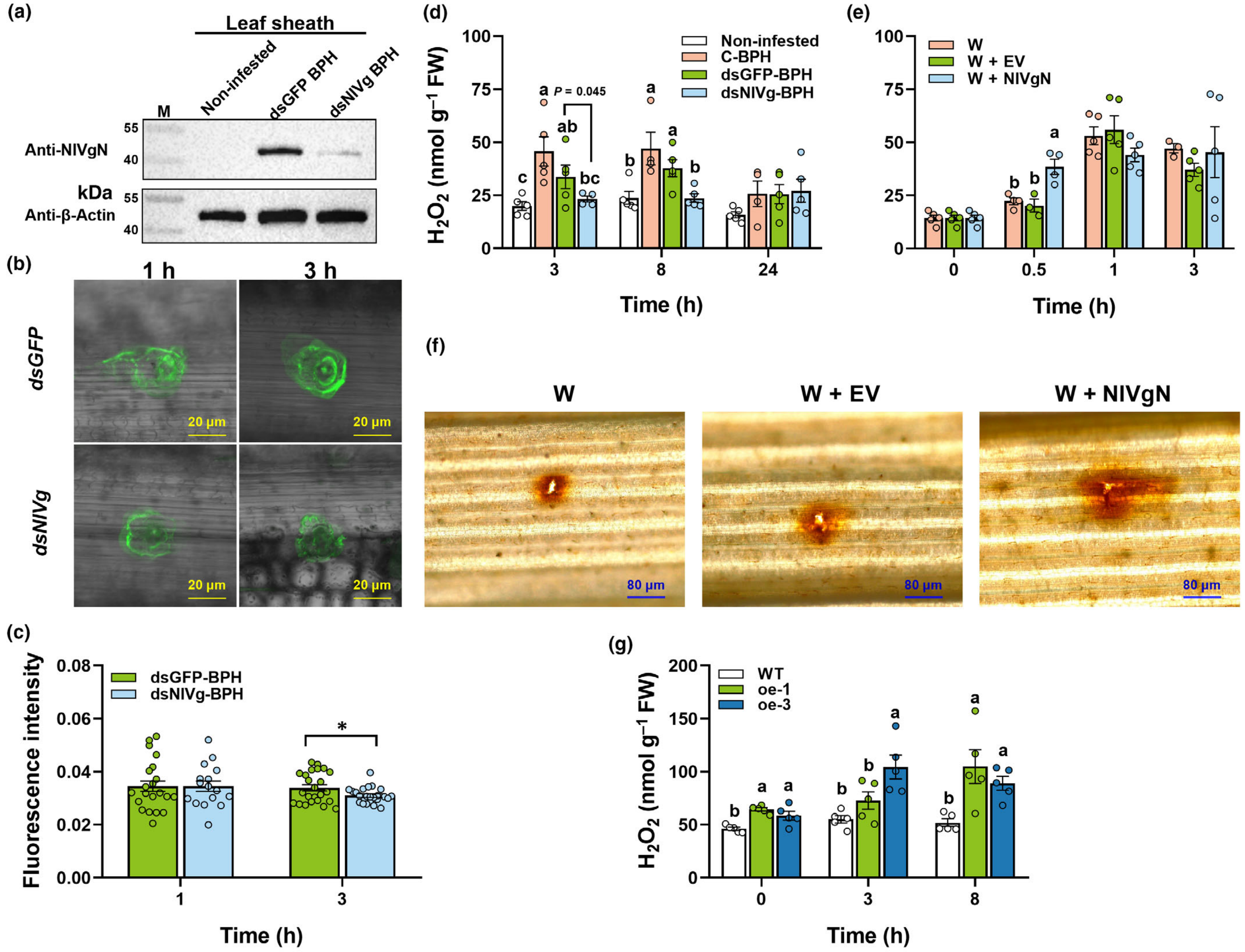

Fig. 2 NlVgN enhances brown planthopper (BPH) feeding-induced concentrations of cytosolic Ca^{2+} and H_2O_2 in rice. (a) Detection of protein fraction NlVgN in rice infested for 24 h by 50 newly emerged BPH female adults (12 to 24 h after emergence) that had been injected with dsRNA of *GFP* (*dsGFP*) or *NlVg* (*dsNlVg*) at fifth-instar nymph stage, or kept noninfested. M indicates molecular weight markers (kDa). (b, c) Confocal microscopic images showing green fluorescence of Fluo-3 AM binding with intracellular Ca^{2+} at BPH feeding sites of rice leaf sheaths (b) and mean fluorescence intensity (+SE, n = 16–24) at feeding sites (c) that were infested for 1 and 3 h by newly emerged female adults that had been injected with *dsGFP* or *dsNlVg* at fifth-instar nymph stage. Asterisk indicates significant difference between treatments: *, $P < 0.05$ (Student's t-test). (d) Mean levels (+SE, n = 4–5) of H_2O_2 in leaf sheaths of TN1 plants that were kept noninfested (Non-infested) or infested for 3, 8, and 24 h by newly emerged BPH female adults that had been injected with *dsGFP*, *dsNlVg*, or kept noninjected (C-BPH) at fifth-instar nymph stage. (e) Mean levels (+SE, n = 3–5) of H_2O_2 in leaf sheaths of TN1 plants that were kept unmanipulated (0 h) or treated for 0.5, 1, and 3 h with the purified recombinant protein NlVgN (W + NlVgN), the purified products of the empty vector (EV) (W + EV) or nothing to fresh wounds. (f) *In situ* detected H_2O_2 accumulation in rice leaves by 3,3′-diaminobenzidine (DAB) staining. Plant leaves were treated for 30 min with wounding (W), W + EV, or W + NlVgN. (g) Mean levels (+SE, n = 5) of H_2O_2 in leaf sheaths of XS11 plants expressing *NlVgN* (line oe-1 and oe-3) and wild-type (WT) plants that were kept noninfested or infested with 10 gravid BPH female adults for 3, 8, and 24 h. Letters indicate significant differences among different treatments ($P < 0.05$, Tukey's HSD *post hoc* test).

