

PCR markers for phosphine resistance in grain beetles: tools for detecting strong resistance genotypes

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Abstract

Phosphine resistant populations of stored product insects have been known for approximately 40 years. Determination of PH₃ resistance in stored product beetles is based on a mortality bioassay that requires 15 days to evaluate the presence or absence of resistant (surviving) adults. We developed PCR-based methods to detect critical point mutations in the highly conserved rph2 locus, the dihydrolipoamide dehydrogenase (DLD) gene that confers PH₃ resistance in geographically disparate populations of the red flour beetle, *Tribolium castaneum*, and the lesser grain borer, *Rhyzopertha dominica*. We first isolated and sequenced DLD genes from known susceptible and strongly resistant populations of *T. castaneum* and *R. dominica*. Then we obtained the corresponding amino acid sequences for each DNA sequence. A single amino acid change in DLD in strong resistant populations of *T. castaneum* and *R. dominica* was identified as P45S in a population of *T. castaneum* from Oklahoma (central USA) and the P49S mutation in a population of *R. dominica* also from Oklahoma. For each species we PCR products from experimental beetles that were digested using the MboI restriction endonuclease, which cleaves the resistant allele sequence and BstNI, which cleaves the sensitive allele sequence by which we could rapidly distinguish resistant genotypes including homozygous strong resistant (RR), heterozygote (RS) and susceptible (SS) for the rph2 locus. Results from our research show that determination of PH₃- resistance in stored product beetles using our PCR amplification, followed by site-specific restriction enzyme digestion of the PCR product, is relatively fast and effective for determining the presence of key resistance alleles from the DLD gene in North American populations of both *T. castaneum* and *R. dominica*.

Keywords: PCR, point mutation, dihydrolipoamide dehydrogenase (DLD), PH₃-resistance, *Tribolium castaneum*, *Rhyzopertha dominica*