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REVIEW

Recent advances in molecular biology research of a rice pest, the brown planthopper

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Abstract

The brown planthopper, *Nilaparvata lugens* Stål, has become a major threat in tropical Asian and China since the rice green revolution of the 1960s. Currently, insecticide application remains the primary choice for controlling this rice insect pest, but heavy use of insecticides poses dangerous risks to beneficial natural enemies and pollinators, and stimulates *N. lugens* reproductivity, and has caused a resurgence of the pest in the major rice-planting regions throughout Asia. Achieving the long-lasting goal of sustainable management of *N. lugens* requires understanding of the molecular basis of outbreaks of the pest and the development of environment-friendly pest-control strategies. Here, we review the recent molecular advances in *N. lugens* research on the aspects of its endosymbionts, virus transmission, insecticide resistance, and interaction between *N. lugens* and rice plants. We also put forward further research directions that may shed some lights on management of the rice pest.

Keywords: *Nilaparvata lugens*, endosymbionts, virus transmission, insecticide resistance, interaction with rice, biological management

1. Introduction

The brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is a typical monophagous insect herbivore that feeds exclusively on rice sap (Xue *et al.* 2014). This insect pest causes serious damage to rice crop through phloem sap sucking and nutrient depletion, which often leads to "hopper burn" (complete drying and wilting of rice plants) in the major rice-planting regions of Asia-Pacific countries. Despite a debate over whether delphacid planthopper species in Asia and Oceania differ, *N. lugens* occurs sporadically in Australia. *N. lugens* is an insect vector that transmits *rice ragged stunt virus* (RRSV) and *rice grassy stunt virus* (RGSV). Even at low population densities, *N. lugens* can cause significant losses in rice production when they transmit these viruses (Fujita *et al.* 2013). Historically, severe *N. lugens* outbreaks occurred during the 1970s in many East and Southeast Asian countries (Matsumura and Sanadamorimura 2010). In China, outbreaks of *N. lugens* have re-occurred since the 1970s, where the most serious outbreak in history occurred in Yangtze River Delta areas in China in 2005 (Cheng and Zhu 2006). Once the outbreak begins, little can be done to control the planthoppers. The outbreaks were closely related to their long-distance

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migration, quick adaptation to resistant rice varieties, and the development of high resistance to insecticides, which led to difficulty in controlling *N. lugens* infestations (Yuan *et al.* 2014). To understand the biological features of *N. lugens*, i.e., interaction with endosymbionts, insecticide resistance, long-distance migration, virus transmission, and plant host selection, that enable frequent *N. lugens* outbreaks in condensed rice paddy fields, it is necessary to explore the functional roles of the important genes of *N. lugens*. In 2014, *N. lugens* genome sequencing was completed, marking it as the first characterized genome of a monophagous sap-sucking arthropod herbivore (Xue *et al.* 2014). *N. lugens* has a 1.14-Gbp genome that contains 27 571 protein-coding genes. Genome-wide comparison with available insect species revealed the major contraction or loss of evolutionarily conserved genes that are involved in chemoreception, detoxification, and digestion mechanisms. These unique genomic features are functionally associated with *N. lugens* exclusive plant host selection. Some genes missing from *N. lugens* in conserved biochemical pathways essential for its survival on the nutritionally imbalanced sap diet are present in the genomes of its microbial endosymbionts, which have evolved to complement the mutualistic nutritional needs of their host. Many species-specific genes in *N. lugens* are an invaluable source for understanding the biological characteristics of *N. lugens*. This review mainly introduces the recent advances in molecular biology research on the aspects of its interaction with endosymbionts, virus transmission, insecticide resistance, interaction with the host plant, and the potential RNA interference (RNAi) strategies in managing *N. lugens*.

