UDC 632.74:632.913.1:632.937.32

QUANTITATIVE ANALYSIS OF THE REGULATORY GENE HSF1 OF *BEMISIA TABACI* UNDER DIFFERENT TEMPERATURES

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Bemisia tabaci (Gennadius) is one of the most important pests in tropical, subtropical and adjacent temperate regions. B. tabaci is a major agricultural pest that is closely watched worldwide. With the widespread application of vegetable greenhouse planting patterns and frequent vegetable and flower transfers, more favorable conditions were created for the occurrence and spread of B. tabaci, making it the major pest in China's agricultural production.

The ability of B-biotype to adapt for new environments is closely related to its tolerance to temperature. Heat shock proteins (HSP s) are the group of proteins produced by cells under the induction of stressors, especially environmental high temperature. Heat shock proteins play an important role in the adaptability of organisms to the environment. This experiment mainly was studied from the heat shock protein of B. tabaci and its regulatory factors (Heat shock factor 1, hsf1). Meanwhile, fluorescence quantitative technology was used to observe the expression of this regulatory factor under different temperature conditions. It is speculated that the HSPs regulatory factor hsf1 is B-biotype B. tabaci and it can induce protection against high temperature stress.

Key words: *Plant pest and quarantine objects in agricultural production, pest invasion, B-biotype of Bemisia tabaci; biological method of plants protection from pests, regulatory factors, hsf1- heat shock factor.*

DOI: https://doi.org/10.32782/agrobio.2020.3.6

Introduction. B-biotype *Bemisia tabaci* (*Gennadius*) belongs to the *Homoptera* family. It is a tiny piercing-sucking pest, mainly concentrated in tropical and subtropical regions (Wei et al., 2014; Chen et al., 2014; Coppola et al., 2013; Dogan et al., 2017; Dorta et al., 2012). Since the successful invasion of *B. tabaci* in the mid-to-late 1990s, B-biotype *B. tabaci* has been outbreaks in Chinese vegetable areas, not only in the greenhouse, but also in large numbers in the field (Luo & Zhang, 2000). In recent years, *B. tabaci* has become one of the typical alien invasive species for its great harm to agricultural production to domestic and foreign researchers (Chu Dong et al., 2008; Farooq & Freed, 2018; Feng et al., 2010; Ferron et al., 2015; Fontes et al., 2012; Freed et al., 2011; Gillespie & Crawford, 2015; Glare & Inwood, 2014).

Temperature is an important environmental factor that affects the growth and development of insects. The change of external environment temperature can directly affect the physiological metabolism of the individual, the activity of various enzymes in the individual and the synthesis and release of hormones (Abdel-Raheem et al., 2016; Alavo et al., 2015; Benz, 2015; Foolad & Panthee, 2012). Under the instantaneous heat shock, it is conducive to the occurrence of B-biotype *B. tabaci* population in the summer high temperature season (Cui, 2007a, 2007b). Studies have shown that the molecular chaperone function of heat shock proteins (HSPs) is an important source of heat resistance (Zhang et al., 2014; Huang et al., 2009; Yang et al., 2016). Heat shock proteins were initially considered to be highly conservative stress proteins produced by organisms under the influence of adverse environmental factors (Javed & Qiu, 2020; Kirkland et al., 2014; Koppert, 2015; Latch & Fallon, 2013). But studies have found that the class of heat shock genes are activated and expressed in non-stimulated cells or expressed at certain stages of biological development (Zhao & Jones, 2012; Binder, 2014; Chandrasekhar et al., 2014; Lovera et al., 2020; Macleod, 2014). Studies have pointed out that organisms may induce the synthesis of such stress proteins under stress environmental conditions such as high temperature, salinity, drought, and osmosis, and function as molecular chaperones in cells (Majeed et al., 2017; Mora et al., 2017; Mustu et al., 2015). The synthesis of more heat shock proteins allows the physiological processes in cells to run smoothly (Mogk et al., 2003; Sangster & Queitsch, 2005).

