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Transcriptome analysis and identification of induced genes in the response of *Harmonia axyridis* to cold hardiness

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ABSTRACT

Harmonia axyridis is an important predatory lady beetle that is a natural enemy of agricultural and forestry pests. In this research, the cold hardiness induced genes and their expression changes in *H. axyridis* were screened and detected by the way of the transcriptome and qualitative real-time PCR under normal and low temperatures, using high-throughput transcriptome and digital gene-expression-tag technologies. We obtained a 10 Gb transcriptome and an 8 Mb gene expression tag pool using Illumina deep sequencing technology and RNA-Seq analysis (accession number SRX540102). Of the 46,980 non-redundant unigenes identified, 28,037 (59.7%) were matched to known genes in GenBank, 21,604 (46.0%) in Swiss-Prot, 19,482 (41.5%) in Kyoto Encyclopedia of Genes and Genomes and 13,193 (28.1%) in Gene Ontology databases. Seventy-five percent of the unigene sequences had top matches with gene sequences from *Tribolium castaneum*. Results indicated that 60 genes regulated the entire cold-acclimation response, and, of these, seven genes were always up-regulated and five genes always down-regulated. Further screening revealed that six cold-resistant genes, E3 ubiquitin-protein ligase, transketolase, trehalase, serine/arginine repetitive matrix protein 2, glycerol kinase and sugar transporter SWEET1-like, play key roles in the response. Expression from a number of the differentially expressed genes was confirmed with quantitative real-time PCR (HaCS_Trans). The paper attempted to identify cold-resistance response genes, and study the potential mechanism by which cold acclimation enhances the insect's cold endurance. Information on these cold-resistance response genes will improve the development of low-temperature storage technology of natural enemy insects for future use in biological control.

1. Introduction

Cold hardy insects in arctic and sub-arctic regions of North America, Scandinavia, Russia and China must survive the low temperatures of their habitats (Bale, 1996; Somme, 1999; Khani and Moharramipour, 2010). Cold acclimation is an important aspect of overwintering adaptation. The Asian lady beetle (*Harmonia axyridis*) is remarkably cold hardy and can overwinter as a diapausing adult (Koch et al., 2004). In nature, a large population of *H. axyridis* overwinters in Northeastern China. In the laboratory, short-time cold acclimation of these insects can significantly promote cold tolerance (Watanabe, 2002; Koch et al., 2004; Labrie et al., 2008; Berkvens et al., 2010).

H. axyridis is extensively distributed throughout Asia. As a ferocious predator of many pests, especially aphids (Koch, 2003), it is considered a natural enemy of several agricultural and forestry pests in Asia (Tang et al., 2014a; Shi et al., 2016). Its voracious appetite, reproductive capacity and strong resistance to cold have allowed it to become an invasive species: the first specimens were collected in North America and Canada in 1994 (Coderre et al., 1995; Brown and Miller, 1998), and the insect was observed in Belgium as early as 2001 (Adriaens et al., 2003). As an invasive species, *H. axyridis* has a detrimental effect on natural environments and indigenous species and has rapidly become dominant in many areas. Nonetheless, it is considered a beneficial organism and has been used to control pests, including aphids, in North America (Koch, 2003) and Canada (Coderre et al., 1995). It is believed that the use of *H. axyridis* in biological control can effectively reduce the harmful environmental impact of pesticide use.

However, the use of *H. axyridis* as a biological control presents the following limitations to large-scale application: cannibalism, the need for artificial feeding and the logistics of low-temperature storage (Watanabe, 2002; Shi et al., 2016). In particular, the beetles cannot be stored at low temperatures for periods that are longer than their natural overwintering conditions (Wu et al., 2016). Little is known about how these beetles endure the low winter temperatures and how the induced cold-hardiness genes function. Although the cold hardiness of this insect has been studied extensively, the molecular mechanisms and genetic regulators of cold adaptation are less well characterized.

Transcriptome and differential gene expression (DGE) analyses based on next-generation deep-sequencing technology provided

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Table 1
Summary statistics of assembly of *Harmonia axyridis* transcriptome.

Parameters	HaRT_trans	HaCS_trans
Total raw reads	57,398,406	56,915,276
Total clean reads	53,875,178	53,590,364
Q20	97.90%	98.03%
GC percentage	43.09%	41.92%
Contig number	70,876	69,975
Contig N50 (bp)	916	1039
Unigene number	42,886	44,741
Unigene N50 (bp)	1005	1070

Table 2
Summary statistics of annotation of all unigenes.

Database	Unigene number	Percentage (%)
NR	28,037	59.7
NT	10,559	22.5
Swiss-prot	21,604	46.0
KEGG	19,482	41.5
COG	9921	21.1
GO	13,193	28.1
All unigenes	46,980	100.0

extensive data with enormous depth and coverage (Oppert et al., 2010; Husseneder et al., 2012). In addition, the application of RNA-Seq analysis, which allows the de novo assembly of short reads from RNA sequences without genome references, was also performed. As well as transcriptome and RNA-Seq analysis were used to find the new gene and its function in insect (Liu et al., 2015; Zhou et al., 2015; Cui et al., 2016; Niu et al., 2016), especially in discovering and studying of stress-induced genes (Dunning et al., 2013; Pan et al., 2015; Osachoff et al., 2016; Wang et al., 2016).