2. Endosymbionts in *N. lugens*

Rice planthoppers harbor a variety of intracellular endosymbionts and benefit from them from multiple aspects, including nutrition metabolism, development, reproduction, immunity defense, virus transmission, and virulence adaptation to resistant rice varieties (Tang *et al.* 2010; Ferrater *et al.* 2013). When the endosymbionts are removed from the insect body, the planthoppers grow and develop slowly, lose reproductive ability, and die prematurely (Douglas 1989). As a monophagous rice pest, *N. lugens* survives on highly specialized and nutritionally imbalanced rice sap (a food that is poor in essential amino acids, nitrogen, and sterols) owing to its endosymbionts, which provide the essential nutrient supplementation to their host in a long-term adaptive relationship (Meer *et al.* 2010). *N. lugens* hosts an obligatory yeast-like endosymbiont, a filamentous ascomycete, in the fat body cells. This fungal symbiont has been isolated from *N. lugens* using density-gradient ultracentrifugation and designated *Entomomyces delphacidicola* str. NLU based on its taxonomic affiliations (Fan *et al.* 2015). *E. delphacidicola* is distributed in all developmental stages of *N. lugens* and is transferred to the next generation *via* transovarial infection (Cheng and Hou 2001). Actin plays a vital role in the transmission, from the entry of *E. delphacidicola* into the epithelial plug of female ovarioles to its delivery to the oocytes (Yukuhiro *et al.* 2014). Eighteen bacterial symbionts representing four phyla (Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes) have been identified in *N. lugens*, and are extensively distributed in natural field populations (Tang *et al.* 2010). *Wolbachia* or *Arsenophonus* are the most dominant endosymbiotic bacteria in *N. lugens* field populations from different regions of China and Southeastern Asian countries including the Philippines, Thailand, Laos, Malaysia, and Vietnam (Qu *et al.* 2013). The two genera are mainly localized in the mycetocytes of the fat body in *N. lugens* and are maternally transmitted *via* the ovarian passage. It has been hypothesized that *N. lugens Wolbachia* participates in manipulating reproduction, while *Arsenophonus* is phylogenetically close to *Nasonia arsenophonus*, which induces male offspring killing (Wang *et al.* 2010). Interestingly, the two bacterial endosymbionts demonstrate an exclusive relationship in their insect host, *N. lugens* (Qu *et al.* 2013). Genome sequencing of *E. delphacidicola* and *Arsenophonus* of *N. lugens* has been completed (Xue *et al.* 2014; Fan *et al.* 2015, 2016). The draft genome sizes of *E. delphacidicola* and *Arsenophonus* are 26.8 and 2.96 Mbp, respectively. *E. delphacidicola* plays nutritional roles in sterol biosynthesis (Noda and Koizumi 2003) and nitrogen recycling for its host, *N. lugens* (Sasaki *et al.* 1996; Hongoh *et al.* 2000). The complement of the *E. delphacidicola* and *Arsenophonus* genome sequencing provided the first genetic evidence supporting the mutualistic interactions between the endosymbionts and their insect host. The most important evidence is that *E. delphacidicola* can synthesize 10 essential amino acids, nitrogen recycling compounds, and steroid biosynthesis intermediates that *N. lugens* cannot; *Arsenophonus* can produce vitamin B for *N. lugens* (Xue *et al.* 2014; Fan *et al.* 2016). In these nutritional metabolism pathways, the genes absent from the *N. lugens* genome are present in *E. delphacidicola* or *Arsenophonus* genomes. These genetic compensatory mechanisms enable *N. lugens* to thrive on the low-nutrient food source of rice phloem sap, thereby facilitating *N. lugens* adaptation to rice host specialization (Chen *et al.* 2011).

3. Virus transmission

N. lugens is an insect vector of rice viruses including RRSV (genus *Oryzavirus*) and RGSV (genus *Tenuivirus*), which

are transmitted in a persistent-propagative manner to rice plants without transovarial passage to progeny (Hibino 1986; Cabauatan *et al.* 2009; Jena *et al.* 2010; Kouji *et al.* 2013). These viruses share a very similar infection route within the *N*. *lugens* body. *N*. *lugens* ingests RRSV or RGSV by feeding on diseased rice plants. The viruses initially enter the midgut epithelial cells and traverse the basal lamina into the gut visceral muscles, spread into the hemolymph, and finally reach the salivary glands (Jia *et al.* 2012; Zheng *et al.* 2014). During sequential sap sucking, the viruses are transmitted to healthy host plants. RRSV causes rice ragged stunt disease, which was first discovered in 1976–1977 in Indonesia and Philippines, and then became prevalent in the major rice-producing regions, especially in Southeast Asia and southern China (Wu J *et al.* 2010; Hiraguri *et al.* 2014). RRSV has icosahedral symmetry with 65–70-nm diameter and is made up of capsid protein double layers (Hagiwara *et al.* 1986; Whitfield and Rotenberg 2015). The RRSV genome is composed of 10 linear double-stranded RNA (dsRNA) segments (GenBank accession no. PRJNA14794) encoding at least seven structural proteins and three nonstructural proteins (Chen *et al.* 2014). Among the RRSV structural proteins, P5 is a putative capping enzyme with guanylyltransferase activity (Li *et al.* 1996). The capsid protein P3 and RNA-dependent RNA polymerase P4A constitute the polyhedral core particles; the major outer capsid protein P8, along with spike protein P9, form double-layer virions (Hagiwara *et al.* 1986; Upadhyaya *et al.* 1996, 1997, 1998; Zhou *et al.* 2010; Jia *et al.* 2012). Nonstructural proteins are not present in the viral particles. Pns6 plays dual roles in RNA silencing as a virus suppressor and in cell-to-cell movement of RRSV (Wu J *et al.* 2010; Wu Z *et al.* 2010). Pns7 is a putative nucleoside triphosphate (NTP)-binding protein that contains two NTP binding motifs. It has been indicated that Pns7 forms a filament structure on the insect cell surface and is probably involved in viral spread in insect vectors (Zhang *et al.* 2017). Pns10 has ATPase activity (Shao 2004). Pns6 and Pns10 constitute the major components of viroplasm and are essential for virus replication and assembly in *N*. *lugens* cells (Jia *et al.* 2012; Chen *et al.* 2014). During viral infection, Pns6 is recruited to the viroplasm by direct interaction with Pns10. A recent study determined that oligomycin sensitivity conferral protein (OSCP), a membrane component of the mitochondrial FoF1 proton adenosine triphosphate (ATP) synthase/ATPase (F-type H^+-ATPase), binds with Pns10 (Huang *et al.* 2016b). Silencing *OSCP* gene expression significantly reduced viral loads in *N*. *lugens*. This finding established an initial link between a nonstructural protein of plant reoviruses and the mitochondrial membrane component of the insect vector, which is meaningful for understanding the functions of mitochondrial membrane protein during viral propagation in insect host cells. The salivary gland plays a crucial role in virus transmission from the insect vector to the plant host. New research has revealed that RRSV infection induces apoptosis in *N*. *lugens* salivary gland cells (Huang *et al.* 2015a). The virus-associated apoptosis is regulated in a caspase-dependent manner. The discovery of RRSV-induced apoptosis occurring in salivary gland cells will be helpful for improving understanding of the virus transmission mechanism from its insect vector to the plant host.