extract in the buffer, or the homogenized dsGFP-BPH ovary extract in the buffer to fresh wounds, all significantly induced the biosynthesis of JA and JA-Ile in plants, as compared to applying the buffer alone to fresh wounds (Fig. 3c–f; Table S5). Importantly, applying the homogenized dsNlVg-BPH ovary extract in the buffer (low levels of NlVgN) to fresh wounds exhibited impaired induction of JA and JA-Ile (Fig. 3e,f). Additionally, plants expressing *NlVgN* showed high constitutive and BPH-induced (infestation for 24 h) levels of JA and JA-Ile (Fig. 3g,h; Table S5). Treatments with the application of recombination protein NlVgN to fresh wounds did not induce the biosynthesis of ET (Fig. S4c; Table S2). Taken together, these findings indicate that NlVgN-induced production of JA and JA-Ile in rice is dependent on damage level or type of the tissue that NlVgN comes in contact with and/or on the effectors and other elicitors derived from BPH feeding or oviposition. These data further show that NlVgN does not affect the biosynthesis of ET in rice.

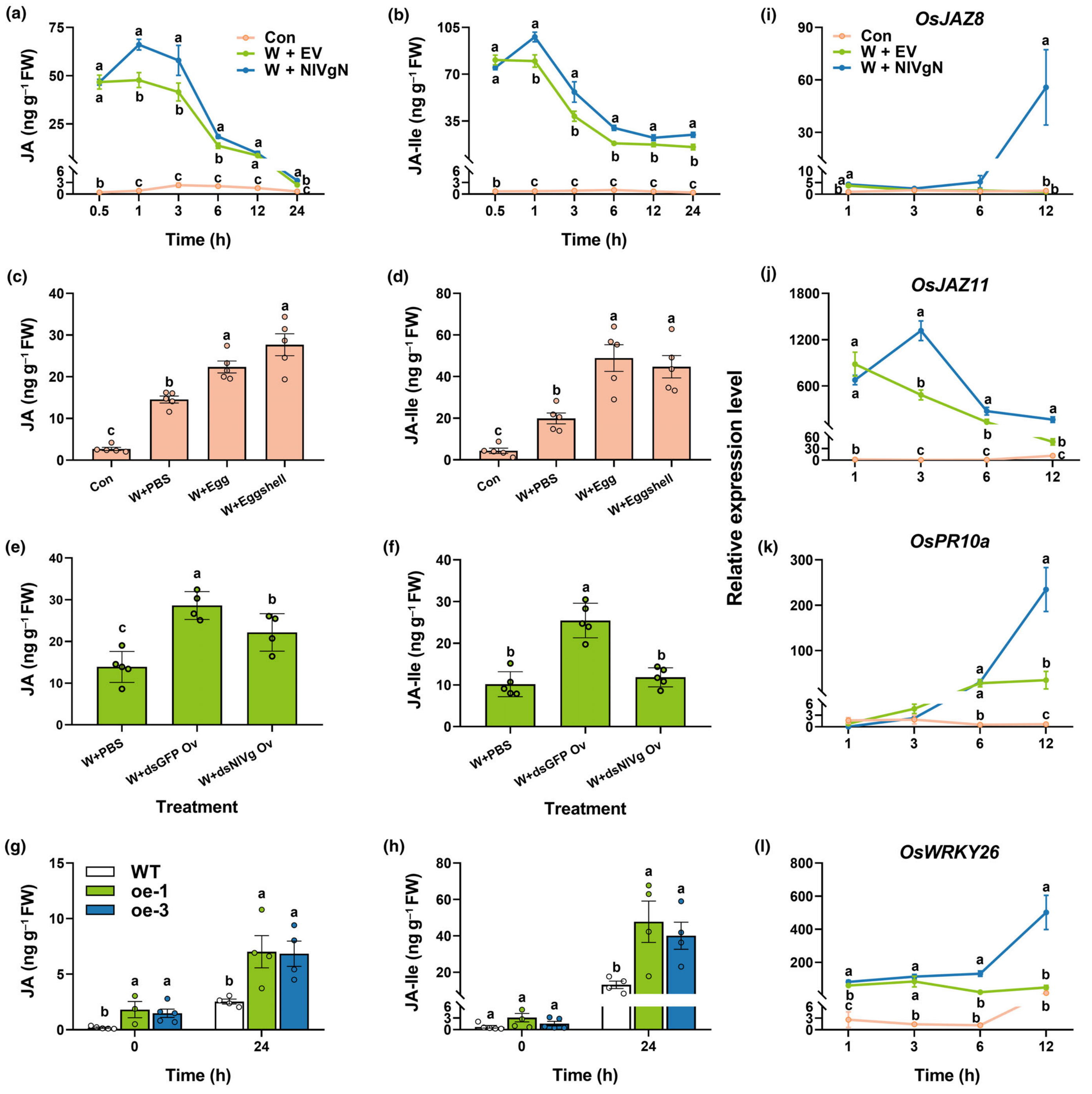

Fig. 3 NlVgN elicits the production of jasmonic acid (JA) and jasmonoyl-isoleucine (JA-Ile) and the expression of defense-related genes in rice. (a, b) Mean levels (+SE, $n = 4$–5) of JA (a) and JA-Ile (b) in rice leaf sheaths that were kept unmanipulated (Con) or treated for 0.5, 1, 3, 6, 12, and 24 h with the purified products of the empty vector (EV) (W + EV) or the purified recombinant protein NlVgN (W + NlVgN) to fresh wounds. (c, d) Mean levels (+SE, $n = 5$) of JA (c) and JA-Ile (d) in rice leaf sheaths that were kept unmanipulated (Con) or treated for 3 h with the phosphate-buffered saline (PBS) (W + PBS) or the extract of homogenized BPH eggs (W + Egg) or eggshells (W + Eggshell) in the buffer to fresh wounds. (e, f) Mean levels (+SE, $n = 4$–5) of JA (e) and JA-Ile (f) in rice leaf sheaths that were treated for 3 h with the PBS or the solution of homogenized ovaries of BPH female adults (4 d after emergence) that were injected with dsRNA of *GFP* (*dsGFP*) (W + dsGFP Ov) or *NlVg* (*dsNlVg*) (W + dsNlVg Ov) at fifth-instar nymph stage to fresh wounds. (g, h) Mean levels (+SE, $n = 3$–5) of JA (g) and JA-Ile (h) in XS11 plants and plants expressing *NlVgN* (line oe-1 and oe-3) that were kept noninfested (0 h) or infested with 10 gravid BPH female adults for 24 h. (i–l) Mean transcript levels (+SE, $n = 3$–5) of *OsJAZ8* (i), *OsJAZ11* (j), *OsPR10a* (k), and *OsWRKY26* (l), in rice leaf sheaths that were kept unmanipulated (Con) or treated for 1, 3, 6, and 12 h with W + EV or W + NlVgN. Letters indicate significant differences among different treatments ($P < 0.05$, Tukey's HSD *post hoc* test).

