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Molecular cloning, characterization and mRNA expression of a ryanodine receptor gene from whitefly, *Bemisia tabaci* MED

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ABSTRACT

The *Bemisia tabaci* (Gennadius) cryptic species complex comprises very destructive insect pests for agricultural crops worldwide. In China, the *B. tabaci* MED species (also known as biotype 'Q'), has supplanted the MEAM1 species (biotype 'B') which is threatening agricultural production around the country. The new anthranilic diamide insecticide, cyantraniliprole, provides one novel step for the management of *B. tabaci* and the development of resistance to other insecticides. Ryanodine receptors of insect are the main target sites of the diamide insecticides. In this study, the full-length cDNA of a ryanodine receptor gene (*BtRyR*) was cloned and characterized from *B. tabaci* MED. The cDNAs of *BtRyR* contain a 15,369-bp open reading frame with encoding 5122 amino acids (GenBank ID: KY244091). *BtRyR* shares 76–83% identity with other insect RyR isoforms and 42–45% identity with vertebrate RyR isoforms. Spatial and temporal expression of *BtRyR* mRNA was at the highest relative level in pseudopupae and head, and at the lowest expression level in egg and abdomen. The expression levels of whole body *BtRyR* mRNA were increased remarkably after insecticide-treatments of adults with cyantraniliprole at 0.01 to 1 mg/l. This structural and expression data on *BtRyR* provides the basis for further understanding the selective action of cyantraniliprole.

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Introduction

The whitefly, *Bemisia tabaci* (Gennadius), is a worldwide devastating agricultural pest that exhibits a high genetic diversity. It has been known to infest >600 host plant species primarily feeding on their phloem (De Barro et al., 2011). In addition to the directly damages, the transmission of >100 different plant viruses indirectly is also a non-negligible problem during the crop growing season (Hogenhout et al., 2008). Within the *B. tabaci* species complex, the Mediterranean (MED or biotype Q) and Middle East-Asia Minor 1 (MEAM1 or biotype B) species are highly invasive and have caused substantial economic damage to crops in China (Luo et al., 2002; Chu et al., 2006). It is consequently the target of repeated insecticide applications such as neonicotinoids, pyrethroids and the juvenile hormone analog pyriproxyfen and resulted in the inefficiency of these insecticides due to the rapid evolution of insecticide resistance (Ahmad et al., 2002; Horowitz et al., 2005; Castle and Prabhaker, 2013; Horowitz and Ishaaya, 2014; Shadmany et al., 2015). In China, the *B. tabaci* MED species has outnumbered the existing MEAM1 population and becoming a dominant species for their higher resistance to several different insecticides and rapid evolution (Luo et al., 2010; Pan et al., 2011).

Calcium (Ca^{2+}) is an important secondary messenger that regulates various cellular and developmental processes such as muscle contraction, synaptic transmission, hormone secretion, fertilization, nuclear pore regulation and transcription (Berridge et al., 2000). The Ryanodine receptors (RyRs) are ligand-gated intracellular calcium channels and mainly located in the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR) of muscle cells and some other non-muscle cells, mediate intracellular Ca^{2+} signaling (Pessah et al., 1985; Lai et al., 1988). In mammals, three RyR isoforms (RyR1, RyR2 and RyR3) have been identified and characterized (Zorzato et al., 1990; Giannini et al., 1995). Moreover, in amphibians, fish and birds, two RyR isoforms (RyRA and RyRB) which are highly related with mammals RyR1 and RyR3, respectively (Ottini et al., 1996). In contrast to the above vertebrates, various insect RyRs has been identified, only one isoform of RyR gene has been detected in insects and nematodes up to now (Tao et al., 2013; Wang et al., 2013; Peng et al., 2016). Anthranilic diamides have been proven to control considerable pest species from different orders effectively (Knight and Flexner, 2007; Yeoh and Lee, 2007; Koppenhöfer and Fuzy, 2008; Peck et al., 2008; Jacobson and Kennedy, 2011). In the field of hemipteran pest management, cyantraniliprole was widely used to control plant hopper, aphid and the whitefly (Sattelle et al., 2008).

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Similarly to chlorantraniliprole, cyantraniliprole molecules were binding to ryanodine receptors and resulting in uncontrolled delivery and depletion of internal calcium, inhibiting muscle contraction (Sattelle et al., 2008). The toxicity levels of cyantraniliprole have been shown to be minimal in mammals while being toxic for several insects, which is a desirable toxicological profile (Lahm et al., 2005; Cordova et al., 2006; Sattelle et al., 2008). The use of cyantraniliprole could be one promising alternative to existing insecticides.