Heat shock proteins can be divided into HSP90, HSP70, HSP60 and small molecule heat shock proteins according to their relative molecular mass (Bob & Buchannan, 2000; Srensen et al., 2003; Liu et al., 2013; Liu et al., 2014; Nazir et al., 2020). Each family of heat shock proteins has many different forms or differently modified protein molecules (Lu et al., 2014; Jacques, 2003; Christine et al., 2002; Evgen 'ev et al., 2004; Nazir et al., 2019).

Studies have shown that heat shock proteins do not di-

rectly participate in the protection of their intracellular environment in these organisms. (Rehner & Buckley, 2015; Ruiu, 2018; Sánchez-rodríguez et al., 2018; Sánchez-rodríguez et al., 2016; Saranraj & Jayaprakash, 2017), but through heat shock transcription factors (Heat shock factors, HSFs) bound to the heat shock element (HSE) of the promoter of the heat shock protein gene.To recruit other transcription factors to form a transcription complex to promote the expression of heat shock protein genes (Zhang & Zhang, 2019; King & MacRae, 2015; Wettstein et al., 2012; Verma & Deepthi, 2016; Xu et al., 2019). HSF is a trans-acting factor about 20 nucleotides upstream of the TATA box of the HSP gene that can bind to the heat shock element HSE and activate the transcription of the heat shock protein gene (Zhang et al., 2012; Chen & Zhang, 2015; Yun et al., 2017). According to its function, it is divided into 4 types, including hsf1, hsf2, hsf3 and hsf4 (Snoeckx et al., 2001; Maaroufi & Tanguay, 2013; Maaroufi & Tanguay, 2015), hsf1 is considered to be the main regulatory factor of cell heat shock protein expression(Moutaoufik et al., 2017a; Moutaoufik et al., 2017b).

Heat shock proteins can improve the body's tolerance to adverse environments and enhance the body's or cell's resistance to subsequent lethal stress. At the same time, it also has the characteristics of protecting and repairing proteins, participating in the body's immunity and cross-protection (Auluck et al., 2002; Nakano & Iwama, 2002). When subjected to heat stress or other environmental stresses, a large number of HSPs bind to denatured proteins, thereby releasing HSF from the HSPs-HSF complex. Free HSF is phosphorylated under the action of protein kinases or other amino acid kinases, and becomes active. The trimer is transferred to the nucleus, binds to the HSE sequence in the upstream promoter region of the heat shock gene in the nucleus, then it is phosphorylated by kinases, and starts the expression of heat shock genes (including hsp70) (Kroeger et al., 1993). When the heat shock protein HSPs accumulate to a certain extent, they bind to HSF, HSF and HSE are separated, and transcription stops, thereby realizing feedback inhibition of heat shock response. After obtaining DNA binding activity, oligomerization, and nuclear localization activities, hsf1 regulates the expression of stress-induced heat shock genes, so that organisms can respond to environmental stresses, such as high temperature, protease inhibitors and other stress environments (Wu, 1995; Pirkkala, 2001).

Materials and methods. *1. Tested materials* **(***B. tabaci***).** The adults of B-biotype *B. tabaci* used in the experiment are long-term breeding populations of the Entomological Laboratory of Henan Institute of Science and Technology. The temperature in the greenhouse is 25–28 ºC, the relative humidity is 60–70 %, the light comes from sodium lamp irradiation, and the alternating day and night sunshine is 12h:12h. At the same time, attention should be paid to regular species detection with primers such as H16 to ensure the unity of insect species. The host plants for feeding *B. tabaci* are cotton, tomato and bean.

2. Main materials. TAE buffer: Purchased from Shanghai Double Helix Biotechnology Co., Ltd., the specification is 400 ml, and stored at room temperature.

Tryptone: Purchased from Beijing Shuangxuan Microbial Culture Medium Product Factory, with a specification of 250 g, and stored in a cool and dry place.

Yeast Dip Powder: Purchased from Beijing Obosing Biotechnology Co., Ltd., the specification is 205 g.

cDNA Synthesis Kit: Primer Script 1st Strand cDNA Synthesis Kit, the item number is D6210A, the specification is 50 times, stored at -20 ºC.

RNA extraction kit: purchased from QIAGEN, REeasy Mini Kit, item number 74104, specification for 50 times, stored at 20 ºC at room temperature.