RGSV causes rice grassy stunt with profuse tillering, yellowing, and stunting syndrome. RGSV incidence was high from 1970 to the early 1980s in East, Southeast, and South Asia. Since the mid-1980s, RGSV incidence has been generally low in Asia (Hibino 1996; Ta *et al.* 2013). However, there were severe epidemics of co-infection with RRSV in southern Vietnam during 2006–2009 (Cabauatan *et al.* 2009; Ta *et al.* 2013). RGSV/RRSV co-infection has high pathogenicity, usually causing death in rice plants before maturity (Shimizu *et al.* 2013). RGSV is a filamentous ribonucleo protein particle 6–8 nm in diameter. They are often circular and of various lengths from 200 to 2 400 nm (Hibino *et al.* 1985; Whitfield *et al.* 2015). The RGSV genome is composed of six single-stranded RNA segments (GenBank accession no. PRJNA14692) encoding 12 proteins with an ambisense coding strategy (Ramirez 2008). RNA 1, 2, 5, and 6 of RGSV are equivalent to RNA 1, 2, 3, and 4, respectively, of *rice stripe virus* (RSV), the type species of the *Tenuivirus* genus (Chomchan *et al.* 2002). RNA 3 and 4 of RGSV are unique to the *Tenuivirus* genus. RGSV complementary sense strand RNA 1 (cRNA1) encodes RNA-dependent RNA polymerase (Toriyama *et al.* 1998). P2 protein encoded by virus genomic (sense) strand RNA 2 (vRNA2) is presumed to function in cell-to-cell movement for systemic infection in rice plants, and has silencing suppressor activity (Chomchan *et al.* 2002; Nguyen *et al.* 2015). The complementary sense strand RNA 5 (cRNA5) encodes a nucleocapsid protein that is a major component of thin filamentous particles; the viral sense strand RNA 5 (vRNA5) encodes a putative silencing suppressor essential to the RGSV lifecycle both in insect vector and rice plant (Chomchan *et al.* 2002). P6 protein encoded by vRNA6 functions in viral cell-to-cell movement as a movement protein (Hiraguri *et al.* 2011). The other protein functions of RGSV remain largely unknown. Understanding the molecular function of RGSV proteins will be helpful for future pest control strategies.