In several previous studies, the cyantraniliprole has shown its superiority to alter the whitefly feeding behavior and weaken the transmission of many plant viruses as a vector finally to achieve the purpose of controlling the damage of plant viruses (Caballero et al., 2013, 2015; Civolani et al., 2014). It was also suggested that cyantraniliprole could be effective to mitigate resistance to other insecticides in *B. tabaci* because it does not provide cross-resistance to the commonly used insecticides for whitefly control (Selby et al., 2013; Grávalos et al., 2015). Besides, we found that cyantraniliprole is one promising agent to control *B. tabaci* by the lethal and sublehtal effects (Wang et al., 2017). So far, there is no report about resistance in *B. tabaci* to cyantraniliprole. However, it is unable to ignore the resistance problem with the extensive application of the chemical on vegetables in the future. In order to carry out further studies to understand the mechanism of insecticide resistance targeting RyR in *B. tabaci*, we cloned and characterized the full-length cDNA for *B. tabaci* MED RyR (*BtRyR*) from adults. Furthermore, we investigated the mRNA expression pattern of *BtRyR*. Our results will provide the basis for functional characterization of *BtRyR* and make a contribution to understand the mechanism of insecticide resistance targeting RyR in *B. tabaci* in future.

Materials and methods

Insects

B. tabaci MED were obtained from the Institute of Vegetables and Flowers in the Chinese Academy of Agricultural Sciences, and established in the laboratory at the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, China. All colonies were maintained on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') under a 16 h: 8 h, light: dark photoperiod at 25–28 °C and 60–80% humidity. Adult whiteflies <7 days old were used for RNA extraction.

RNA extraction and cDNA synthesis

The total RNA was extracted from developmental stages (eggs with 2000 per sample, 1st to 3th instar nymphs with 1200 per sample each nymph stage, pseudopupae with 500 per sample and adults with 200 per sample), body parts (head with 600 per sample, thorax with 600 per sample, abdomen with 600 per sample, leg with 1000 per sample and wing with 1000 per sample) of adults with the Trizol kit (Invitrogen, CA, USA) following the manufacturer's instructions. The potential remaining genomic DNA was removed and the first-strand cDNA was synthesized from 1 μg total RNA using the Prime Script™ 1st Strand cDNA Synthesis Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China) for reverse transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR).

Cloning and sequence analysis

Combine with previous report (Liu et al., 2013) and our transcriptome data (unpublished), the Open Reading Frame (ORF) of RyR gene was predicted by utilizing the tool of ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). To validate the reliability of the assembled sequence, seven pairs of specific primers (Table 1) were designed to amplify complete open reading frames (ORFs) of the RyR gene from *B. tabaci* MED. The cDNA fragments assembling and multiple sequence alignment were performed with DNAMAN (DNAMAN 5.2.2, Lynnon BioSoft). ExPASy Proteomics Server (http://cn.expasy.org/tools/pi_tool.html) was used to compute isoelectric point and molecular weight of deduced protein sequences. The signal peptide was predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). The matured RyR protein sequences from *B. tabaci*, and other Hemipteran pest species were aligned by using ClustalX 1.83 and a phylogenetic tree was constructed in MEGA5.1 using the neighbor-joining method with 1000-fold bootstrap resampling. Transmembrane domains were predicted using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). The regions of putative motifs were predicted by ExPASy ScanProsite (http://prosite.expasy.org/scanprosite/) or alignment to other published RyRs.

qRT-PCR analysis of BtRyR expression profiles

The relative transcription levels of *BtRyR* in different developmental stages (eggs, 1st to 3rd instar nymph, pseudopupae and adults) and various body parts from the adults (such as head, thorax, abdomen, leg and wing) were examined using qRT-PCR. Total RNA for each sample was

Table 1
Primers used for RT-PCR and qRT-PCR.

Primer name		Primer sequence (5′-3′)	Position	Length of fragment (bp)
BtRYR1	Forward	ATGACGGAGGCAGACGGAGG	1	2621
	Reverse	AGTCTGTCTCGGATGTTCTCA	2621	
BtRYR2	Forward	AAGTATGTGCCTCCTGAAGAA	2407	2735
	Reverse	CATTGGTGTGGTCGTAAGC	5141	
BtRYR3	Forward	CCTCTATCCTCAGCTATTCTTC	5047	2455
	Reverse	CTTCATCGGACTCTGGTAACG	7501	
BtRYR4	Forward	CGGCGTCCTGAATGCTTAG	7324	2892
	Reverse	GTGGTTACCTGCTGTGAGATTA	10,215	
BtRYR5	Forward	TCGGAGCATTCTCTTCAACT	9908	2555
	Reverse	GCCATTCACTACATTACCTTCT	12,462	
BtRYR6	Forward	CATTGGTGTTGCTAGTCAAGTT	12,210	2993
	Reverse	TGGCTAAGTTGTGTTCCTGTT	15,202	
BtRYR7	Forward	GTGCCTCTCGCCATCTTCA	14,257	1113
	Reverse	TCAACCTCCTCCACCACCCAA	15,369	
qBtRYR	Forward	CAGCAATCATCCGAGGCAACCA		
	Reverse	CTTCGCTGGAGGCTTGTGAACC		
Actin	Forward	TCTTCCAGCCATCCTTCTTG		
	Reverse	CGGTGATTTCCTTCTGCATT		
EF-1α	Forward	TAGCCTTGTGCCAATTTCCG		
	Reverse	CCTTCAGCATTACCGTCC		

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