

二斑叶螨抗螺螨酯品系GST基因的克隆与表达分析

吕娟娟¹, 王进军², 张寿芳¹, 沈慧敏^{1,*}

(1. 甘肃农业大学草业学院, 草业生态系统省部共建教育部重点实验室, 中-美草地畜牧业可持续发展中心, 兰州 730070; 2. 西南大学植物保护学院, 昆虫学及害虫控制工程重点实验室, 重庆 400716)

Cloning and expression profiling of glutathione S transferase genes in the spirodiclofen-resistant strain of *Tetranychus urticae*

LÜ Juan-Juan¹, WANG Jin-Jun², ZHANG Shou-Fang¹, SHEN Hui-Min^{1,*}

(1. Sino-U.S. Centers for Grazingland Ecosystem Sustainability, Key Laboratory of Grassland Ecosystem Education Ministry, College of Prataculture, Agricultural University, Lanzhou 730070, China; 2. Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, China)

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摘要 【目的】揭示二斑叶螨 *Tetranychus urticae* 对螺螨酯的分子抗性机理。【方法】利用RT-PCR克隆了二斑叶螨的谷胱甘肽-S-转移酶 (glutathione S-transferase, GST) 基因 cDNA 全长序列, 采用生物信息学软件分析了克隆基因的编码蛋白特性; 利用实时荧光定量PCR方法分析GST基因在二斑叶螨的螺螨酯抗性敏感品系中的表达差异。【结果】克隆获得的谷胱甘肽-S-转移酶2个基因分别被命名为 *TuGSTd1* 和 *TuGSTd2* (GenBank登录号分别为: KC445659和KC445660)。序列分析发现, *TuGSTd1* 的开放阅读框长度为648 bp, 编码215个氨基酸, 分子量约为24.47 kDa, 理论等电点为5.49; *TuGSTd2* 的开放阅读框为648 bp, 编码215个氨基酸, 分子量约为24.57 kDa, 理论等电点为6.33。系统发育分析表明这两个基因与桔全爪螨 *Panonychus citri* Delta家族的GST基因的氨基酸序列一致性为93%。实时荧光定量PCR结果表明, *TuGSTd1* 和 *TuGSTd2* 在二斑叶螨抗螺螨酯品系中的相对表达量分别为敏感品系的5.60和3.75倍。【结论】 GST基因在二斑叶螨抗螺螨酯品系中的相对表达量均显著高于敏感品系, 据此推测GST基因的过量表达可能与其对螺螨酯的抗性形成有关。

关键词: 二斑叶螨 抗螺螨酯品系 GST 基因克隆 表达量 荧光定量PCR

Abstract: 【Aim】 In order to clarify the resistance mechanism of *Tetranychus urticae* to spirodiclofen at the molecular level. 【Methods】 The full-length cDNAs of GST genes of *T. urticae* were cloned by RT-PCR, the structure and function of the coded proteins were analyzed by bioinformatic software, and the expression levels of GST genes in the spirodiclofen-resistant (Sp-R) and susceptible (SS) strains of *T. urticae* were assayed by quantitative real-time PCR. 【Results】 Two GST genes were cloned and named as *TuGSTd1* and *TuGSTd2*, which were deposited in GenBank under the accession no. KC445659 and KC445660, respectively. The open reading frame of *TuGSTd1* is 648 bp in length, encoding 215 amino acids with the predicted molecular mass of 24.47 kDa and the theoretical pI of 5.49, while that of *TuGSTd2* is 648 bp in length, encoding 215 amino acids with the predicted molecular mass of 24.57 kDa and the theoretical pI of 6.33. Phylogenetic analysis showed that these two GST genes have 93% amino acid sequence identity with Delta class GSTs of *Panonychus citri*. Quantitative real-time PCR showed that the relative expression levels of *TuGSTd1* and *TuGSTd2* in Sp-R were 5.60 and 3.75 times as high as those in SS, respectively. 【Conclusion】 The relative expression levels of GST genes in Sp-R were higher than those in SS, suggesting that the up-regulated expression of GST genes is probably related with the development of resistance to spirodiclofen in *T. urticae*.

Key words: *Tetranychus urticae* spirodeclofen-resistant strain glutathione S-transferase (GST) gene cloning expression level real-time fluorescent quantitative PCR

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引用本文:

吕娟娟,王进军,张寿芳等. 二斑叶螨抗螺螨酯品系GST基因的克隆与表达分析[J]. 昆虫学报, 2013, 56(4): 438-445.

Lü Juan-Juan, Wang Jin-Jun, Zhang Shou-Fang et al. Cloning and expression profiling of glutathione S transferase genes in the spirodiclofen-resistant strain of *Tetranychus urticae*[J]. ACTA ENTOMOLOGICA SINICA, 2013, 56(4): 438-445.

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