4. Insecticide resistance

Beginning in the 1960s, rice green revolution technologies (genetically improved cultivars, chemical fertilizers, and pesticides) rapidly replaced traditional rice farming in many

Asian counties (Bottrell and Schoenly 2012). However, an unanticipated problem arising with the modern agronomic practices was large-scale outbreaks of *N. lugens* in favorable growing environments under heavy use of insecticides and nitrogen fertilizer. Overuse of chemical insecticides including organophosphates, carbamates, pyrethroids, and neonicotinoids greatly stimulated *N. lugens* reproduction potential and harmed natural enemies, which gave rise to planthopper resurgence and additional environmental risks, and imposed an economic burden on rice growers. Insecticide resistance has become increasingly common (Gorman *et al.* 2008; Wang *et al.* 2008; Matsumura and Sanadamorimura 2010; Heong *et al.* 2013). In the last century, i.e., the 1970s to 1980s, farmers in China universally used conventional organophosphate and carbamate insecticides to control *N. lugens*. However, the chemical applications were not highly efficient against the pest, and worsened the development of resistance (Wen *et al.* 2009). For example, sub-lethal doses of triazophos induced high fecundity on *N. lugens* adults and caused the resurgence of the second generation of *N. lugens* (Zhuang *et al.* 1999; Zhu *et al.* 2004). A reason for this is that triazophos stimulated the expression levels of the reproduction-related genes, e.g., *vitellogenin*, in the ovaries of *N. lugens* adult females (Ge *et al.* 2009; Bao *et al.* 2010). In the 1980s, buprofezin, an insect growth regulator that inhibits chitin deposition and biosynthesis, was extensively used to control *N. lugens* because there was no cross-resistance with common insecticides (Uchida *et al.* 1985). However, *N. lugens* field populations evolved high levels of resistance to buprofezin in 2011–2012. The consequent loss of efficacy and utility of buprofezin was reported in 2013 (Zhang X *et al.* 2015). Since the introduction of the first commercialized imidacloprid by Bayer in 1991, neonicotinoids have become the fastest-growing class of insecticides for *N. lugens* control due to the high efficacy and long-lasting activity on the insect's nicotinic acetylcholine receptors (nAChRs), which mediate fast cholinergic synaptic transmission in the insect central nervous system (Matsuda *et al.* 2001; Wang *et al.* 2009; Puinean *et al.* 2010; Bass *et al.* 2011). Nevertheless, *N. lugens* field populations have developed high to extremely high levels of resistance to imidacloprid over large areas in China since 2005, which has led to control failure (Gorman *et al.* 2008; Wang *et al.* 2009; Cheng 2015). Currently, the second-generation neonicotinoid thiamethoxam was commonly used for controlling *N. lugens* in China (Zhang X *et al.* 2015). However, the obviously increased resistance to this insecticide was observed in a variety of *N. lugens* field populations in the major rice growing regions in 2012–2014 (Zhang X *et al.* 2015).

Many studies on the molecular mechanisms of *N. lugens* insecticide resistance have made great progress. Metabolic enzyme-dependent mode, i.e., overexpression of a detoxifying enzyme through gene upregulation or amplification, and insecticide target site mutation are thought to be the major resistance mechanisms. Three main detoxification enzyme families, namely the cytochrome P450 monooxygenases (P450s), glutathione *S*-transferase (GST), and esterase, are implicated in insecticide resistance in *N. lugens*. The resistance to imidacloprid stems from a single point mutation in nAChR α subunits that decreased imidacloprid affinity on receptors in a resistant *N. lugens* laboratory strain (Liu *et al.* 2005). However, in *N. lugens* field populations, imidacloprid resistance is primarily attributable to enhanced oxidative detoxification by the overexpression of one or more P450 genes that can selectively metabolize and inactivate insecticides (Wen *et al.* 2009; Puinean *et al.* 2010; Ding *et al.* 2013). For example, two *N. lugens* P450 genes, *CYP6ER1* and *CYP6AY1*, were overexpressed up to 40- and 17.9-fold in imidacloprid-resistant *N. lugens* strains, respectively (Bass and Field 2011; Ding *et al.* 2013). GST participates in conferring pyrethroid resistance in *N. lugens*. The underlying resistance mechanism is mediated through gene amplification and elevated activity of GST, which detoxifies lipid peroxidation products, rather than through the direct metabolism of pyrethroid molecules (Vontas *et al.* 2002; Bass and Field 2011). *N. lugens* resistance to organophosphorus/carbamates correlates with the amplification of a *carboxylesterase* gene, which results in enhanced gene expression level and esterase activity (Small and Hemingway 2000; Vontas *et al.* 2000). A recent study reported that *CYP303A1*, *CYP426A1*, *CYP4CE1v2*, *CYP18A1*, *CYP4DE1*, and *CYP304H1v4* are potentially involved in triazophos, imidacloprid, or deltamethrin resistance in *N. lugens* (Lao *et al.* 2015), which provides new clues to understanding the resistance mechanisms toward various insecticides. Insecticide target site mutation contributes to insecticide resistance in pest management. Fipronil is the first phenylpyrazole insecticide acting on γ-aminobutyric acid receptor (GABA receptor) and has been extensively used for controlling *N. lugens*. However, *N. lugens* has developed high resistance to fipronil due to a GABA receptor subunit mutation (Feng *et al.* 2015). Insecticide resistance is a long-standing and complex problem influencing insect control and pest management (Sparks and Nauen 2015). Due to the continuing expansion of insecticide resistance worldwide, promoting biological control tactics that integrate natural enemies and other nonchemical strategies along with plant resistance would be helpful for reducing insecticide use and the risk of planthopper outbreaks.