Gel Extraction Kit: purchased from QIAGEN, item number D2500-01, specification for 50 times, stored at room temperature.

DEPC: purchased from Shanghai Solebold Technology Co., Ltd., the specification is 100 ml, stored at 5 ºC and protected from light.

3. Preserving B. tabaci. In the laboratory, I cut off the blue gun head with scissors and put the big mouth on one end of the rubber tube, then I wrap the other end with gauze, and put the small mouth on, put the small mouth in the cage. I hold the sucker with my mouth, and then the worms are blown into the prepared polypropylene centrifuge tubes (1.5 ml) for later use. Each tube should have more than 200 heads. I take them to the laboratory and put them in a cryogenic processor for 1 hour at low temperature, then freeze them in liquid nitrogen and store -80 ˚C. Store in an ultra-low temperature refrigerator for later RNA extraction. Set 3 replicates for each treatment.

4. Instrument sterilization. We prepared the reagents and utensils used in the experiment. Because RNAse is everywhere in the air, it is very easy to degrade in the extraction process. All utensils used in the experiment should be strictly sterilized. The pipette gun head and centrifuge tube should be imported for sterilization. The glass and metal utensils used in the experiment should be dried at 180 ºC for 8 hours or baked at 250 ºC for more than 3 hours In the process of RNA extraction, gloves should be changed frequently. The sterilization steps are as follows:

Preparation of DEPC water: Add 600ul DEPC per 600 ml of water to make DEPC water, shake it overnight and the next day, autoclave for 30 minutes to obtain Treated Water.

Treat with 1 ml tip, 1.5 ml tube and grinding rod, use 1/1000 of water (made by mixing DEPC and distilled water in a ratio of 1/1000), soak 1ml tip, 1.5 ml tube and grinding rod overnight. Make sure that the DEPC water fills the entire cavity. The next day, I dry the tip and tube and sterilize them with high temperature and high pressure for 30 minutes. I take it out and place it on the clean bench for later use.

5. Extraction method of total RNA. A) Treat the sample at 7 ºC. I Take the leaves with eggs, place them in the ultra-low temperature processor, set the temperature to 7 ºC, treat them for one hour, I take the eggs under the microscope, repeat three times, each centrifuge tube 150 eggs, and then put it in liquid nitrogen for a few seconds, place it on a clean bench, add 1000 ul Trizol, and let it stand at room temperature for 5 minutes.

B) Add 200 ul of chloroform, shake for 15 s, and let stand for 2 minutes.

C) Centrifuge at 4 ºC, rotate at 12000 for 15 minutes, and take the middle layer.

D) Add 500ul is opropanol, gently mix the liquid in the tube, and let it stand at room temperature for 10 minutes.

E) Centrifuge at 4 ºC, rotate at 12000 for 10 minutes, and discard the supernatant.

F) Add 1000 ul of 75 % ethanol, gently wash the precipitate, centrifuge at 4 ºC, and rotate at 7,500 for 5 minutes. Discard the supernatant.

G) Let it dry, add an appropriate amount of DEPC water to dissolve it (65 ºC for 10–15 minutes).

6. Steps to synthesize cDNA. We use RACE technology to obtain full-length cDNA, I prepare the following mixture in a sterilized centrifuge tube, and perform it on an ultra-clean workbench. The amount of reagent Oligo DT primer is 1 ul, the amount of dNTP mixture is 1 ul, the amount of template RNA is 6 ul, Rnase Free water consumption Up to 10 ul. After preparation, after incubating at 65 ºC for 5 minutes, I quickly take it to the ultra-clean workbench, put it on ice in advance, and then add the following reverse transcription reaction solution to the centrifuge tube. The amount of reagent 10*RT buffer is 2 ul, Mgcl2 the dosage is 4 ul, the dosage of DTT is 2 ul, the dosage of Rnase OUT is 0.5 ul, the dosage of sterile water is 1 ul and the dosage of SssII is 0.5 ul.