2. Materials and methods

2.1. Experimental samples and sample treatment

Beetles were maintained at 25 ± 1 °C with 70 ± 5% humidity. The population was established under the lab conditions of Hangzhou Normal University and raised for more than three generations. Insects were raised in rectangular plastic boxes (15 × 12 × 7 cm) at densities of 20 to 30 insects per cage and were fed with bean aphid (*Aphis medicaginis*) once per day. Folded paper was placed in the boxes to provide spawning substrates. After females spawned, eggs were removed to insect cages (aluminum alloy with 60 mesh gauze net system, 5 × 30 × 30 cm) for development and breeding.

Insects were exposed to normal conditions and different periods under low-temperature conditions, with all treatments replicated three. The populations of *H. axyridis* exposed to normal conditions (room temperature) were the RT group and termed HaRT_Trans or RT in transcriptome and DGE analysis, respectively. The population under different treatments of *H. axyridis* was exposed to the low temperature of 5 °C for 2 h, 12 d and 150 d – the corresponding treatments were termed "CS_A" and "CS_B", "DF_A" and "DF_B", "DFLT_A" and "DFLT_B" in DGE sequencing and analysis. At the same time, the library preparation for RNA under three treatment groups which 5 °C for 2 h, 12 d and 150 d, and every thirty *Harmonia axyridis* adults were isolated and mixed as "HaCS_Trans" sample for transcriptome sequencing and analysis.

2.2. RNA isolation and library preparation for transcriptome analysis

Total RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Three micrograms of total RNA was used as the input material for sequencing. All sample RNAs were detected with RIN values >8 using the 2100 Bioanalyzer (Agilent Technologies). The sequencing library was generated using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. mRNA was purified from total RNA using poly(T)-oligo-conjugated magnetic beads. Fragmentation was performed by heating the RNA in fragmentation buffer (Illumina) containing divalent cations. First-strand complementary DNA (cDNA) was performed using random oligonucleotides and SuperScript II reverse transcriptase (Invitrogen). Second-strand cDNA synthesis and RNA degradation were performed using DNA Polymerase I and RNase H. The overhangs were converted to blunt ends through the exonuclease activity of the polymerase, and the double-

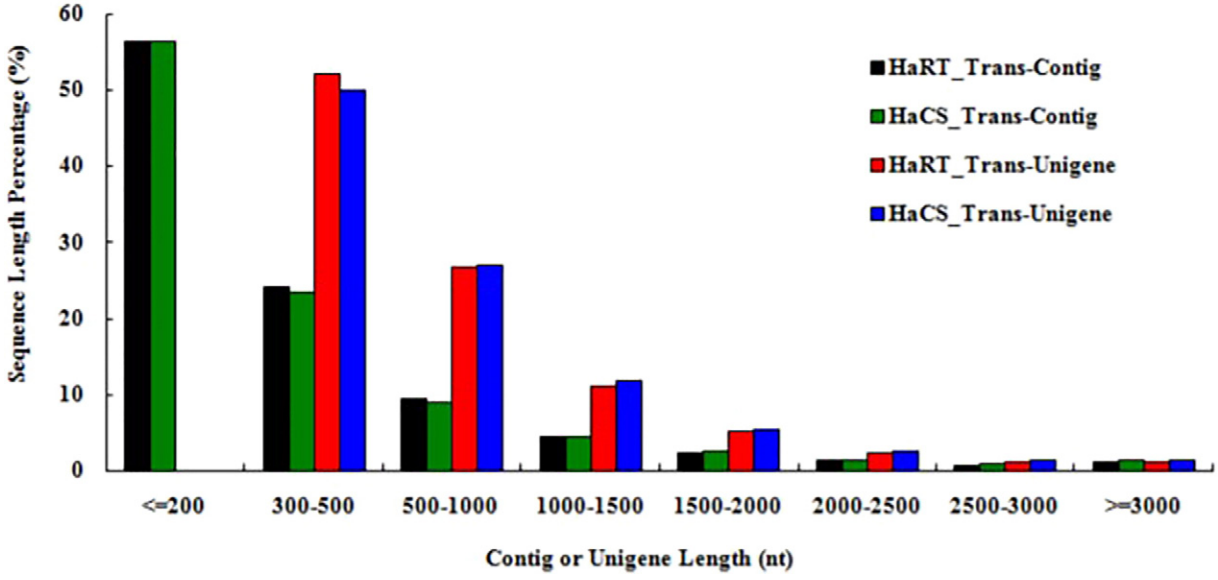

Fig. 1. Length distribution of *Harmonia axyridis* transcriptome unigenes and contigs. Sizes of all unigenes and contigs were calculated: the length (nt) is plotted on the x-axis and the number on the y-axis.