5. Interaction of *N. lugens* and rice plant

In the interaction between insects and their host plants,

insect saliva plays an important role in determining the compatibility with the plant host. The saliva contains abundant bioactive components that have an array of functions from countering plant defense mechanisms to nutrient digestion, thus allowing insects to feed and survive successfully. *N. lugens*, like most plant sap-sucking insects, secretes gelling and watery saliva (Huang *et al.* 2016a). The gelling saliva forms a salivary sheath around the stylets, which provides mechanical stability and lubrication and protects the insect from chemical defenses. The watery saliva is mainly secreted into the rice phloem and is believed to contain bioactive proteins, i.e., effectors for overcoming plant defense responses. A recent work on *N. lugens* saliva revealed its protein composition and function in its interaction with rice plants (Huang *et al.* 2016a). In a follow-up study, comparative analysis of the secreted saliva of three rice planthopper species, namely the monophagous *N. lugens*, the polyphagous white-backed planthopper *Sogatella furcifera*, and the small brown planthopper *Laodelphax striatellus*, revealed a diverse salivary protein composition that included planthopper-ubiquitous salivary proteins and species-specific proteins (Huang *et al.* 2017). The diversity and specificity of the salivary proteins in the three rice planthoppers reflect their evolutionary strategies, which are consistent with the monophagous or polyphagous habit, necessitating particular components to participate in interactions with their plant hosts. As recognition of *N. lugens* salivary components deepens, an increasing number of salivary protein functions have been understood over the past few years. It was found that an annexin-like protein and a salivary sheath protein are necessary for salivary sheath formation (Huang *et al.* 2015b, 2016a). Several salivary proteins, including salivap3, carbonic anhydrase, and the catalase-like protein Kat-1, play key roles in the feeding behavior and survival of *N. lugens* on rice plants (Petrova and Smith 2015; Huang *et al.* 2016a, 2017). More recently, an endo-β-1,4-glucanase and an EF-hand calcium-binding protein were identified to function as effectors in *N. lugens* saliva, mediating defense responses in rice (Ji *et al.* 2017; Ye *et al.* 2017). Huang *et al.* (2017b) found that a saliva component called mucin-like protein was essential for *N. lugens* virulence and host adaptation (Petrova and Smith 2015; Huang *et al.* 2016a, 2017); later, it was found that the protein can induce the immunity response in plants (Petrova and Smith 2015; Huang *et al.* 2016a, 2017) . These new findings provide insights into the evolutional adaptation of *N. lugens* to its unique plant host, rice. The *N. lugens*-specific salivary protein components would be an important molecular target in pest management.

In response to the biotic stresses caused by *N. lugens* feeding and virus transmission, rice plants have developed sophisticated defense strategies, including antixenosis to repel attacks from pests, and antibiosis to disrupt pest development (Daisuke *et al.* 2013). The rice hormone signaling pathways and mitogen-activated protein kinase (MAPK) cascades constitute a major herbivore-induced defense signaling network. *N. lugens* attack elicits a series of phytohormones such as jasmonic acid, salicylic acid, jasmonoyl-isoleucine, and ethylene in rice (Hu *et al.* 2012). These phytohormones positively or negatively modulate rice resistance to *N. lugens* by releasing insect-triggered rice volatile compounds and inducing the expression of defense-related molecules, e.g., transcription factors (*OsERF1*, *OsWRKY53*), polyphenol oxidases, peroxidase, and trypsin proteinase inhibitors (Howe and Jander 2008; Lu *et al.* 2011). A *Bphi008a* gene and several *OsMPK* genes of MAPK cascades mediate resistance in the response of rice to *N. lugens* feeding (Hu *et al.* 2011, 2012). As rice germplasm is resistant to insect pests (Pathak 1971), host-plant resistance has been utilized as an efficient method of controlling *N. lugens* to reduce direct damage of feeding and indirect damage of viral transmission. Deploying improved rice varieties by introducing resistance genes into susceptible cultivars is an environment-friendly approach to rice breeding. *Bph1* and *bph2* were the first two resistance genes screened *via* a rice breeding program at the International Rice Research Institute (IRRI). To date, 28 *N. lugens* resistance genes (designated *BPH1-28*) have been identified, and most have been mapped to rice chromosomes in cultivated and wild rice species (Kabir and Khush 1988; Nemoto *et al.* 1989; Kawaguchi *et al.* 2001; Liu *et al.* 2001; Murai *et al.* 2001; Renganayaki *et al.* 2002; Sharma *et al.* 2004; Yang *et al.* 2004; Kim and Sohn 2005; Chen *et al.* 2006; Jena *et al.* 2006; Su *et al.* 2006; Sun *et al.* 2006; Rahman *et al.* 2009; Qiu *et al.* 2010; Yara *et al.* 2010; Hu *et al.* 2012; Myint *et al.* 2012; Yang *et al.* 2012; Cheng *et al.* 2013; Daisuke *et al.* 2013; He *et al.* 2013; Tamura *et al.* 2014; Wu *et al.* 2014). These genes confer resistance by inhibiting *N. lugens* sucking from rice phloem, and reducing growth and survival rates of *N. lugens* population, or confer resistance *via N. lugens* tolerance behavior, e.g., *Bph28* (Cheng *et al.* 2013; Wu *et al.* 2014). Thus far, only *Bph3*, *Bph14*, and *Bph26* have been isolated by molecular cloning and characterized. *Bph14* and *Bph26* have been identified as immune receptors belonging to the coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein family, which might function in recognizing the invasion of sap-sucking insects and pathogens, triggering the plant defense signaling pathway and callose deposition (Du *et al.* 2009; Tamura *et al.* 2014). *Bph3* is a G-type LecRK gene cluster encoding three plasma membrane-localized lectin receptor kinases (OsLecRK proteins) that act together in conferring a high level of *N. lugens* resistance in rice (Liu *et al.* 2015). OsLecRK proteins are considered potential