NlVgN induces the expression of defense-related genes and defense response of rice to BPH infestation

Because NlVgN triggers the production of JA, JA-Ile, and H_2O_2 in rice (Figs 2, 3), we hypothesized that treating plants with recombinant protein NlVgN will not only alter the expression of defense-related genes, but also affect the performance of BPH and the behavioral response of *A. nilaparvatae*, an egg parasitoid of rice planthoppers. As predicted, the transcript level of three JA-responsive genes, *OsJAZ8* (Yamada *et al.*, 2012; Xu *et al.*, 2021), *OsJAZ11* (Xu *et al.*, 2021), and *OsPR10a* (Ersong *et al.*, 2021), and one defense-related gene, *OsWRKY26* (Li *et al.*, 2021), in rice, were up-regulated after NlVgN treatment (Fig. 3i–l; Table S5). The survival and the mass of BPH nymphs and newly emerged BPH female adults fed on rice plants that were treated by applying recombinant protein NlVgN to fresh wounds were similar to those fed on plants that were treated by applying purified elution products of the empty vector to fresh wounds (Fig. S4d–f; Table S2). By contrast, the hatching rate of BPH eggs and the number of eggs laid by 15 gravid BPH females (for 24 h) were significantly lower on plants that were twice treated with NlVgN, as compared to EV-treated plants. One-time treatment with NlVgN did not influence the hatching rate of BPH eggs and the number of eggs laid by 15 gravid BPH females (Fig. 4a,b; Table S5). When the JA pathway was impaired in rice, the effect of two-time treatment with NlVgN on the hatching rate of BPH eggs and the number of eggs laid by gravid BPH females disappeared (Fig. 4c,d; Table S4). Expressing *NlVgN* in plants also reduced the hatching rate of BPH eggs (Fig. 4e,f; Table S4).

Furthermore, volatiles emitted from plants treated with purified recombinant protein NlVgN to fresh wounds were more attractive to female *A. nilaparvatae* wasps than volatiles from plants treated with EV to fresh wounds ($\chi^2 = 10.509$, $P = 0.001$, Fig. 5a). Volatile collections and analyses revealed that the total amount of volatiles emitted from plants treated with purified recombinant protein NlVgN to fresh wounds was significantly higher than the total amount of volatiles from plants treated with EV to fresh wounds or from unmanipulated plants. Compared with nonmanipulated control plants, applying EV to fresh wounds enhanced levels of four volatile compounds, 2-heptanone, 2-heptanol, α-thujene, and linalool, whereas 11