The total volume is 20 ul. After preparation, the reverse transcription reaction is carried out on a PCR instrument. The setting program is 50 minutes at 50 ºC, 5 minutes at 85 ºC, reverse transcription into cDNA, and then run PCR. The amount of reagent mix is 12.5 ul, the amount of primer 1 is 1 ul, the amount of primer 2 is 1 ul, the amount of cDNA is 1ul, and the amount of sterilized water is 9.5 ul. There are two pairs of primers, one is β-tub-F\R, and the other is P98685-wactR\ P98684-wactF. The PCR program is 5minutes at 95 ºC, 20 second at 55 ºC, 20 second at 72 ºC, 35 cycles, when the time is up, run the electrophoresis and see the electrophoresis diagram.

7. Real-time fluorescence quantitative PCR technology. Through RACE technology, a part of the hsf1 gene sequence (about 900 bp) of *B. tabaci* was amplified. The DNAMAN software analysis verified that the amplified gene sequence accorded with the relevant characteristics of hsf1, and the similarity with the conservative gene sequence of hsf1 reached 66.55 %, can be used for fluorescence quantitative verification. Real-time fluorescent quantitative PCR technology is a method to measure the total amount of products after each polymerase chain reaction (PCR) cycle with fluorescent chemicals in the DNA amplification reaction.In the process of PCR amplification, the PCR process is detected in real time by fluorescent signals.

Results. Through different temperature gradient induction treatments, using spss (13.0) software to analyze statistics, and the significance determination using the new multiple range test (Duncan method), it can be concluded that the highest expression temperature of hsf1 regulatory factors of *Bemisia tabaci* at 9 ºC, followed by 39 ºC the expression differences at other different temperatures were not significant (Fig. 1).

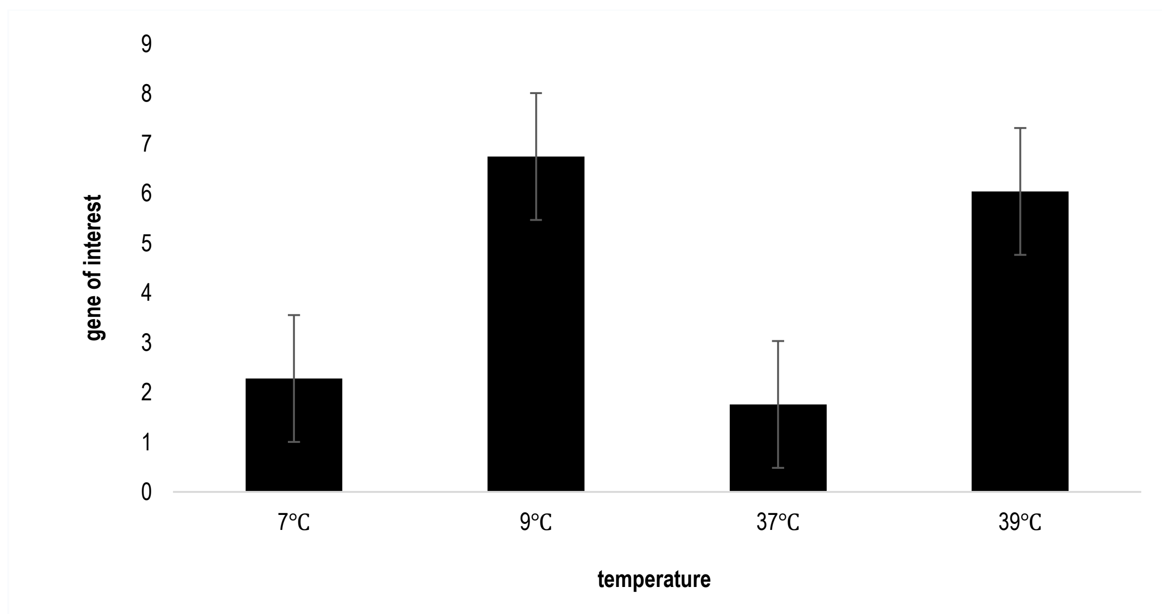

Fig. 1. Hsf1 expression of B-biotype *B. tabaci* at different temperatures.