cell surface receptors for insect- or plant pathogen-derived elicitors and prime pattern-triggered immunity responses, leading to resistance against planthoppers (Liu *et al.* 2015). The identification and characterization of *Bph3*, *Bph14*, and *Bph26* provide insights for better understanding of the co-evolution of rice pests and the innate immune system of rice plants under natural selection. At present, resistance genes have been incorporated into rice varieties and released in rice production. However, rice varieties harboring a single major resistance gene quickly render the resistance ineffective, i.e., resistant cultivars carrying *Bph1*, *bph2*, *Bph3*, *bph4*, *bph8*, or *Bph9* genes have remained viable for a few years after the varieties were released for *N. lugens* control (Myint *et al.* 2009; Daisuke *et al.* 2013). Polygenic resistance is expected to have more durable or higher resistance than a single major gene. Nevertheless, pyramiding *Bph1* and *bph2* genes into a susceptible commercial *Oryza sativa japonica* cultivar exhibited the same resistance level to that of a *Bph1*-single introgression line (Sharma *et al.* 2004). Although pyramided lines carrying two or more resistance genes, i.e., *Bph14* and *Bph15*, *Bph12* and *Bph16*, and *Bph25* and *Bph26*, have stronger antixenotic and antibiotic effects on *N. lugens* (Hu *et al.* 2012; Myint *et al.* 2012; Qiu *et al.* 2012), attention should be paid to the deployment of pyramided varieties, as it is uncertain whether pyramided lines can lead to rapid adaptation to the resistant varieties (Daisuke *et al.* 2013). *N. lugens* populations easily overcome single gene-derived resistance, thus the effectiveness of resistant rice cultivars against this rice pest is restricted. Development of advanced genetic engineering approaches, e.g., silencing functional gene expression of *N. lugens*, is a better means of achieving stable resistance against this rice pest than a breeding strategy that seeks high resistance from a single major gene.