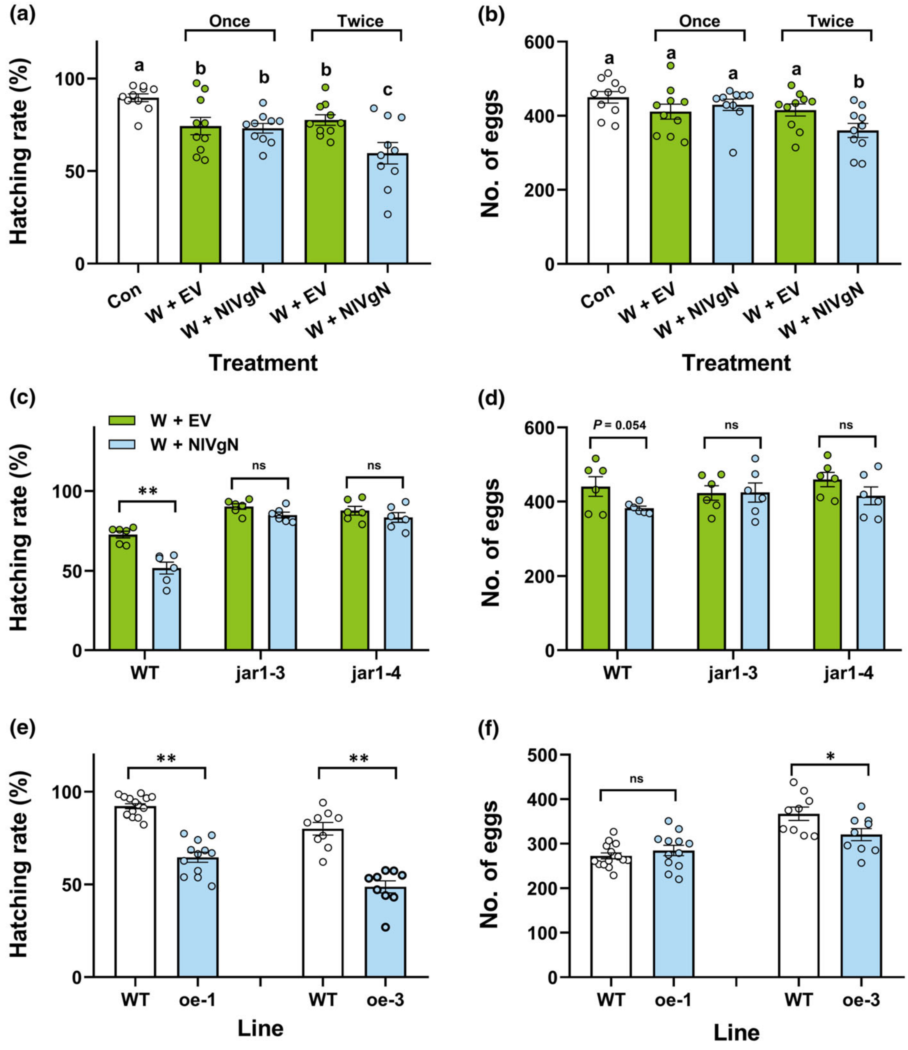

Fig. 4 NlVgN induces direct defenses of rice against brown planthopper (BPH). (a, b) Mean hatching rate (+SE, *n* = 9–15) of BPH eggs (a) and mean number (+SE, *n* = 9–15) of eggs laid by gravid BPH females for 24 h (b) on plants that were kept unmanipulated (Con), treated with the purified products of the empty vector (EV) (W + EV) or the purified recombinant protein NlVgN (W + NlVgN) to fresh wounds. Once and twice indicate that plants were treated with W + EV or W + NlVgN one time and two times, respectively. Letters indicate significant differences among treatments ($P < 0.05$, Tukey's HSD *post hoc* test). (c, d) Mean hatching rate (+SE, *n* = 6) of BPH eggs (c) and mean number (+SE, *n* = 6) of eggs laid by gravid BPH females for 24 h (d) on WT plants and plants with knocked out *OsJAR1* (jar1-3 and jar1-4) that were treated with W + EV or W + NlVgN two times. NS, not significant. (e, f) Mean hatching rate (+SE, *n* = 6) of BPH eggs (e) and mean number (+SE, *n* = 6) of eggs laid by gravid BPH females for 24 h (f) on plants that expressed with *NlVgN* (line oe-1 and oe-3) or not (wild-type, WT). The experiments on the hatching rate of BPH eggs on WT plants vs one of the two transgenic lines, oe-1 and oe-3, were performed separately. Asterisk indicates significant differences between different treatments (*, $P < 0.05$; **, $P < 0.01$; Student's *t*-test). ns, not significant.

volatile compounds, α-pinene, sesquithujene, α-cedrene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, sesquisabinene A, (E)-β-farnesene, α-curcumene, zingiberene, and two as yet unknown compounds, were released in higher amounts from recombinant protein NlVgN-treated plants compared with plants treated with EV to fresh wounds (Fig. 5b; Table S6). Taken together, the data imply that NlVgN induces the expression of defense-related genes and enhances the direct and indirect defense responses to BPH infestation.

Silencing *NlVg* impairs BPH feeding, survival, and fecundity

Consistent with results reported in Shen *et al.* (2019), knockdown of *NlVg* significantly increased the body size and mass of BPH female adults (Fig. S5a–c; Table S3), but resulted in oocyte malformations and drastically reduced the number of mature eggs in the ovaries (Fig. S5d,e), as well as the number of eggs laid by female adults (Fig. S5f; Table S2). Knockdown of *NlVg* also decreased the amounts of honeydew secreted by newly emerged female adults of BPH compared with those secreted by newly emerged females of BPH injected with *dsGFP* (dsGFP-BPH) and BPH females that were not injected (C-BPH) (Fig. 6a; Table S7). Moreover, compared with dsGFP-BPH and C-BPH, dsNlVg-BPH showed lower survival rates on rice plants and artificial diet 6–10 d and 5–10 d, respectively, post-injection (Fig. 6b,c; Table S7). The data confirm that NlVg plays an important role in the feeding, development, survival, and especially fecundity of BPH.

VgNs in other rice planthoppers also function as elicitors

Rice plants suffer from attacks by several planthopper species. The main ones are BPH, WBPH, and SBPH. We wondered whether VgNs in the other two rice planthoppers also induce defense responses in rice. We therefore investigated the change in levels of JA and JA-Ile in rice plants when they were treated with egg or ovary extracts of WBPH or SBPH. Similar to results found for BPH (Fig. 3c–f), applying the homogenized fresh egg or ovary extract of WBPH or SBPH to fresh wounds resulted in higher levels of JA and JA-Ile than applying the buffer to fresh wounds (Fig. 7a–d; Table S7). When *Vg* was knocked down in WBPH (Fig. S6a; Table S3) or SBPH (Fig. S6b; Table S3), the same treatments with WBPH or SBPH ovary extract did not or only weakly induced the production of JA-Ile (Fig. 7c,d). Taken together, these findings show that VgN from WBPH and SBPH also functions as an elicitor that induces defense responses in rice.