Fluorescence quantitative PCR technology (RT-PCR) was used to quantitatively detect the expression levels of heat shock protein genes and hsf1 regulatory factors of *B. tabaci* at different temperatures. The results showed that the hsf1 of *B. tabaci* at different growth stages was uniform at low temperature (Fig. 2). There is a significant amount of expression, but there is no significant amount of expression at high temperature. At the same time, it can be seen that hsf1 expression is induced at low temperature. This can clearly indicate that heat shock proteins are induced and protected by hsf1 regulatory factors.

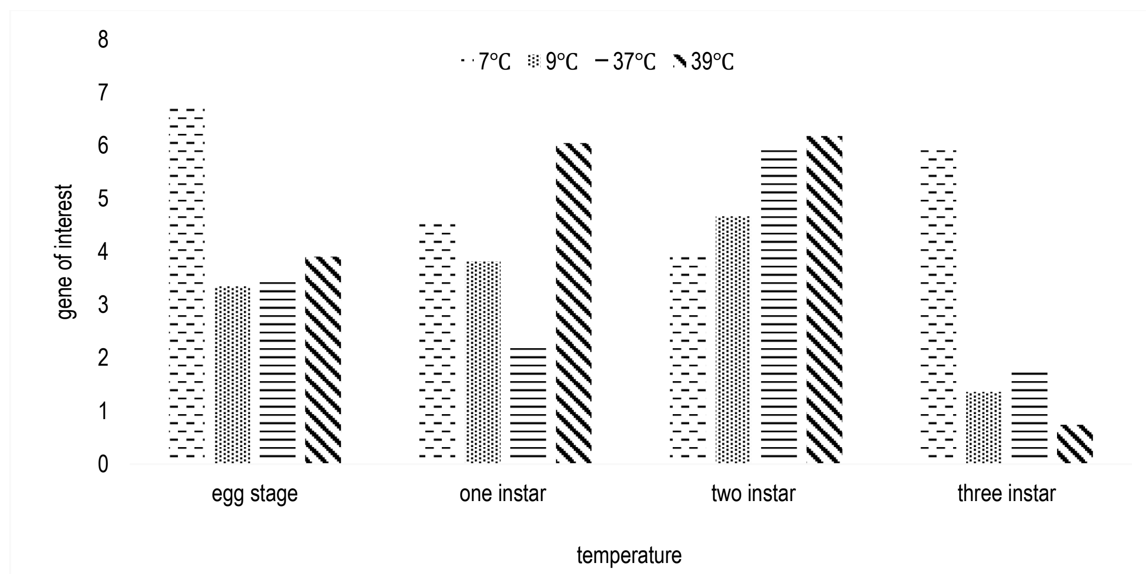

Fig. 2. Differences in expression of hsf1 in different growth stages of *B. tabaci* under various temperatures.

Discussion. In nature, insects are very sensitive to temperature changes and it is difficult to avoid the stress of temperature changes. The adaptive process mechanism of insect tolerance to temperature stress can predict the origin, distribution and dynamics of populations (Holder & Keyhani, 2015; Hoog & Rao, 2015; Jaber & Ownley, 2018). Summer high temperature and dryness are favorable conditions for the exponential growth of the *B. tabaci* population and rampant damage (Shen & Ren, 2007).

The results of this study suggest that hsf1 in *B. tabaci* may be positively correlated with its heat resistance (Geng et al., 2018; Ghanim, 2014; Gianesello et al., 2017; Gilbertson et al., 2015; Guo et al., 2018). The invading *B. tabaci* with stronger heat resistance is subject to environmental pressure in the process of adapting to the new environment, so that it can be selected in the harsh environment. Under environmental stress, it can induce more heat shock protein gene expression, thereby gaining stronger stress resistance.

In the process of long-term adaptation and evolution of insects, the long-term selection of extreme temperatures will affect the temperature adaptability of species. It is difficult for insects to avoid the stress of temperature changes (Kaksonen & Roux, 2018; Kanakala & Ghanim, 2016; Kanakala & Ghanim, 2019; Lei et al., 2020), so that their populations can obtain environmental stress that can be maintained and inherited (Hesketh et al., 2018; Hipp et al., 2017; Hu et al., 2019). When B-biotype *B. tabaci* is subjected to temperature stress, especially extreme temperature stress, heat shock protein factors are rapidly expressed, which can improve the heat tolerance of B-type *B. tabaci* under high temperature, and finally survive the competition (Lü & Wan, 2011).