6. Biological management of *N. lugens* by gene interference

An ongoing challenge in achieving long-lasting control of *N. lugens* is minimizing chemical insecticide use and developing ecologically sound strategies for breeding rice varieties with more sustainable resistance. The *Bacillus thuringiensis* (Bt) transgenic crop is considered an environmentally friendly insect-pest management strategy. Bt insecticidal toxins have been successfully adopted in transgenic plants for certain advantages, they are highly toxic and highly specific to insect pests, i.e., Lepidopteran, Dipteran, and Coleopteran. Bt transgenic technology has limitations in resisting phloem-sucking hemipteran insect species. A recent study reported that transgenic rice plants expressing a modified *cry30Fa1* gene from Bt exhibited high-efficiency insecticidal activity against *N. lugens* (Wang *et al.* 2016). New transgenic rice plants expressing novel crystal proteins or other insecticidal proteins will enrich *N. lugens* resistance germplasm resources and increase in importance in the coming years. Currently, RNAi-based technology has shown great potential in protecting crops against agriculturally important insect pests; this is due to its feasibility and safety, allowing the suppression of the specific insect pest genes and the protection of non-target organisms, thus providing species-species management (Whyard *et al.* 2009). For phloem-sucking insects, RNAi is an attractive option because dsRNA can be delivered *via* transgenic expression in plants, transiently expressed by viral vectors, or exogenously applied by soil drench to plants (Whitfield and Rotenberg 2015). In RNAi, the Achilles heel target genes that are vital to insect growth, development, or survival as well as to plant viral infection, proliferation, or transmission can be used to develop transgenic rice plants, conferring durable and broad-spectrum resistance against phloem-sucking rice pests and their transmitted rice viruses. The availability of *N. lugens* genome and transcriptome data provides a potential target gene source for transgenic rice plants engineered specifically to resist *N. lugens* (Xue *et al.* 2010, 2014; Huang *et al.* 2016a). A series of functionally important genes or gene families have been identified and characterized in *N. lugens*, which include the innate immune-related genes (Bao *et al.* 2013); P450 genes (Lao *et al.* 2015); serine proteinase genes (Bao *et al.* 2014); digestion-related genes; detoxification-related *GST* genes (Zhou *et al.* 2013); chemoreception-related chemosensory, olfactory, and gustatory genes (Xue *et al.* 2014); chitin metabolism-related chitinase-like, chitin deacetylase, and β-*N*-acetylhexosaminidase genes (Xi *et al.* 2014a, b, 2015); fecundity-related genes (Zhai *et al.* 2013; Qiu *et al.* 2016); secreted salivary protein genes (Huang *et al.* 2016a); and seminal fluid protein genes (Yu *et al.* 2016a). *N. lugens* possess a highly RNAi-sensitive system (Xu *et al.* 2013). Successful RNAi by targeting *N. lugens* genes has led to strong and long-lasting phenotypic effects, i.e., increased lethality, delayed development, decreased egg hatching and offspring of the population, tissue/organ morphologic defects, and altered feeding behavior, which make available a valuable gene repertoire for breeding new rice varieties with satisfactory resistance against *N. lugens* in the field. Among the characterized genes of *N. lugens*, two *insulin receptor* genes, *NlInR1* and *NlInR2*, could be potential RNAi targets. They act as wing-morph switches in determining wing developmental plasticity in planthoppers (Xu *et al.* 2015). Knockdown of *NlInR2* generated a strong bias towards long-winged morphs; *NlInR1* knockdown resulted in short-winged morphs. It has been speculated that *NlInR2* is a negative regulator of the *NlInR1* signaling cascade (Xu and Zhang 2017). This finding provides the first evidence