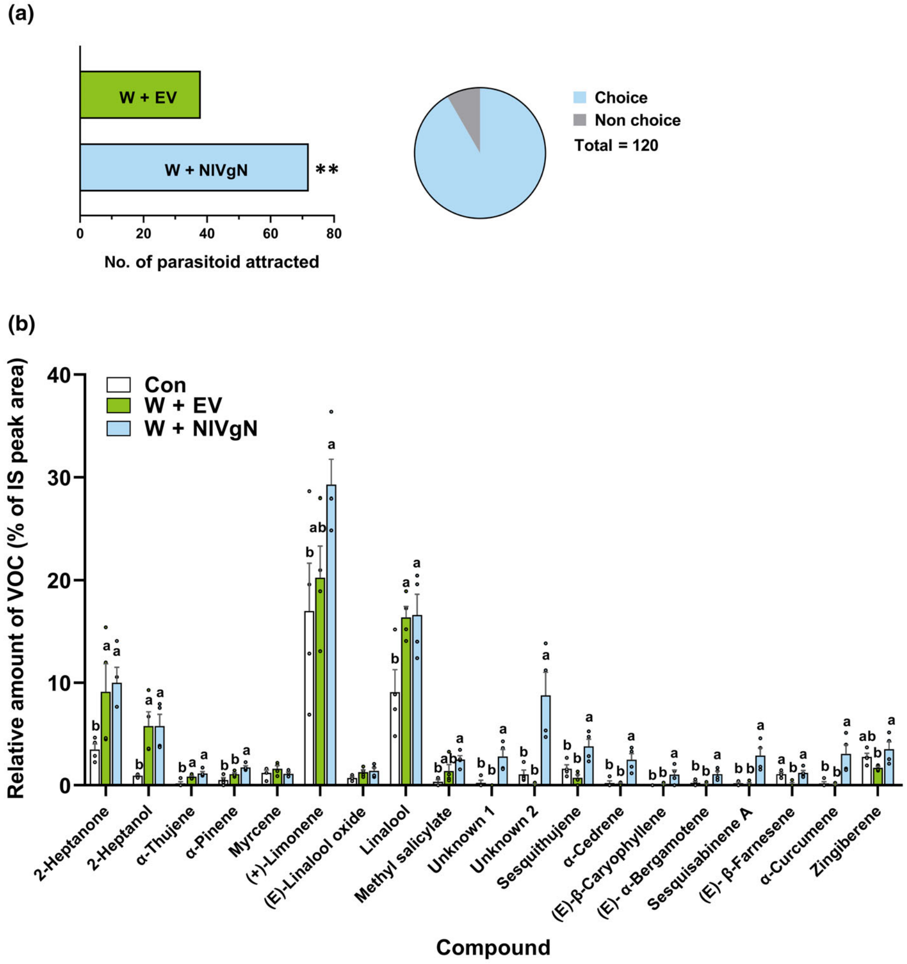

Fig. 5 NlVgN induces indirect defenses of rice against brown planthopper (BPH). (a) Number of newly emerged *Anagrus nilaparvatae* female adults attracted by volatiles emitted from rice plants treated with the purified products of the empty vector (W + EV) or the purified recombinant protein NlVgN (W + NlVgN) to fresh wounds. Pie chart indicates the number of *A. nilaparvatae* female adults that make a choice or not within 5 min. Asterisk indicates significant differences between different treatments: **, $P < 0.01$ (χ^2 test). (b) Mean amount (% of IS peak area, +SE, $n = 4$) of volatiles emitted from rice plants that were kept unmanipulated (Con) or treated with W + EV or W + NlVgN. Letters indicate significant differences among different treatments ($P < 0.05$, Tukey's HSD *post hoc* test).

Discussion

The evolutionary arms race between plants and herbivorous insects has resulted in numerous clever defense traits in plants, and equally ingenious counter adaptations in specialized insects (Farmer, 2014). Plant defenses against insects are often inducible (Karban & Baldwin, 1997), and in order for the plants to launch the most appropriate defense they need to recognize their specific attackers. This is possible thanks to insect specific elicitors. In turn, the insect herbivores are under strong selective pressure to avoid excreting such indicative elicitors. This implies that only compounds that insects cannot avoid producing can serve as reliable elicitors. This is indeed the case for the two most studied types of HAMPs, fatty acid-amino acid conjugates like volicitin (Yoshinaga *et al.*, 2010; Hettenhausen *et al.*, 2014) and inceptins, which are peptide fragments from chloroplastic ATP synthase γ-subunit proteins (Schmelz *et al.*, 2006). Both types of elicitors are formed in caterpillar buccal cavities during feeding and cannot be avoided unless the insects adapt their diet (De Moraes & Mescher, 2004) or change digestive enzyme activity (Schmelz *et al.*, 2012), respectively. Recently, the first plant receptor to allow this specific recognition of HAMPs was identified (Steinbrenner *et al.*, 2020).

Here, we identified a new type of elicitor, the small N-terminal subunit of Vg protein from the BPH, NlVgN. It is uniquely different from other elicitors in that it is introduced into the plants, not only via the saliva, but also, in large quantities, during oviposition. Based on our results, we propose that the difference in responses to gravid BPH females and BPH nymphs is due to the difference in the source (quantities) of NlVgN, in addition to the difference in damage inflicted by nymphs and gravid females. NlVgN enters the plants at feeding sites via BPH saliva, as well as oviposition sites via the eggs. Its role in for defense induction was confirmed by the observation that knockdown of *NlVg* in the insect significantly decreased BPH feeding-induced levels of cytosolic Ca^{2+} and H_2O_2 but did not affect JA and JA-Ile levels. Moreover, wounding plus the application of extracts containing NlVgN, induced the biosynthesis of JA and JA-Ile in plants, whereas knocking down *NlVg* reduced the levels of JA and JA-Ile induced by the ovary extract. Importantly, exogenous application of the recombinant NlVgN or expressing *NlVgN* in the rice plants themselves was sufficient to elicit the production of H_2O_2, JA, and JA-Ile. This implies that NlVgN from saliva induces the production of cytosolic Ca^{2+} and H_2O_2, whereas NlVgN from eggs induces the production of cytosolic Ca^{2+}, H_2O_2, as well as JA and JA-Ile. This appears to be the first report on the role of Vg in inducing plant defense responses, but, intriguingly, vitellin produced by the cattle tick *Boophilus microplus* has been previously found to act as an elicitor of immune responses in sheep (Tellam *et al.*, 2002).