RT-PCR technology can not only effectively detect gene mutations (Hanan et al., 2020; Hayet et al., 2018), but also accurately detect the expression of oncogenes, which can be used for early diagnosis, classification, staging and prognosis of tumors, and use RT-PCR to detect the expression of various globin genes difference is an effective method for the diagnosis of thalassemia (Yu, 2003; Glare & Inwood, 2014; Goettel, 2015; Hanan et al., 2020). The expression level of specific genes can reflect the growth and survival status of cells. Quantitative analysis of specific gene transcription levels has become a core part of gene function research (Chen, 2003; Wang, 2007; Boulan et al., 2015; Brodsky, 2012; Brown et al., 2015; Chi et al., 2019; Czosnek et al., 2017; Fiallo-Olivé et al., 2020). Finally, China has established a fluorescent quantitative PCR method, and quantitative detection of genetically modified products has been carried out at some ports (Li et al., 2009).

Conclusion. The strong adaptability to temperature stress of *B. tabaci* is an important reason for its successful invasion and colonization. To study the regulation and function of invasive *B. tabaci* hsf1, reveal the mechanism of *B. tabaci* invasion, verify and improve the theoretical hypothesis of *B. tabaci* invasion mechanism. Further reveal the internal mechanism of *B. tabaci* invasion and its adaptability to temperature stress, enrich and perfect the previous researchers' proposals. The hypothesis of *B. tabaci* invasion and adaptive mechanism provides atheoretical basis for biological invasion control research.

The results of this study provide a basis for the research on the resistance and adaptability of *B. tabaci*, and further verify that the conserved functional gene heat shock protein can be used as one of the methods to study the development of biological systems. To study the production of whitefly heat shock protein, and the law of change can be understand the relationship between its growth and development and various influencing factors, and comprehensive provide new ideas for prevention and treatment. In this study, it was found that different temperatures and humidity had different responses to the heat shock of *B. tabaci*.

In practical production, chemical pesticides with strong endoinhalation were usually used to effectively control *B. tabaci* when the heat shock reaction was strong. Based on the change law of greenhouse temperature and humidity, the research is carried out to prevent and control.

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КІЛЬКІСНИЙ АНАЛІЗ РЕГУЛЯТОРНОГО ГЕНА hsf1 BEMISIA TABACI ПРИ РІЗНИХ ТЕМПЕРАТУРАХ

Bemisia tabaci (Gennadius) – один з найважливіших шкідників рослин у тропічних, субтропічних та прилеглих помірних регіонах. B. tabaci – головний шкідник сільського господарства, за яким пильно стежать у всьому світі. Завдяки широкому застосуванню посадки овочів у теплицях та частим суміщенням овочів і квітів були створені більш сприятливі умови для появи та поширення B. tabaci, що робить його головним шкідником у сільськогосподарському виробництві Китаю.

Здатність B-біотипу адаптуватися до нових середовищ тісно пов'язана з його толерантністю до температури. Білки теплового шоку (HSP) – це група білків, що виробляються клітинами під індукцією стресових чинників, особливо високої температури навколишнього середовища. Білки теплового шоку відіграють важливу роль в адаптації організмів до навколишнього середовища. Цей експеримент проводився в основному щодо вивчення білка теплового шоку B. tabaci та його регуляторних чинників (фактор теплового шоку 1, hsf1). Водночас, була використана кількісна технологія флуоресценції для спостереження за вираженням цього регуляторного чинника за різних температурних умов. Існує припущення, що регуляторним чинником HSPs hsf1 є B-біотип B. tabaci, і він може стимулювати захист від стресу високої температури.

Ключові слова*: шкідники рослин у сільськогосподарському виробництві, інвазія шкідника, біологічний захист рослин від шкідників, B-біотип білокрилки тютюнової, регуляторні чинники, hsf1 – фактор теплового шоку.*

Дата надходження до редакції: 03.11.2020 р.