of a molecular basis of the regulatory mechanism underlying wing polyphenism in *N. lugens*. As important as wing polyphenism, sexual dimorphism is an evolutionarily conserved feature in insects. A more recent work revealed cross-talk between sexual differentiation and wing polyphenism in *N. lugens*. Knockdown of a sex determination-related gene, *NlTra-2*, in *N. lugens* nymphs generated infertile males and caused females to develop into infertile pseudomales with undeveloped ovaries. More interestingly, dysfunction of *NlTra-2* in female adults produced long-winged female offspring, indicating that *NlTra-2* is involved in wing polyphenism in female offspring. Silencing *NlTra-2* downregulated *NlFoxO* expression and upregulated *NlAkt* expression, which are critical genes in the insulin signaling pathway. Furthermore, the long-winged morphs caused by *NlTra-2* knockdown could be reversed by silencing *NlFoxO* and *NlAkt*, leading to short-winged morphs (Zhuo *et al.* 2017). This finding of binary regulation of the *NlTra-2* gene will greatly improve understanding of the mechanistic basis of sexual differentiation and wing polyphenism in insects. Some other function-important genes, e.g., an ecdysone receptor gene, which regulates molting and metamorphosis during *N. lugens* development, is a good potential target for RNAi-mediated pest control (Wu *et al.* 2012; Yu *et al.* 2014). Furthermore, a large number of function-known genes would be good candidates for developing RNAi-based transgenic rice lines with resistance against *N. lugens*. For example, an ion transport peptide regulates wing expansion and cuticle melanization (Yu *et al.* 2016b); dicer-1, bicaudal-C, and a pancreatic lipase-related protein-2 are vital for oocyte maturation (Zhang *et al.* 2013; Zhang B X *et al.* 2015; Xu *et al.* 2017); chitin synthase-1, chitinase, chitin deacetylase, and β-*N*-acetylhexosaminidase are required for chitin metabolism (Wang *et al.* 2012; Xi *et al.* 2014a, b, 2015); a salivary sheath protein is essential for the interaction of *N. lugens* and rice plants (Huang *et al.* 2015b); a mitochondrial membrane protein is a RRSV-interacting protein that plays important roles in viral proliferation (Huang *et al.* 2016b). In RNAi-based pest control of sap-sucking insects, the dsRNA delivery mode will be a major challenge. Injecting dsRNA into target pests is impractical for field-level pest control; spraying with dsRNA may not be an applicable approach for phloem-sucking insects. Using plants as a dsRNA delivery method is a promising strategy that has been applied for RNAi-based pest control against several hemipteran species (Christiaens and Smagghe 2014), e.g., transgenic rice plant lines expressing dsRNA from the *N. lugens* ecdysone receptor gene have been produced (Yu *et al*. 2014). In this practice, feeding on the dsRNA-transgenic plant lines resulted in significant reduction of transcript levels of target mRNAs in *N. lugens*, suggested successful delivery of dsRNA uptake in *N. lugens* (Yu *et al.* 2014). Viruses have been utilized as efficient gene silencing vectors for expressing insecticidal dsRNA in plants (Hajeri *et al.* 2014). This approach lies in introducing exogenous dsRNA constructs into the virus genome. Once the viral infection is established in the host plant, the recombinant virus serves as a continues RNAi source (Burand and Hunter 2013). Virus-based, plant-mediated dsRNA uptake is perhaps an alternative delivery system for controlling *N. lugens*. As previously described, *N. lugens* is an insect vector transmitting two rice viruses, RRSV and RGSV, that are serious menaces to rice production. It is necessary to develop an efficient strategy to control rice viral diseases, i.e., disrupting the insect-mediated transmission of viruses. Successful attempts at expressing virus-derived dsRNA have been made in transgenic rice plants, conferring resistance against rice reoviruses and tenuiviruses, including *rice dwarf virus* (RDV), *rice black streaked dwarf virus* (RBSDV), *rice gall dwarf virus* (RGDV), RSV, and RGSV (Shimizu *et al.* 2009, 2011a, b, 2012, 2013). The RNAi strategy of interfering with viral gene expression caused a significant decrease in virus titer and transmission over the lifecycle of the insect vectors and induced strong resistance in rice plants. It would be effective for controlling other rice-infecting viruses such as *southern rice black-streaked dwarf virus* (SRBSDV) and RRSV (Sasaya *et al.* 2013). With the increase in approaches of dsRNA delivery and persistence, the future objective is to move research findings from the laboratory to the rice field in keeping the environment healthy and productive (Whitfield *et al.* 2015). It would prove a big step forward in applications for controlling plant viruses and their insect vectors on a broader scale (Christiaens and Smagghe 2014).

7. Conclusion and prospects

Frequent *N. lugens* outbreaks are closely associated with the cultivation of high-yield rice varieties, insecticide overuse, and long-distance migration of this insect pest. However, the rice pest problem is so complex and geographically widespread that it requires comprehensive and overall understanding of the occurrence mechanisms from molecular to rice ecosystem level. In this review, we mainly present the recent advances in molecular biology with respect to *N. lugens* interaction with endosymbionts, virus transmission, insecticide resistance, interaction with the host plant, and the potential RNAi strategies in managing *N. lugens*. These research findings will be greatly useful for developing sustainable and ecologically friendly rice agriculture through the breeding of resistant rice varieties by targeting the insect host and its endosymbionts or transmitted rice viruses. We also suggest further research directions that may shed light on rice pest management:

(1) Comparing the geographical population differences of *N. lugens* in South and Southeast Asian countries to develop molecular markers for understanding *N. lugens* migration routes in Asia. (2) Exploring new approaches for managing the rice pest by inhibiting its fungal and bacterial endosymbionts based on the complementarity of insect-symbiont nutritional pathways. (3) Understanding the transmission mechanisms of rice viruses to prevent virus transmission from the three main rice planthopper species, i.e., *N. lugens*, *S. furcifera*, and *L. striatellus*, to their host plants. (4) Finding the resistance mechanisms of important insecticides and providing new targets to develop safer green pesticides. (5) Given that *N. lugens* is an ideal insect model of RNAi, future research should emphasize enhancing dsRNA delivery efficiency and persistence in the rice crop field. (6) The clustered regularly interspaced short palindromic repeats/caspase-9 (CRISPR/Cas-9) gene editing system is a powerful and fast-growing technology that shows potential utility for controlling insect pests by knocking out the expression of target genes, e.g., the sex-determining genes and wing dimorphism genes in *N. lugens*. Compared to the current pest management strategies, the CRISPER-based gene drive technique is more precise, cheaper, and allows direct manipulation of pests; therefore, it is considered an attractive means of agricultural pest management. (7) It is important to further define the virulent variation mechanisms of *N. lugens* for the breeding and applications of persistently resistant rice varieties.

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