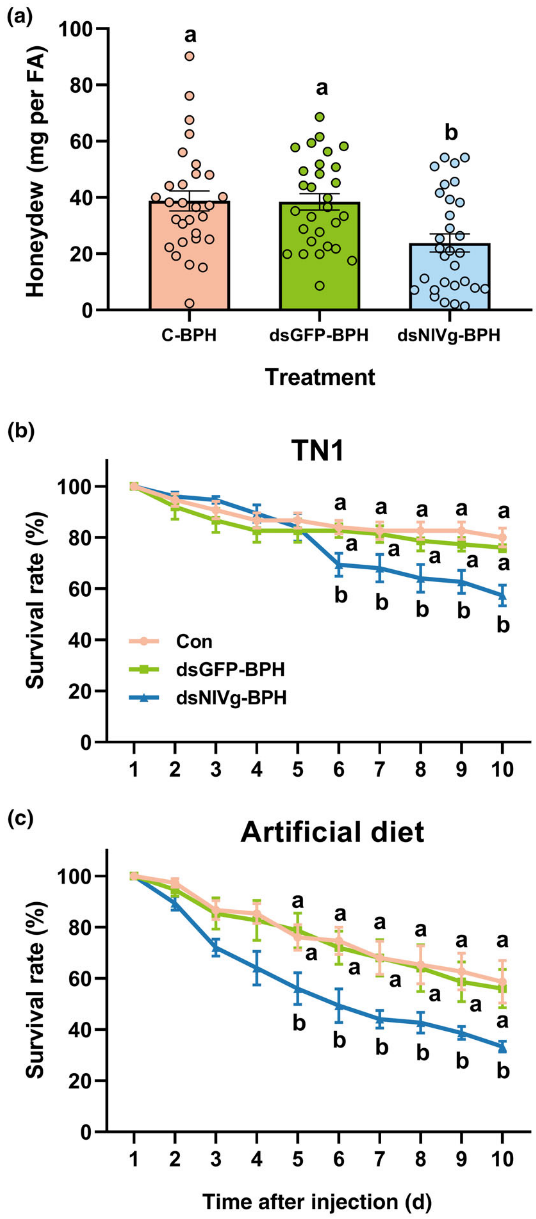

Fig. 6 Knockdown of *NlVg* impairs the feeding capacity and survival of brown planthopper (BPH) female adults. (a) Mean amount of honeydew per day (+SE, $n = 30$) secreted by a newly emerged BPH female adult (FA, 12–24 h after emergence) that was injected with dsRNA of *GFP* (*dsGFP*) or *NlVg* (*dsNlVg*), or kept noninjected (C-BPH) at fifth-instar nymph stage. (b, c) Mean survival rates (+SE, $n = 5$) of newly emerged BPH female adults that were injected with *dsGFP*, *dsNlVg* or kept noninjected (C-BPH) at fifth-instar nymph stage, 1–10 d (2–11 d post-injection) after they fed on rice variety TN1 (b) or artificial diet (c). Letters indicate significant differences among treatments ($P < 0.05$, Tukey's HSD *post hoc* test).

Interestingly, it was recently reported that the C-terminus of Vg, VgC (111 kDa), in SBPH, when secreted into rice plants, serves as an effector (Ji *et al.*, 2021). This was concluded from the fact that *Vg*-silenced SBPH nymphs consistently elicited higher H_2O_2 production, whereas expression of the domains in VgC in rice protoplasts or of VgC in *Nicotiana benthamiana* leaves significantly hindered the accumulation of chitason-induced H_2O_2. Given the fact that silencing *NlVg* decreased the level of NlVgC and that feeding by BPHs with silenced *NlVg* induced lower H_2O_2 levels in rice than corresponding control BPHs, VgC in BPH is unlikely to function as an effector, different from SBPH. Indeed, there is a key difference in the Vg cleavage patterns between the two planthoppers; in BPH, mature Vg is cleaved into a small N-terminal subunit (48.33 kDa, NlVgN) and a large

