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Anais



Functional Validation of the Laccase 2 Gene from the Cotton Boll Weevil, Anthonomus grandis (Coleoptera:Curculionidae)

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The insect cuticle pigmentation and sclerotization is a complex process. Laccase 2 is a phenoloxidase involved in reactions of the cuticle tanning pathway. Lack of this protein has been reported to decrease pigmentation and hardness in the cuticle of insects from different orders. Here we present the validation of a Laccase 2 (Lac2) gene from the Cotton Boll Weevil, Anthonomus grandis (Coleoptera:Curculionidae), the most economically important insect-pest in cotton crop in Brazil. A sequence of Lac2 gene was partially cloned (1355 bp) based on contigs from the A. grandis transcriptome. In order to evaluate the relative expression of the Lac2 gene in different instars, different reference genes for qPCR were assessed. From the 9 genes evaluated, GAPDH and β -Tubulin showed to be the most suitable for all conditions. Lac2 gene showed a decreasing expression during the larval instars and a significant increase during pupal and adult phases. The cloned Lac2 sequence was used as template for the synthesis of a 300-bp double-stranded RNA (dsRNA), here called dsAgraLac2. A. grandis 3rd instar larvae were microinjected either with dsAgraLac2, or dsGFP, or 0,02M Tris buffer, ph 8.0. Two experimental sets were established: qPCR analyses were performed 48 hours and 14 days after injection (48-hai and 14-dai, respectively). Phenotype changes were observed 20 days after injection. Deformed adult-like insects were observed in the dsAgraLac2-treated larvae after 14 to 20 days and the expression of Laccase2 gene was ca. 15 times lower when compared to adult control insects. Some 14-dai insects have not started the pupal phase and had 3 times lower expression, as well as the 48-hai larvae. These data show 100% mortality for the dsAgraLac2-treated boll weevils, either resulting in deformed adult-like or development-arrested larvae. The results reassures Laccase2 as an important gene for development and cuticle formation, and as a promising target for the insect-pest control.

Palavras-chave: Gene silencing; Insect cuticle; Pest Control

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