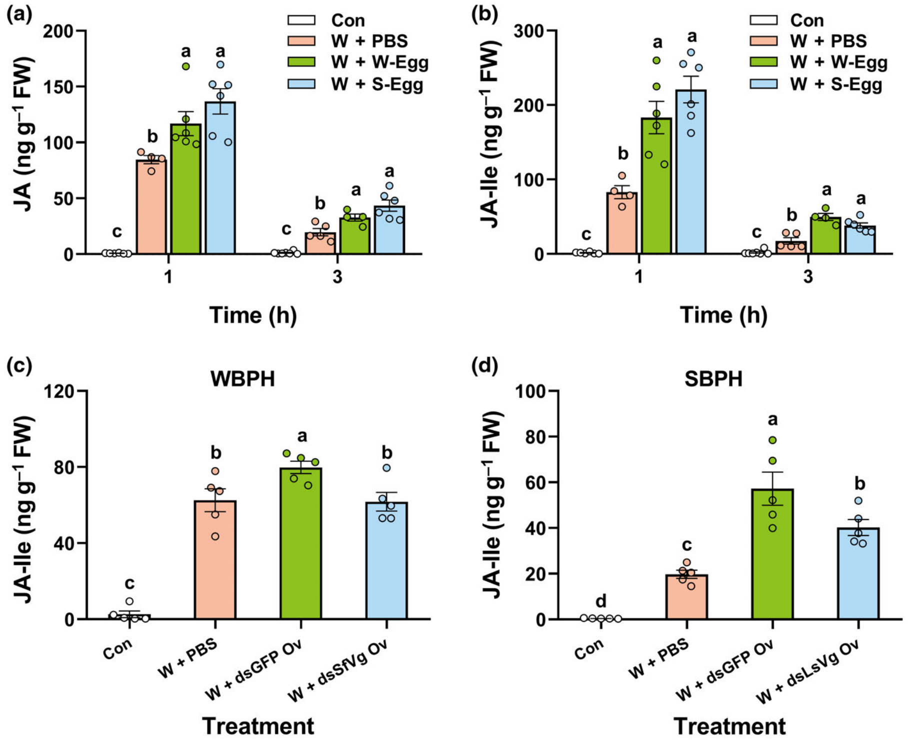

Fig. 7 VgN in white-backed planthoppers (WBPH) and small brown planthopper (SBPH) also elicits the production of jasmonic acid (JA) and jasmonoyl-isoleucine (JA-Ile). (a, b) Mean levels (+SE, $n = 4$–7) of JA (a) and JA-Ile (b) in rice leaf sheaths that were kept unmanipulated (Con) or treated with the phosphate-buffered saline (PBS) (W + PBS) or the solution of the homogenized WBPH eggs (W + W-Egg) or SBPH eggs (W + S-Egg) (for 1 and 3 h) to fresh wounds. (c, d) Mean levels (+SE, $n = 5$) of JA-Ile in rice leaf sheaths that were kept unmanipulated (Con) or treated for 1 h with W + PBS or the solution of homogenized ovaries of WBPH (c) or SBPH (d) female adults (4 d after emergence) that were injected with dsRNA of *GFP* (dsGFP) (W + dsGFP Ov), *SfVg* (dsSfVg) (W + dsSfVg Ov), or *LsVg* (dsLsVg) (W + dsLsVg Ov), respectively, at fifth-instar nymph stage. Letters indicate significant differences among different treatments ($P < 0.05$, Tukey's HSD *post hoc* test).

C-terminal subunit (179.24 kDa, NlVgC) (Tufail *et al.*, 2010), whereas in SBPH, mature Vg is first cleaved into LsVgN (42 kDa) and a large subunit (178 kDa) and then the latter is further cleaved into a 67 kDa subunit and a large C-terminal subunit (111 kDa, LsVgC) (Huo *et al.*, 2018). It would be interesting to determine whether the difference in size (179.24 kDa vs 111 kDa) and amino acid sequence (they share 77.4% similarity, Fig. S7) between NlVgC and LsVgC explains the difference in their functions. Indeed, changes in amino acid sequence can affect the activity of effectors in insects and pathogens (Rao *et al.*, 2019; Yuan *et al.*, 2021; Shih *et al.*, 2023). It should also be noted that silencing *Vg* (decreasing levels of both VgC and VgN) significantly decreased the induction of JA-Ile production by SBPH and WBPH ovary extracts when applied to mechanically wounded plants (Fig. 7c,d). Our results suggest that the function of VgC as an effector in SBPH and possibly in WBPH has no or only a minor effect on the role of VgN as an elicitor.

The reason why NlVgN from different sources differently affected the production of JA and JA-Ile might be related to the type and extent of damage caused and the compounds that enter into plants during BPH feeding vs oviposition. Brown planthopper is a piercing-sucking herbivore whose feeding only causes minor tissue damage and only little NlVgN will enter via the stylet sheaths. By contrast, during oviposition BPH causes considerably more damage to tissues as it makes cuts with its ovipositor to lay egg clusters inside the tissues (Fig. 1b–d). As a consequence, NlVgN from the egg surface comes in direct contact with damaged tissues and thereby induces considerably stronger responses in rice plants than the small quantities of NlVgN deposited during feeding. Moreover, the fluids deposited by BPH also contain effectors and other elicitors, which are probably different between saliva and eggs/oviposition fluids. To date, one elicitor, a mucin-like protein (Shangguan *et al.*, 2018), and several effectors, such as an endo-β-1,4-glucanase (Ji *et al.*, 2017), an EF-hand calcium-binding protein (Ye *et al.*, 2017), and 6 other proteins (Rao *et al.*, 2019), from BPH saliva have been reported. The different combinations of these effectors and elicitors can explain why rice plants respond somewhat differently to NlVgN during feeding and oviposition.

We investigated the effect of NlVgN treatment of rice plants on the performance of BPH on these plants. When plants were treated with NlVgN twice, NlVgN-induced defenses decreased the hatching rate of BPH eggs and the number of eggs laid by gravid BPH females, but when the plants were treated only once with NlVgN, there was no effect on any BPH performance parameter. Treating plants with NlVgN only once did not affect the performance of BPH, probably because of the small amounts of NlVgN in the 40 µl treatment solution, which is similar to the amount of VgN from the surface of only 28 BPH eggs (Fig. S8). The smaller amounts of NlVgN can still play a role in priming of plant defenses as reported for other compounds (Conrath *et al.*, 2015). This remains to be tested. The effect of NlVgN-induced defenses on the hatching rate was also observed in plants expressing *NlVgN*. Moreover, the effect of two-time treatment with NlVgN on the hatch rate of BPH eggs and the number of eggs laid by gravid BPH females disappeared in rice lines with impaired JA pathway. These data suggest that the JA pathway elicited by NlVgN plays an important role in this process. Future research will have to elucidate which defensive compounds cause the death of BPH eggs.

In rice, JA- and ET-mediated signaling pathways also regulate the biosynthesis of inducible volatiles (Lou *et al*., 2005; Tong *et al*., 2012; Lu *et al*., 2014). NlVgN did not affect the production of ET in rice. Hence, the fact that exogenous application of NlVgN increased the amounts of volatiles emitted from rice plants was probably due to NlVgN activation of JA and JA-Ile signaling. Compared with control plants, NlVgN-treated plants produced higher levels of nine volatile compounds, all of which were also induced by gravid BPH female infestation (Xiao *et al*., 2012; Lu *et al*., 2014). However, the NlVgN-induced volatile blend was not exactly the same as the blend induced by gravid BPH females; 2-heptanone, linalool, limonene, and methyl salicylate were induced by infestation with gravid BPH females but not by NlVgN (Tong *et al*., 2012). Again, this discrepancy is probably due to effectors and other elicitors in BPH saliva and eggs/oviposition fluids. The changes in volatile emissions imply that NlVgN is not limited to direct defenses, but also involves volatile-mediated indirect defense that results in the attraction of parasitoids. Of the nine volatile compounds that showed increases after NlVgN treatment, (*E*)-β-caryophyllene has been reported to be attractive to *A. nilaparvatae* (Xiao *et al*., 2012), and to other parasitoids in other plant systems, such as *Cotesia marginiventris* (Cresson) and *Cotesia sesamiae* (Cameron) in maize (Tamiru *et al*., 2017), and *Peristenus spretus* (Chen et van Achterberg) and *Aphidius gifuensis* (Ashmead) in cotton (Zhang *et al*., 2020). Therefore, the higher attractiveness of NlVgN-treated plants to this egg parasitoid was probably due to increases in this and possibly other volatiles.

We further show that the role of VgN in inducing rice defenses is not limited to BPH. Extracts with homogenized fresh eggs of two other planthoppers commonly found on rice, WBPH or SBPH, also induced the biosynthesis of JA and JA-Ile when applied to mechanically wounded plants. Moreover, when *Vg* in WBPH or SBPH was knocked down, the induction of JA-Ile by their respective ovary extracts in mechanically wounded plants significantly decreased. VgN is also found in the saliva and eggs of WBPH and SBPH (Huang *et al*., 2018) and the similarity of the amino acid sequence of VgN among the three planthoppers is high (84.38%; Fig. S9). Taken together, these results indicate that the VgN derived from the three planthoppers functions as a common elicitor during the interaction between rice and planthoppers. Whether VgNs in other herbivores also function as elicitors remains to be determined.

In this study, we also confirm that NlVgN plays an important role in BPH growth, development, and fecundity. Moreover, silencing *NlVg* impaired BPH feeding and decreased its survival on rice plants and artificial diet. The increase in body size and mass of dsNlVg-BPH female adults is probably at least in part related to the failure of egg formation, which prevents them from laying eggs like normal female adults (laying eggs decreases their body size and mass). By confirming the key importance of Vgs for planthoppers, we also expose the vulnerability of *NlVg* and similar genes in other insects, making them excellent targets for gene-silencing strategies to control pests (Christiaens *et al*., 2020). This is evident from our silencing experiment, which resulted in increased BPH mortality and almost completely impaired egg production.

In summary, our study shows that VgN, the small N-terminal subunit of Vg from rice planthoppers, readily enters rice tissues during planthopper feeding and oviposition. VgN from the saliva of rice planthoppers, together with the damage caused by planthopper feeding, possibly in combination with effectors and other elicitors, induces the production of cytosolic Ca^{2+} and H_2O_2, whereas VgN from eggs, accompanying the damage caused during oviposition and possible other chemical factors in oviposition fluids, induces the production of cytosolic Ca^{2+}, H_2O_2, JA, and JA-Ile. The activated JA signaling pathway decreases the hatching rate of BPH eggs and the number of eggs laid by BPH female adults and increases the emission of volatiles from rice, which enhances the attractiveness of rice plants to the egg parasitoid *A. nilaparvatae*. Our study not only identifies VgN from rice planthoppers as a potent elicitor but also provides a compelling example of how an elicitor combined with varying herbivore-inflicted damage types can cause differential defense responses in plants. The importance of VgNs for the planthoppers makes them stable and reliable indicators of planthopper presence and possible targets for molecular pest control strategies.

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Competing interests

None declared.

Author contributions

JZ, WY, TCJT, and YL conceived and designed the experiments. JZ, WY, WH, XJ, PK, WX, and YJ performed the experiments. JZ, WY, WH, and YL analyzed the data. JZ, TCJT, and YL wrote the manuscript. All authors have read and approved the final manuscript. JZ and WY contributed equally to this work.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Information on transformation vectors and SDS-PAGE analysis of purified recombinant NlVgN.

Fig. S2 Identification of rice plants expressing *NlVgN* and their growth phenotypes.

Fig. S3 Silencing efficiency of *NlVg* by RNA interference (RNAi) and the effect of knocking down of *NlVg* on transcript levels of *NlVg-like1* and *NlVg-like2*.

Fig. S4 Effects of NlVgN secreted from brown planthopper (BPH) feeding or recombinant NlVgN protein on the production of jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), or ethylene (ET) in rice, and on the survival and mass of BPH.

Fig. S5 Knockdown of *NlVg* impairs the development, and fecundity of brown planthopper (BPH) female adult.

Fig. S6 Silencing efficiency of *SfVg* and *LsVg* by RNA interference (RNAi).

Fig. S7 Alignment of C-terminal subunit amino acid sequences of vitellogenins (Vgs) of brown planthopper (BPH) and small brown planthopper (SBPH).

Fig. S8 Relative content analysis of the purified recombinant NlVgN.

Fig. S9 Multiple alignment of N-terminal subunit amino acid sequences of vitellogenins (Vgs) of three planthoppers.

Methods S1 Supporting information methods.

Table S1 Primers used for gene clone, double-stranded RNA (dsRNA) synthesis, and vector construction.

Table S2 Analysis of variance with data from figures in supporting information.

Table S3 Student's *t*-test analysis with data from figures in supporting information.

Table S4 Student's *t*-test analysis with data from figures in main text.

Table S5 Analysis of variance with data from figures in main text.

Table S6 Analysis of variance with data from Fig. 5(b).

Table S7 Analysis of variance with data from Figs 6 and 7.

Table S8 Primers used for quantitative real-time PCR.